Reference Series in Phytochemistry
Series Editors:
J.-M. Mérillon · K. G. Ramawat

SPRINGER
REFERENCE

Jean-Michel Mérillon
Kishan Gopal Ramawat *Editors*

Bioactive Molecules in Food

Springer

Reference Series in Phytochemistry

Series Editors
Jean-Michel Mérillon
Faculty of Pharmaceutical Sciences
Institute of Vine and Wine Sciences
University of Bordeaux
Villenave d'Ornon, France

Kishan Gopal Ramawat
Department of Botany, University College of Science, M. L. Sukhadia University
Udaipur, Rajasthan, India

This reference works series provides a platform for all information on plant metabolites and phytochemicals, their chemistry, properties, applications, and methods. By the strictest definition, phytochemicals are chemicals derived from plants. However, the term is often used to describe the large number of secondary metabolic compounds found in and derived from plants. These metabolites exhibit a number of nutritional and protective functions for human welfare such as colorants, fragrances and flavorings, amino acids, pharmaceuticals, hormones, vitamins and agrochemicals. Besides food, fibers, fuel, cloth and shelter, a vast number of wild plants can hence provide important sources for medicines, especially in developing countries for their traditional health systems. Natural products have inspired and provided the foundation to the bulk of FDA-approved compounds and there is tremendous increase in natural products and natural products derived compounds that have been registered against many prevailing diseases. Natural product industry has shown tremendous growth and is expected to continue to do so in the near future. The present series compiles reference information on various topics and aspects about phytochemicals, including their potential as natural medicine, their role as chemo-preventers, in plant defense, their ecological role, their role in plants as well as for pathogen adaptation, and disease resistance. Volumes in the series also contain information on methods such as metabolomics, genetic engineering of pathways, molecular farming, and obtaining metabolites from lower organisms and marine organisms besides higher plants. The books in the series are hence of relevance in various fields, from chemistry, biology, biotechnology, to pharmacognosy, pharmacology, botany, or medicine. Each volume is edited by leading experts and contains authoritative contributions by renowned authors.

More information about this series at http://www.springer.com/series/13872

Jean-Michel Mérillon
Kishan Gopal Ramawat
Editors

Bioactive Molecules in Food

With 238 Figures and 206 Tables

Springer

Editors
Jean-Michel Mérillon
Faculty of Pharmaceutical Sciences
Institute of Vine and Wine Sciences
University of Bordeaux
Villenave d'Ornon, France

Kishan Gopal Ramawat
Department of Botany
University College of Science
M. L. Sukhadia University
Udaipur, Rajasthan, India

ISSN 2511-834X ISSN 2511-8358 (electronic)
ISBN 978-3-319-78029-0 ISBN 978-3-319-78030-6 (eBook)
ISBN 978-3-319-78031-3 (print and electronic bundle)
https://doi.org/10.1007/978-3-319-78030-6

Library of Congress Control Number: 2018964906

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

We are pleased to present a three-volume treatise on *Bioactive Molecules in Food*. Bioactive molecules are present in all the food consumed, and we come to know more about them with the availability of more and more sophisticated tools and techniques. As we know more about benefits of food and their molecules, we intend to improve food quality and fortify the food for human welfare and disease prevention. The aim of the book is to present the state of information about bioactive molecules present in various foods we consume daily and their effect on our physical and mental state of body. The concept of functional foods is of recent origin, but in old civilizations like that of China and India, there are sayings about consumption of selected food and their effect on well-being. Hence history is being revisited.

Selection, cultivation, and domestication of plants for human welfare is almost 9,000 years old which is reconfirmed by a recent report from the remains of domesticated crop plants at an archaeological site in southwest Amazonia. Plants are easy to catch as compared to animals, and hunting evolved with evolution of tools and techniques with civilization of men. Thus, fruits, tubers, bulbs, and seeds were easy to gather and consumed by early men. Some chapters deal with cultivation and improvement of nutrients in food plants.

The debate about food consumption and vegetarian and nonvegetarian diet or consumption of various fatty acids in oils (PUMA, MUFA, omega-3 FA) may continue for another decade. With the new facts coming out day by day, this scene changes for a while and then resets to a new concept. Whatever the diet regimen, a cereal, a pulse, and a fruit make an essential part of all diets. With the advent of new and sophisticated technology, more information available about bioactive molecules present in the food, best combinations of food components to meet nutritional requirement, and fate of ingested molecules as well as their beneficial effects are known. Major part of the book is devoted to this aspect.

Consumers have become very attentive to quality, safety, and health benefits of food. This has direct repercussion on food industry to develop such foods, especially processed and packaged foods and particularly in terms of calorie, quality, nutritional value, and bioactive molecules present in them. Market for nutraceuticals and food supplements is growing at rapid pace with increasing middle class in developing countries and elderly population in developed countries. Therefore, this is a very

timely compilation of information about bioactive molecules present in our daily food.

Longevity of life is associated with preferential consumption of selected food which can prevent diseases and keep overall health in good condition. Society is keen to know the benefits of organic food, bioactive molecules present in their food, and possibility of food fortified with beneficial molecules. This has direct impact on research related to this field by all those involved in food cultivation, processing, and production including meat quality. Certain chapters are devoted to this aspect in the book.

The book is divided into ten parts (I. Favorable Regimen for Good Health and Epidemiological Studies, II. Proteins and Their Biological Activity, III. Lipids and Their Biological Activity, IV. Food Carbohydrates and Derived Compounds, V. Food Carotenoids, VI. Food Polyphenols, VII. Functional Foods, VIII. Agricultural Practices and Food Quality, IX. Methods of Food Analysis and Quality Control, X. Miscellaneous Case Studies) covering entire gamut of bioactive molecules present in daily foods like cereals (carbohydrates), pulses, and other sources (peptides and proteins); oils (lipids, fatty acids); mushrooms, wine, and fruits and vegetables (carotenoids, polyphenols, etc.); and fibers and methods of analysis of some foods. Concepts like French paradox, Mediterranean diet, healthy diet of eating fruits and vegetables, vegan and vegetarian diet, and functional foods are also described with suitable case studies.

Well-recognized international specialists in their respective fields of research contributed these chapters. This book will be useful to all those working in the field of botany, phytochemistry, pharmacy, drug delivery, molecular biology, forestry, biotechnology, industrial food, and medical products. This work is arranged in 78 well-illustrated chapters.

Because of the voluminous work for the treatise, this project was spread over almost 2 years, from concept to print. We would like to acknowledge the cooperation, patience, and support of our contributors who have put their serious efforts to ensure the high scientific quality of this book with up-to-date information. We are thankful to the staff at Springer, namely, Drs. S. Costa, S. Blago, and N. Clifford, for their professional support in this project.

Prof. Jean-Michel Mérillon
Prof. Kishan Gopal Ramawat

Contents

Volume 1

Volume 3

About the Editors

Prof. Dr. Jean-Michel Mérillon received his M.Pharma. (1979) and Ph.D. (1984) from the University of Tours, France. He joined the university of Tours as assistant professor in 1981, became associate professor in 1987. In 1993 he moved to the faculty of Pharmacy, University of Bordeaux, France, accepting a position as full professor. He has been the director of the research group on biologically active plant substances for over 15 years, at the Institute of Vine and Wine Sciences (University of Bordeaux, France), which comprises 25 scientists and research students. The group has been working on phenolic compounds from vine and wine for many years, mainly complex stilbenes and their involvement in health. He is involved in developing teaching on plant biology, natural bioactive compounds and biotechnology. Prof. Mérillon has published more than 160 research papers in internationally recognized journals, and has coedited books and reference works on secondary metabolites and biotechnology. In 2004, he founded the technology transfer unit "Polyphenols Biotech", providing support for R&D programs for SMEs and major groups from the cosmetic, pharmaceutical, agricultural and health-nutrition sectors. He is currently the manager of this unit.

Prof. Dr. Kishan Gopal Ramawat is former professor and head of the Botany Department, M.L. Sukhadia University, Udaipur, India, and has a long-standing research experience. He received his Ph.D. in Plant Biotechnology in 1978 from the University of Jodhpur, India, and afterward joined the university as a faculty member. In 1991, he moved to the M.L. Sukhadia University in Udaipur as associate professor and became professor in 2001. He served as the head of the Department of Botany (2001–2004, 2010–2012), was in charge of the Department of Biotechnology (2003–2004), was a member of the task force on medicinal and aromatic plants of the Department of Biotechnology, Government of India, New Delhi (2002–2005), and coordinated UGC-DRS and DST-FIST programs (2002–2012).

Professor Ramawat had done his postdoctoral studies at the University of Tours, France, from 1983 to 1985 and later returned to Tours as visiting professor (1991). He also visited the University of Bordeaux 2, France, several times as visiting professor (1995, 1999, 2003, 2006, 2010) and, in 2005, in Poland in an academic exchange program (2005). Through these visits in France, Prof. Ramawat and Prof. Mérillon established a strong connection, which has resulted in productive collaborations and several book and reference work publications.

Professor Ramawat has published more than 170 well-cited peer-reviewed papers and articles and edited several books and reference works on topics such as the biotechnology of medicinal plants, secondary metabolites, bioactive molecules, herbal drugs, and many other topics. His research was funded by several funding agencies.

In his research group, Prof. Ramawat has supervised doctoral thesis of 25 students. He is an active member of several academic bodies, associations, and editorial boards of journals. Botany Department, M.L. Sukhadia University, Udaipur, India.

Contributors

Christian Izuchukwu Abuajah Department of Food Science and Technology, University of Uyo, Uyo, Nigeria

Ome Kalu Achi Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Nigeria

Milica G. Aćimović Department of Alternative Crops and Organic Agriculture, Institute of Field and Vegetable Crops, Novi Sad, Serbia

Abiola Fatimah Adenowo Department of Biochemistry and Microbiology, University of Zululand, Kwadlangezwa, South Africa

Abdelkareem Abdall Ahmed Department of Physiology and Biochemistry, Faculty of Veterinary Sciences, University of Nyala, Nyala, Sudan

Saeed Akhtar Institute of Food Science and Nutrition, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan

Natalia Manzatti Machado Alencar Department of Food and Nutrition, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil

Asgar Ali Centre of Excellence for Postharvest Biotechnology (CEPB), School of Biosciences, University of Nottingham, Semenyih, Malaysia

Emilio Alvarez Parrilla Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez. Anillo Envolvente del PRONAF y Estocolmo s/n. Cd. Juárez, Chihuahua, Mexico

Ibrahim Alzorqi Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham, Semenyih, Malaysia

Armin Amanpour Department of Food Engineering, Faculty of Agriculture, Cukurova University, Adana, Turkey

Luis M. Anaya-Esparza Laboratorio de Microbiología de Alimentos, Departamento de Ciencias Pecuarias y Agrícolas, Universidad de Guadalajara, Centro Universitario de los Altos, Tepatitlán de Morelos, Jalisco, Mexico

Laboratorio Integral de Investigación en Alimentos, Instituto Tecnológico de Tepic, Tepic, Nayarit, Mexico

Juan C. Angeles Hernandez Departamento de Nutricion Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de Mexico, Toluca, Estado de Mexico, Mexico

Anjali Division of Biochemistry, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu, Jammu and Kashmir, India

Sudheer K. Annepu Divison of Crop Improvement, ICAR-Directorate of Mushroom Research, Solan, Himachal Pradesh, India

Vanessa Aparecida Marcolino Federal Institute of Paraná (IFPR), Paranavaí, Parana, Brazil

Eugenio Aprea Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all'Adige, Trento, Italy

Jaya Arora Department of Botany, University College of Science, M. L. Sukhadia University, Udaipur, India

Pablo Arranz-Martínez Department of Production and Characterization of Novel Foods, Institute of Food Science Research (CIAL,CSIC-UAM), Madrid, Spain

Shalini S. Arya Food Engineering and Technology Department, Institute of Chemical Technology, Mumbai, India

Naomi U. Asamudo Department of Microbiology, University of Uyo, Uyo, Nigeria

Edward Rob Atwill Western Institute for Food Safety and Security, School of Veterinary Medicine, University of California Davis, Davis, CA, USA

René R. Balandrán-Quintana Centro de Investigación en Alimentación y Desarrollo, A.C., Coordinación de Tecnología de Alimentos de Origen Vegetal, Hermosillo, Son., México

Habeeb Adebodun Bankole Department of Biochemistry, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria

Barbara Baraniak Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Lublin, Poland

Helena Rudge de Moraes Barros Department of Food Science and Experimental Nutrition, University of São Paulo, São Paulo, Brazil

Elvira Barroso Department of Production and Characterization of Novel Foods, Institute of Food Science Research (CIAL,CSIC-UAM), Madrid, Spain

Andressa Mara Baseggio Department of Food Science, Faculty of Food Engineering, University of Campinas, Campinas, Brazil

Alaa El-Din A. Bekhit Department of Food Sciences, University of Otago, Dunedin, New Zealand

Catherine Bennetau-Pelissero Department of Life Science and Health, University of Bordeaux, Bordeaux, France

Department Feed and Food, Bordeaux Sciences Agro, Gradignan, France

Asani Bhaduri Cluster Innovation Centre, University of Delhi, Delhi, India

Hina F. Bhat Division of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu and Kashmir, India

Z. F. Bhat Division of Livestock Products Technology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, Jammu and Kashmir, India

Cristine Vanz Borges Department of Chemistry and Biochemistry, Institute of Biosciences, Sao Paulo State University – UNESP, Botucatu, São Paulo, Brazil

Masood Sadiq Butt National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad, Pakistan

Conceição Calhau Nutrição e Metabolismo, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal

ProNutri - Clinical Nutrition and Disease Programming, CINTESIS - Center for Research in Health Technologies and Information Systems, Porto, Portugal

Maria A. Carluccio Institute of Clinical Physiology (IFC), National Research Council, Lecce, Italy

Olga Castañer Cardiovascular Risk and Nutrition Research Group (CARIN), Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain

CIBER of the Physiopathology of Obesity and Nutrition (CIBEROBN), Institut Hospital del Mar d'Investigacions Mediques (IMIM), Barcelona, Spain

Octavio Castelan Ortega Departamento de Nutricion Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de Mexico, Toluca, Estado de Mexico, Mexico

Anoma Chandrasekara Department of Applied Nutrition, Faculty of Livestock Fisheries and Nutrition, Wayamba University of Sri Lanka, Gonawila, Sri Lanka

Mathilde Charles BIOFORTIS Sensory and Consumer, Saint Herblain, France

Manish Kumar Chatli Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Kotamballi N. Chidambara Murthy Central Research Laboratory, Ramaiah Medical College and Hospital, MSRIT Post, Bengaluru, India

Wee Sim Choo School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Selangor, Malaysia

Marta Corzo-Martínez Department of Production and Characterization of Novel Foods, Institute of Food Science Research (CIAL,CSIC-UAM), Madrid, Spain

Dayanand Dalawai Department of Botany, Karnatak University, Dharwad, India

Vijayalaxmi S. Dandin Department of Botany, Karnatak University, Dharwad, India

Mamali Das Department of Biotechnology, Alagappa University [Science Campus], Karaikudi, Tamil Nadu, India

Theophilus Clavell Davies Department of Geology, University of Nigeria, Nsukka, Nigeria

Ludmilla de Carvalho Oliveira Department of Food Technology, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil

Victor de Freitas REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Mario Fernández de Frutos Madrid Institute for Advanced Studies in Food, Madrid, Spain

J. Abraham Domínguez Avila Centro de Investigación en Alimentación y Desarrollo A.C. Carretera a La Victoria km 0.6, Hermosillo, Sonora, Mexico

Natalia Drabińska Department of Chemistry and Biodynamics of Food, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland

Som Dutt Division of Crop Physiology, Biochemistry and Post Harvest Technology, ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

Carlos Eduardo Barão Federal Institute of Paraná (IFPR), Paranavaí, Parana, Brazil

Ana Évora REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Ana Faria REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Nutrição e Metabolismo, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal

ProNutri - Clinical Nutrition and Disease Programming, CINTESIS - Center for Research in Health Technologies and Information Systems, Porto, Portugal

Azeez Ayomide Fatai Department of Biochemistry, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria

Milad Fathi Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

Iva Fernandes REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Isabel C. F. R. Ferreira Mountain Research Centre (CIMO), Polytechnique Institute of Bragança, Bragança, Portugal

Instituto Politécnico de Bragança, Bragança, Portugal

Montserrat Fitó Cardiovascular Risk and Nutrition Research Group (CARIN), Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain

CIBER of the Physiopathology of Obesity and Nutrition (CIBEROBN), Institut Hospital del Mar d'Investigacions Mediques (IMIM), Barcelona, Spain

Richard J. FitzGerald Department of Biological Sciences, University of Limerick, Limerick, Ireland

José David Flores-Félix Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain

Spanish-Portuguese Institute for Agricultural Research (CIALE), Salamanca, Spain

Bartosz Fotschki Department of Biological function of food, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland

Thumballi R. Ganapathi Plant Cell Culture Technology Section, Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, India

Paula García-Fraile Institute of Microbiology ASCR,v.v.i, Prague, Czech Republic

Lab 210-213, Edificio Departamental de Biología, Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain

Flavia Gasperi Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all'Adige, Trento, Italy

Maria Inés Genovese Department of Food Science and Experimental Nutrition, University of São Paulo, São Paulo, Brazil

Siddhesh B. Ghag UM-DAE Centre for Excellence in Basic Sciences, Mumbai, Maharashtra, India

Giovanna Giovinazzo Institute of Sciences of Food Production (ISPA), National Research Council, Lecce, Italy

Hector Alonzo Gomez-Gomez Department of Chemistry and Biochemistry, Institute of Biosciences, Sao Paulo State University – UNESP, Botucatu, São Paulo, Brazil

Gustavo A. González Aguilar Centro de Investigación en Alimentación y Desarrollo A.C. Carretera a La Victoria km 0.6, Hermosillo, Sonora, Mexico

Manuel Gonzalez Ronquillo Departamento de Nutricion Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de Mexico, Toluca, Estado de Mexico, Mexico

Francesco Grieco Institute of Sciences of Food Production (ISPA), National Research Council, Lecce, Italy

Ricardo J. R. Guilherme QOPNA, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Moni Gupta Division of Biochemistry, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu, Jammu and Kashmir, India

Sachin Gupta Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, India

Janet A. Gutiérrez-Uribe Tecnologico de Monterrey, School of Engineering and Science, Department of Bioengineering and Science, Campus Puebla, Puebla, Mexico

Pádraigín A. Harnedy Department of Biological Sciences, University of Limerick, Limerick, Ireland

Sumio Hayakawa Department of Cellular and Molecular Medicine, Medical Research Institute, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan

Álvaro Hernáez Cardiovascular Risk and Nutrition Research Group (CARIN), Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain

CIBER of the Physiopathology of Obesity and Nutrition (CIBEROBN), Institut Hospital del Mar d'Investigacions Mediques (IMIM), Barcelona, Spain

Muhammad Imran National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

Department of Diet and Nutritional Sciences, Faculty of Health and Allied Sciences, Imperial College of Business Studies, Lahore, Pakistan

Muhammad Jawad Iqbal National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad, Pakistan

Mahdi Irani Gum Group Knowledge Base Company, Technology Development Center, Ferdowsi University of Mashhad, Mashhad, Iran

Mamoru Isemura Tea Science Research Center, University of Shizuoka, Suruga-ku, Shizuoka, Japan

Tariq Ismail Institute of Food Science and Nutrition, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan

Seid Mahdi Jafari Department of Food Materials and Process Design Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Anna Jakubczyk Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Lublin, Poland

Basil Jarvis Daubies Farm, Ross-on-Wye, UK

Department of Food and Nutritional Sciences, School of Chemistry, Food and Pharmacy, The University, Reading, UK

Suellen Jensen Klososki Federal Institute of Paraná (IFPR), Paranavaí, Parana, Brazil

Alejandro Jiménez-Gómez Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain

Spanish-Portuguese Institute for Agricultural Research (CIALE), Salamanca, Spain

Sandeep Baban Kalse Department of Post Harvest Engineering, Post Graduate Institute of Post Harvest Management, Killa Roha, Maharashtra, India

Kristina Kappel Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany

Monika Karaś Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Lublin, Poland

Mehri Karim Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

Mutiu Idowu Kazeem Department of Biochemistry, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria

Hasim Kelebek Department of Food Engineering, Faculty of Engineering and Natural Sciences, Adana Science and Technology University, Adana, Turkey

Soroush Rahimi Khoigani Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

Nevzat Konar Faculty of Architecture and Engineering, Department of Food Engineering, Siirt University, Siirt, Turkey

Aleksandra Kozłowska First Faculty of Medicine, Department of Social Medicine and Public Health, Medical University of Warsaw, Warsaw, Poland

Urszula Krupa-Kozak Department of Chemistry and Biodynamics of Food, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland

Omer Kucuk Winship Cancer Institute of Emory University, Atlanta, GA, USA

Dinesh Kumar Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Pavan Kumar Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Yogesh Kumar ICAR – Central Institute of Post-Harvest Engineering and Technology (CIPHET), Ludhiana, Punjab, India

Anam Layla Institute of Food Science and Nutrition, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan

Aurélien V. Le Gouic Department of Biological Sciences, University of Limerick, Limerick, Ireland

Xunde Li Western Institute for Food Safety and Security, School of Veterinary Medicine, University of California Davis, Davis, CA, USA

Giuseppina Pace Pereira Lima Department of Chemistry and Biochemistry, Institute of Biosciences, Sao Paulo State University – UNESP, Botucatu, São Paulo, Brazil

José Moisés Laparra Llopis Madrid Institute for Advanced Studies in Food, Madrid, Spain

Monica Rosa Loizzo Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Cosenza, Italy

Ronan Lordan Department of Biological Sciences, University of Limerick, Limerick, Ireland

Aline Carbonera Luvizon Faculdade Sudoeste Paulista – FSP, Avaré, São Paulo, Brazil

Terrence Madhujith Faculty of Agriculture, Department of Food Science and Technology, University of Peradeniya, Peradeniya, Sri Lanka

Om Prakash Malav Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Sivakumar Manickam Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham, Semenyih, Malaysia

Cláudia Marques Nutrição e Metabolismo, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal

ProNutri - Clinical Nutrition and Disease Programming, CINTESIS - Center for Research in Health Technologies and Information Systems, Porto, Portugal

Faculty of Medicine, University of Porto, Porto, Portugal

Edwin E. Martínez Leo Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico

Natália Martins Mountain Research Centre (CIMO), Polytechnique Institute of Bragança, Bragança, Portugal

REQUIMTE/LAQV, Faculty of Pharmacy, University of Porto, Porto, Portugal

Susan Mason Department of Wine Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand

Nuno Mateus REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Meeta Mathur Department of Botany, Mithibai College, University of Mumbai, Mumbai, India

Samira Mebdoua Laboratoire d'amélioration Intégrative des Productions Végétales, Ecole Nationale Supérieure Agronomique, Alger, Algeria

Nitin Mehta Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Rogério Mendes Portuguese Institute for the Sea and Atmosphere, Lisbon, Portugal

Ana María Mendoza-Wilson Centro de Investigación en Alimentación y Desarrollo, A.C., Coordinación de Tecnología de Alimentos de Origen Vegetal, Hermosillo, Son., México

Igor Otavio Minatel Department of Chemistry and Biochemistry, Institute of Biosciences, Sao Paulo State University – UNESP, Botucatu, São Paulo, Brazil

Faculdade Sudoeste Paulista – FSP, Avaré, São Paulo, Brazil

Shabir Ahmad Mir Department of Food Technology, Islamic University of Science and Technology, Awantipora, Jammu and Kashmir, India

Remya Mohanraj Houston Community College, Houston, TX, USA

P. Monika Department of Biotechnology, Ramaiah Institute of Technology, MSRIT Post, Bengaluru, India

Efigenia Montalvo-González Laboratorio Integral de Investigación en Alimentos, Instituto Tecnológico de Tepic, Tepic, Nayarit, Mexico

James D. Morton Department of Wine Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand

Vahid Mosayebi Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

Hosakatte Niranjana Murthy Department of Botany, Karnatak University, Dharwad, India

Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju, Republic of Korea

Hassan Hussein Musa Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan

Taha Hussein Musa Key Laboratory of Environmental Medicine, Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu, China

Ricardo Fernández Musoles Valencian International University, Valencia, Spain

Emine Nakilcioglu-Tas Department of Food Engineering, Faculty of Engineering, Ege University, Bornova, Izmir, Turkey

Androniki Naska Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Constantina Nasopoulou Department of Food Science and Nutrition, School of the Environment, University of the Aegean, Myrina, Lemnos, Greece

Yumiko Oishi Department of Cellular and Molecular Medicine, Medical Research Institute, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan

Maria Beatriz P. P. Oliveira REQUIMTE/LAQV, Faculty of Pharmacy, University of Porto, Porto, Portugal

Cemal Orhan Department of Animal Nutrition, Faculty of Veterinary Science, Firat University, Elazig, Turkey

Jorge Osorio Avalos Departamento de Nutricion Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de Mexico, Toluca, Estado de Mexico, Mexico

Semih Otles Department of Food Engineering, Faculty of Engineering, Ege University, Bornova, Izmir, Turkey

Kee-Yoeup Paek Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju, Republic of Korea

Ibrahim Palabiyik Faculty of Agriculture, Department of Food Engineering, Namik Kemal University, Tekirdağ, Turkey

Kasi Pandima Devi Department of Biotechnology, Alagappa University [Science Campus], Karaikudi, Tamil Nadu, India

Arti Parihar Bellingham Technical College, Bellingham, WA, USA

Mordhwaj S. Parihar School of Studies in Zoology and Biotechnology, Vikram University, Ujjain, Madhya Pradesh, India

So-Young Park Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju, Republic of Korea

Maike Passon Institute of Nutritional and Food Sciences, Molecular Food Technology, University of Bonn, Bonn, Germany

Niti Pathak Department of Botany, Deshbandhu College, University of Delhi, Delhi, India

Kakoli Pegu Food Engineering and Technology Department, Institute of Chemical Technology, Mumbai, India

Alejandra Pérez-Vega Cardiovascular Risk and Nutrition Research Group (CARIN), Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain

CIBER of the Physiopathology of Obesity and Nutrition (CIBEROBN), Institut Hospital del Mar d'Investigacions Mediques (IMIM), Barcelona, Spain

Chia-Wei Phan Mushroom Research Centre, University of Malaya, Kuala Lumpur, Malaysia

Department of Pharmacy, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Tatiana Colombo Pimentel Federal Institute of Paraná (IFPR), Paranavaí, Parana, Brazil

Haniyeh Rasouli Pirouzian Department of Food Science and Technology, Tabriz University of Medical Sciences, Tabriz, Iran

Paolo Polidori School of Pharmacy, University of Camerino, Camerino (MC), Italy

Valeria Polzonetti School of Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy

Alejandra M. Preciado Saldaña Centro de Investigación en Alimentación y Desarrollo A.C. Carretera a La Victoria km 0.6, Hermosillo, Sonora, Mexico

Stefania Pucciarelli School of Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy

Zahra Rafiee Department of Food Materials and Process Design Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Ashwani K. Rai Department of Botany, Banaras Hindu University, Varanasi, India

Pinky Raigond Division of Crop Physiology, Biochemistry and Post Harvest Technology, ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

Kishan Gopal Ramawat Department of Botany, University College of Science, M. L. Sukhadia University, Udaipur, India

Seyed Mohammad Ali Razavi Food Hydrocolloids Research Centre, Department of Food Science and Technology, Ferdowsi University of Mashhad (FUM), Mashhad, Iran

Guillermo Reglero Department of Production and Characterization of Novel Foods, Institute of Food Science Research (CIAL,CSIC-UAM), Madrid, Spain

Department of Production and Development of Foods for Health, IMDEA-Food Institute, CEI (UAM-CSIC), Madrid, Spain

Natália Crialeison Balbo Vall Ribeiro Department of Food Science, Faculty of Food Engineering, University of Campinas, Campinas, Brazil

Raúl Rivas Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain

Spanish-Portuguese Institute for Agricultural Research (CIALE), Salamanca, Spain

Associated R&D Unit, USAL-CSIC (IRNASA), Salamanca, Spain

Lab 210-213, Edificio Departamental de Biología, Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain

Lizbeth E. Robles Jimenez Departamento de Nutricion Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de Mexico, Toluca, Estado de Mexico, Mexico

Sílvia M. Rocha QOPNA, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Delia B. Rodriguez-Amaya Faculty of Food Engineering, University of Campinas, Campinas, Sâo Paulo, Brazil

Universidade Federal da Fonteira Sul, Laranjeiras do Sul, Parana, Brazil

Rafael A. Rojas Herrera Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico

Cristina M. Rosell Food Science Department, Institute of Agrochemistry and Food Technology (IATA-CSIC), Paterna, Valencia, Spain

Michele Rosset Federal Institute of Paraná (IFPR), Colombo, Parana, Brazil

Jacqueline Ruiz Canizales Centro de Investigación en Alimentación y Desarrollo A.C. Carretera a La Victoria km 0.6, Hermosillo, Sonora, Mexico

Vikineswary Sabaratnam Mushroom Research Centre, University of Malaya, Kuala Lumpur, Malaysia

Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

Prajakta D. Sadawarte Food Engineering and Technology Department, Institute of Chemical Technology, Mumbai, India

Kazim Sahin Department of Animal Nutrition, Faculty of Veterinary Science, Firat University, Elazig, Turkey

Nurhan Sahin Department of Animal Nutrition, Faculty of Veterinary Science, Firat University, Elazig, Turkey

Ângelo C. Salvador QOPNA, Department of Chemistry, University of Aveiro, Aveiro, Portugal

CICECO- Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Liliana Santos-Zea Tecnologico de Monterrey, School of Engineering and Science, Centro de Biotecnología-FEMSA, Monterrey, Mexico

Vicki Schlegel Department of Food Science and Technology, University of Nebraska Lincoln, Lincoln, NE, USA

Ute Schröder Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany

Maira R. Segura Campos Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico

Serkan Selli Department of Food Engineering, Faculty of Agriculture, Cukurova University, Adana, Turkey

Manzoor Ahmad Shah Department of Food Science and Technology, Pondicherry University, Puducherry, India

Ramesh Kumar Sharma Food and Environment Issues, Bikaner, Rajasthan, India

M. Shivapriya College of Horticulture, University of Horticultural Sciences, GKVK, Bengaluru, India

Vincenzo Sicari Department of Agricultural Science, Mediterranean University of Reggio Calabria, Reggio, Calabria, Italy

Helena Silva Portuguese Institute for the Sea and Atmosphere, Lisbon, Portugal

Armando J. D. Silvestre CICECO- Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Brajesh Singh Division of Crop Physiology, Biochemistry and Post Harvest Technology, ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

Brij Pal Singh Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India

Charalampos Siotos Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Subajiny Sivakanthan Faculty of Agriculture, Department of Agricultural Chemistry, University of Jaffna, Jaffna, Sri Lanka

Carmen G. Sotelo Instituto de Investigaciones Marinas (CSIC), Vigo, Spain

Soma Srivastava Division of Agricultural Engineering and Renewable Energy, Central Arid Zone Research Institute, Jodhpur, India

Anna Stachniuk Laboratory of Separation and Spectroscopic Method Applications, Center for Interdisciplinary Research, The John Paul II Catholic University of Lublin, Lublin, Poland

Surya Sudheer Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham, Semenyih, Malaysia

Hafiz Ansar Rasul Suleria UQ School of Medicine, Translational Research Institute, The University of Queensland, Brisbane, QLD, Australia

Department of Food, Nutrition, Dietetics and Health, Kansas State University, Manhattan, KS, USA

Baby Summuna Division of Plant Pathology, Faculty of Agriculture, SKUAST-Kashmir, Jammu, Jammu and Kashmir, India

Yasuo Suzuki Department of Nutrition Management, Faculty of Health Science, Hyogo University, Kakogawa, Hyogo, Japan

Shrikant Baslingappa Swami Department of Post Harvest Engineering, Post Graduate Institute of Post Harvest Management, Killa Roha, Maharashtra, India

Dorota Szostak-Węgierek Faculty of Health Science, Department of Clinical Dietetics, Medical University of Warsaw, Warsaw, Poland

Elson Yi-Yong Tan Mushroom Research Centre, University of Malaya, Kuala Lumpur, Malaysia

Department of Pharmacy, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Hiroki Tanabe Department of Nutritional Sciences, Faculty of Health and Welfare Science, Nayoro City University, Nayoro-City, Hokkaido, Japan

B. Tejashree Department of Biotechnology, Ramaiah Institute of Technology, MSRIT Post, Bengaluru, India

Omer Said Toker Faculty of Chemistry and Metallurgical, Department of Food Engineering, Yildiz Technical University, İstanbul, Turkey

Carlos Torres Department of Production and Characterization of Novel Foods, Institute of Food Science Research (CIAL,CSIC-UAM), Madrid, Spain

Alexandros Tsoupras Department of Biological Sciences, University of Limerick, Limerick, Ireland

Rosa Tundis Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Cosenza, Italy

Julieta Valussi Cardiovascular Risk and Nutrition Research Group (CARIN), Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain

CIBER of the Physiopathology of Obesity and Nutrition (CIBEROBN), Institut Hospital del Mar d'Investigacions Mediques (IMIM), Barcelona, Spain

Luis Vázquez Department of Production and Characterization of Novel Foods, Institute of Food Science Research (CIAL,CSIC-UAM), Madrid, Spain

Gustavo R. Velderrain Rodríguez Centro de Investigación en Alimentación y Desarrollo A.C. Carretera a La Victoria km 0.6, Hermosillo, Sonora, Mexico

V. Venugopal Former Scientific Officer Food Technology Division, Bhabha Atomic Research Center, Mumbai, India

Visiting faculty, Department of Food Science and Technology, Kerala University of Fisheries and Ocean Sciences (KUFOS), Kochi, Kerala, India

Akhilesh Kumar Verma Department of Livestock Products Technology, College of Veterinary Science, Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya evam Go Anusandhan Sansthan (DUVASU), Mathura, Uttar Pradesh, India

Véronique Verrez-Bagnis Ifremer, Nantes, France

Shilpa Vij Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India

Mónica A. Villegas Ochoa Centro de Investigación en Alimentación y Desarrollo A.C. Carretera a La Victoria km 0.6, Hermosillo, Sonora, Mexico

Javier Villela-Castrejón Tecnologico de Monterrey, School of Engineering and Science, Centro de Biotecnología-FEMSA, Monterrey, Mexico

Silvia Vincenzetti School of Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy

Marco Vinceti Research Center for Environmental, Genetic and Nutritional Epidemiology (CREAGEN), University of Modena and Reggio Emilia, Modena, Italy

Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA

Anshu Wali Division of Biochemistry, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu, Jammu and Kashmir, India

Jianbo Xiao Institute of Chinese Medical Sciences, State Key Laboratory of Quality Research in Chinese Medicine, Macau University, Taipa, Macau

Institut für Pharmazie und Lebensmittelchemie, Universität Würzburg, Würzburg, Germany

Deepika Yadav Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India

Ioannis Zabetakis Department of Biological Sciences, University of Limerick, Limerick, Ireland

Ewelina Zielińska Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Lublin, Poland

Damian Zieliński Department of Ethology and Animal Welfare, University of Life Sciences in Lublin, Lublin, Poland

Part I

Favorable Regimen for Good Health and Epidemiological Studies

Health Benefits of Dietary Phenolic Compounds and Biogenic Amines

1

Hector Alonzo Gomez-Gomez, Cristine Vanz Borges, Igor Otavio Minatel, Aline Carbonera Luvizon, and Giuseppina Pace Pereira Lima

Contents

Abstract

The bioavailability of dietary phytochemicals may be influenced by several factors as food matrix, cultivation conditions, host microbiota, and physiological state. Phenolic compounds and polyamines have been associated with various

H. A. Gomez-Gomez · C. V. Borges · G. P. P. Lima (✉)
Department of Chemistry and Biochemistry, Institute of Biosciences, Sao Paulo State University – UNESP, Botucatu, São Paulo, Brazil
e-mail: gpplima@ibb.unesp.br

I. O. Minatel
Department of Chemistry and Biochemistry, Institute of Biosciences, Sao Paulo State University – UNESP, Botucatu, São Paulo, Brazil

Faculdade Sudoeste Paulista – FSP, Avaré, São Paulo, Brazil

A. C. Luvizon
Faculdade Sudoeste Paulista – FSP, Avaré, São Paulo, Brazil

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_27

health benefits. However, their role in human health is dependent on interactions with the gut microbiota and conversion to further bioactive compounds. Dietary compounds have a wide range of effects that are play after the binding and/or interaction between these compounds and other molecules.

Keywords
Phenolic compounds · Polyphenols · Polyamines · Biogenic amines · Fruit · Vegetable · Beverage · Gut microbiota

Abbreviations

ADC	Arginine decarboxylase
AIH	Agmatine iminohydrolase
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CANSpdDC	Carboxynorspermidine decarboxylase
CANSpdDH	Carboxynorspermidine dehydrogenase
CPA	N-carbamoylputrescine amidohydrolase
DAO	Diamine oxidase
DDC	3,4-dihydroxyphenylalanine decarboxylase
DOPA	3,4 Dihydroxyphenylalanine
E4-P	Erythrose 4-phosphate
GABA	γ-aminobutyric acid
HDC	Histidine decarboxylase
ODC	Ornithine decarboxylase
PAL	Phenylalanine ammonia liase
PAO	Polyamine oxidase
PEP	Phosphoenolpyruvate
POD	Peroxidase
PPO	Polyphenol oxidases
ROS	Reactive oxygen species
SAM	S- adenosylmethionine
SAMDC	S-adenosylmethionine decarboxylase
SAT1	Spermidine/spermine-N1-acetyltransferase
SPDS	Spermidine synthase
SPMS	Spermine synthase
TAL	Tyrosine ammonia-lyase
TGFβ	Transforming growth factor-β

1 Introduction

Phenolic compounds are natural compounds occurring in vegetables, fruits, and derived beverages that humans usually obtain through the diet. Structurally, phenolic compounds are characterized by the presence of one or several phenolic groups, conferring their complexity and allowing to classify them into flavonoids

(polyphenol) or nonflavonoids. This large class of compounds has a well-established antioxidant activity and other beneficial effects on human health. However, its interactions in human body after dietary ingestion should be better addressed. Polyamines are a group of aliphatic amines, ubiquitously produced by humans and vegetables. These compounds are able to bind to many cellular macromolecules as DNA, RNA, and proteins, conferring them the ability to promote cell proliferation, signal transduction, and membrane stabilization. Phenolics and polyamines are produced by vegetables, whereas in human organism, only polyamines can be synthesized. Considering the possible interaction of these dietary components, this chapter summarizes some phytochemicals in vegetables and the mechanisms by which these compounds are absorbed, metabolized, and affect physiological functions in humans.

2 Phenolic Compounds

Phenolic compounds constitute an important class of secondary metabolites, notably in function of its recognized antioxidant activity, which confers quality to the food and beneficial potential to the human health. These antioxidants neutralize the free radicals, by inhibiting or interrupting the chain of initiation/propagation of oxidative reactions, converting the free radicals in less harmful molecules, and repairing the oxidative damages in human cells [1]. The functionality and stability of these phytochemicals in the human body depends not only on the quantity, but also on their location in the food matrix, on the presence of other bioactive compounds and, mainly, on the binding and/or interaction between these compounds and other molecules. Interestingly, the interaction between polyphenols and biogenic amines metabolic pathways should be pointed out to emphasize the role of both molecules on the healthy or harmful characteristics of a certain food.

Derived from the secondary plant metabolism, phenolic compounds constitute a group of molecules with a diversity of functions in the plant and different beneficial properties for health [2, 3]. The antioxidant activity and the free radical scavenging capacity depend on their structure, number of hydroxyl groups (OH), and position on the structure [4]. These heterogeneous characteristics are due to the complex metabolic pathways, for which the phenolic compounds are synthesized and the shikimic acid pathway is the most important biosynthesis route [5, 6].

By the shikimate pathway (present in plants and absent in animals), the binding of primary metabolism occurs (carbohydrates production) with the secondary metabolism, using phosphoenolpyruvate (PEP) and erythrose 4-phosphate (E4-P, pentose phosphate pathway), synthesizing chorismate, from which aromatic amino acids can be synthesized, such as phenylalanine, tyrosine, and tryptophan. These amino acids are used in the protein synthesis and also as secondary metabolites, including phenolic compounds, meaning that this biosynthetic route may contribute as a regulator of primary and secondary plant metabolism [7–9].

Phenylalanine ammonia lyase (PAL, EC 4.3.1.24) catalyzes the synthesis of trans-cinnamic acid from phenylalanine. In addition to PAL, tyrosine ammonia-lyase

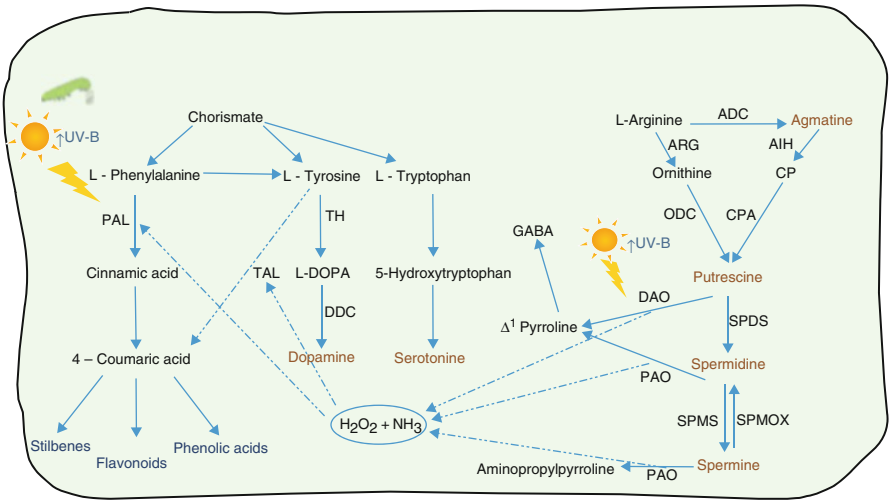


Fig. 1 Interaction of polyamines (primary metabolism) and phenolics (secondary metabolism) in plants. *ARG* arginase, *AIH* agmatine iminohydrolase, *ADC* arginine decarboxylase, *ODC* ornithine decarboxylase, *CP* N-carbamoyl putrescine, *CPA* N- carbamoylputrescine amidohydrolase, *SPDS* spermidine synthase, *SPMS* spermine synthase, *SPMOX* spermine oxidase, *DAO* diamine oxidase, *PAO* polyamine oxidase, *PAL* phenylalanine ammonia-lyase, *TAL* tyrosine ammonia-lyase, *TH* tyrosine hydroxylase, *DDC* dopa decarboxylase

(TAL, EC 4.3.1.23) has great importance in this metabolic route. TAL catalyzes the conversion of tyrosine to p-coumaric acid [9]. On the other hand, tyrosine can be converted in DOPA (3,4 dihydroxyphenylalanine) by tyrosine dehydrogenase and in dopamine (biogenic amine) by 3,4-dihydroxyphenylalanine decarboxylase (DDC; EC 4.1.1.27) (Fig. 1). Both enzymes are relevant for phenolic compounds synthesis.

On the other hand, phenolic compounds may be oxidized by polyphenol oxidases (PPO, EC 1.10.3.2 or EC 1.14.18.1), such as tyrosinase, cresolase, catecholase, diphenolase, and phenolase or peroxidases (POD, EC 1.11.1.7). High POD and PPO activities are related to the production of browning compounds, with decrease in the commercial value and loss of quality, including nutritional benefits [10, 11]. To reduce oxidative enzymes activities (PPO and POD), it may be necessary for physical and chemical treatments to guarantee the nutritional quality, maintaining some phytochemicals contents, such as anthocyanins, other phenolic compounds, and antioxidant activity [12–15].

3 Polyamines/Biogenic Amines

Polyamines and/or biogenic amines have been investigated in food matrixes due to the interest regarding the nutritional paradox and its possible action as antioxidants. Previous studies indicate a possible role of polyamines, present abundantly in food of the Mediterranean diet, with the prolongation of human life [16]. Diamines, as

putrescine, and polyamines (spermidine and spermine) can be formed through the decarboxylation of specific amino acids [17]. The biogenic amines are the aliphatic, cationic organic compounds of low molecular weight, formed after amino acids decarboxylation or by amination and transamination of aldehydes and ketones [18, 19], which include histamine, serotonin, tyramine, 2-phenylethylamine, tryptamine, putrescine, cadaverine, and agmatine. In raw vegetables, the amines are formed from the activity of some enzymes, while in processed foods they are formed by the action of microorganisms, which can often be, depending on the amine found and on the concentration, a signal of contamination [20].

In mammals, the polyamines can be synthesized, obtained from the diet, or even, synthesized for intestinal microorganisms. In mammals, the putrescine biosynthesis comes from the ornithine decarboxylation by the action of ornithine decarboxylase (ODC) (EC 4.1.1.17), forming the diamine putrescine. In plants, there is an alternative pathway by the action of arginine decarboxylase (ADC, EC 4.1.1.19) forming putrescine. Arginine is decarboxylated by ADC, forming agmatine, which forms N- N- carbamoylputrescine by the action of agmatine iminohydrolase (AIH, EC 3.5.2.12). The enzyme N-carbamoylputrescine amidohydrolase (CPA, EC 3.5.1.53) converts the product in putrescine (Fig. 1). The suppression of the putrescine synthesis from arginine could increase the amount of available arginine available for the synthesis of nitric oxide, which is responsible for maintaining normal vascular functions [21], retarding the progression of vascular diseases associated to the aging process. The diamine cadaverine is produced by microorganisms and originated from lysine decarboxylation, by lysine decarboxylase [22].

Both in mammals and plant cells, spermidine is formed from the addition of aminopropyl group in putrescine by the action of spermidine synthase (SPDS, EC 2.5.1.16) and spermine synthase (SPMS, EC 2.5.1.16) and adds another aminopropyl group to the spermidine, forming spermine. In parallel, S-adenosylmethionine (SAM) is decarboxylated by SAM decarboxylase (SAMDC, EC 4.1.1.50) and provides aminopropyl groups to the formation of spermidine and spermine. All three, ODC, ADC, and SAMDC, are regulatory enzymes (rate-limiting) of the polyamines synthesis [23, 24].

The polyamines oxidation occurs via amines oxidases. Diamine oxidase (DAO, EC 1.4.3.6) oxides putrescine or cadaverine in primary amino groups, forming 4- aminobutanal, which cyclics, forming delta-1-pirroline and hydrogen peroxide, and polyamine oxidase (PAO, EC 1.5.3.3) oxides spermidine and spermine. Tri- and tetramines oxidations produce hydrogen peroxide (Fig. 1). During the polyamines oxidation, there is also a formation of γ-aminobutyric acid (GABA), a nonprotein amino acid that acts as a endogenous molecular marker, with inhibitory action of neurotransmitters in animal cells [25]. The food enriched with GABA became popular due to their functional effects and regarding the health in the arterial pressure regulation, cardiac frequency, relieve of pain, and anxiety [26].

Polyamines present a series of functions in cells from prokaryotes and eukaryotes organisms. In plants, they have an important role in the growth,

differentiation, synthesis of nucleic acids and proteins, membrane stability, resistance, senescence, programmed cells death, adaptive responses to the environment, among others [27–30]. In addition, these molecules in mammals are involved in some diseases as Alzheimer and cancer [31]. Studies demonstrate increase in polyamines levels in the urine and serum [32], as well as induce the activity of enzymes involved in the polyamines synthesis, as ODC in tumor cells [33].

Biotic and abiotic stresses lead to oxidative stress, which is, many times, quickly manifested with accumulation of reactive oxygen species (ROS). ROS are important signaling molecules, but are toxic in high concentrations. ROS affect many cell functions by damaging the nucleic acids, oxidizing proteins, and causing lipid peroxidation [34]. The polyamines have a double role against the ROS accumulation in stressful conditions and are considered osmoprotectors and ROS scavengers. The polyamines putrescine, spermidine, and spermine are positively charged at physiological pH and can bond to membrane cell groups negatively charged and prevent damages caused by stress factors in plants [35]. In addition, polyamines can interact with negatively charged molecules as DNA and RNA through electrostatic bonding [36]. This bonding stabilizes the DNA structure, mainly by bonding with spermidine and spermine [37, 38]. Spermidine and spermine (not putrescine), when in high concentrations (above 10 mM), provide protection against damages from DNA radiation [39]. However, in sub-mM levels, it is ineffective and can slightly promote damages caused by gamma radiation. Other studies affirm that the polyamines have the ability to induce the DNA compaction and aggregation, besides acting as a scavenger of free radicals, mainly OH^- generated by gamma irradiation [39, 40], thus becoming a DNA protector cell.

The antioxidant action of some biogenic amines and polyamines correlates to the quantity of amino groups in their chemical structures. Studies have shown that amines as dopamine present a higher in vitro antioxidant capacity (through DPPH test), comparing to other natural antioxidants, as ascorbic acid [41]. In addition, it has been claimed that the increased intake of polyamines present in foods led to augmented intracelular amounts of these metabolites, promoting vascular health in humans. The increase in intracelular polyamines suppresses the enzymatic activity required for their synthesis.

Some biogenic amines (histamine, tryptamine, tyramine, putrescine, and cadaverine) are undesirable in high concentrations in the food, because they can promote respiratory problems, headaches, cardiac palpitations, hypertension, or hypotension, among other allergic diseases. Simultaneously, some polyamines (e.g., putrescine, spermidine, and spermine) in high concentrations can cause the proliferation of cancer cells [42]. The association of polyamines to cancer growth/progression may be related to polyamines catabolism. The excessive ingestion of food rich in polyamines, with a consequent increase of its content in the cells, can induce apoptosis, possibly due to the increasing peroxide content which is the product of the degradation of di- and polyamines by the oxidative enzymes DAO and PAO [43], leading to the cell death.

The intake of polyamines by feed and to control of some diseases has been studied by many researches groups, with the intention of decreasing or preventing damages provoked by these amines. Cipolla et al. [44] observed, in patients with prostate cancer, that reducing polyamine diet and partial intestinal decontamination was beneficial to improving the life quality and pain control. The authors also describe that 3 months after the end of this treatment, the pain came back.

4 Processing Methods – Effects in the Content of Phenolic Compounds and Polyamines

Changes in the phenolic compounds and polyamines compositions are of great complexity because they vary according to the structure and the type of analyzed food, besides the cooking method used to its preparation. The most of the vegetables and some fruits are preferably consumed after some type of thermal processing, which causes favorable changes in the flavor and texture, increasing the food palatability. Previous studies indicate that the retention of phytochemicals and the antioxidant properties after the cooking vary considerably among the different vegetables, among the methods used in their preparation, and mainly, among the specific analyzed compounds [45, 46]. The phenolic compounds are generally considered stable to the heat, and the occasional loses in the different thermal processes are probably attributed to lixiviation. Studies indicate that rutine and other polyphenols present in vegetables (e.g., asparagus) do not degrade when heated. The rutine's stability to heat was confirmed by recovering the compounds from the water used in the boiling process [47]. Generally, methods that do not use water, e.g., steaming, lead to an increase in the total phenolic compounds, flavonoids and phenolic acids in comparison to the *in natura* vegetables [48]. This can be explained by the rupture of complexes among the phenolic compounds and other compounds (e.g., proteins) generated by the extraction by steam, resulting in a better availability of these compounds [49].

The polyamines are original from amino acids, such as arginine and ornithine, which act as precursors and can suffer decarboxylation processes by the action of putrefactive bacteria. This explains why a higher concentration of polyamines is found after the fermentation of products as sauerkraut, some sausages, cheeses, and wines [50–52]. In addition, it is known that the processing of vegetables, such as the heating or freezing, can rupture the cell wall, leading to the liberation of these compounds that were free or binding to the membrane, inducing an increase of bioavailability of some compounds [49, 53]. However, for polyamines there are no differences on the content, in organic or conventional green beans, after thermal processing [46]. Besides this, organic green beans presented higher spermidine and spermine contents. These data can be important when the relation of polyamines with diets for cancer patients is studies, as described by [44], or even when it is wanted to obtain food with antioxidants, since these amines are considered free radicals scavengers [54]. Thus, studies with polyamines acting as antioxidants and their relation to diets should be deepened, due to the duality of these substances function.

5 Organic Versus Conventional Fruits and Vegetables

Several studies highlight the benefits of ingesting of organic foods, due to the higher content of some bioactive compounds and higher antioxidant activity, besides being free of chemical residues [46, 55–57]. The plant exposition to a stressful environment, as attack by insects or fungi, can induce the production of natural defense substances, as well as the phenolic compounds [58, 59]. The organic plant-based food contains higher polyamines levels (that can be protection mechanisms against many types of stress) in comparison to the conventional farming [57]. Studies with fruits indicate an increased polyamine content and a protective effect against damages caused by biotic and abiotic factors in the membranes [60]. However, this profile was not verified in collard green, where the highest putrescine levels were found in the conventionally cultivated vegetables [57].

Currently, the pharmaceutical industry has searched for organic plants with higher contents of bioactives. It is known that the interaction between different food metabolites (as biogenic amines and polyphenols) and their biosynthetic pathways can contribute to the healthy or harmful characteristics of the own food. In a research developed in our laboratory using two jambu cultivars (*Acmella oleracea* cv. Jambuarana and Nazaré), a medicinal plant used in the cosmetic and pharmaceutical industries and also consumed by the population from North region of Brazil, the polyamines levels was affected by the cultivation practice. Organic jambu (both varieties) showed higher spermine content in leaves and spermine and spermidine in inflorescences compared with the conventional fertilization. Besides the polyamines levels, the phenolic compounds content was increased by the organic cultivation [61]. The results show that the organic cultivation can induce positive alterations regarding the antioxidants levels.

Biotic or abiotic stress can induce high phenolic compound contents by increasing the activity of the enzymes. Phenolic compounds act as cell protectors against free radicals generated by stress [62, 63]. Some plant-based foods produced under moderate stress, such as organic farming, tend to show higher antioxidant activity due to phytochemical levels, such as phenolics, compared with conventional ones. Organic food may contain more phenolic compounds due to the absence of agrochemicals, which collaborates in the defense against some types of stress [30]. An increased 139% in the total phenolic content of organic tomatoes in relation to conventional farming, which may be a consequence of increased PAL activity, was observed during development [64]. Even after the processing, this quality seems to be maintained. Organic tomato juice presents higher phenolic compounds levels and higher antioxidant activity when compared to those conventionally grown [65].

6 Beneficial Effects of Phenolic Compounds

Phenolic compounds play an important role in sensory characteristics and are responsible for some organoleptic properties such as aroma, color, taste, bitterness, and astringency in fruits and vegetables, as well as their derivatives [66, 67].In addition,

they are directly or indirectly related to quality and health benefits. Flavonoids, anthocyanins, tannins, and resveratrol (stilbene) have proven action against different diseases in humans [68–71]. Quercetin shows beneficial properties and is found in onion, apple, brassicas, among other vegetables. This flavonoid has antidiabetic, anti- inflammatory, and anticancer activity [72]. Other flavonoids, such as kaempferol and catechin, act as antioxidants. Studies demonstrated the inhibition of tumor cell growth, reducing the progression of breast cancer in a rat model after treatment with both flavonoids [73, 74].

Some species of the genus *Citrus*, *Brassica*, *Allium,* and *Mallus* contain kaempferol, a flavonoid with potential to be used in individuals with cardiovascular diseases. In vitro studies showed its action on the decrease of cellular apoptosis induced by LDL cholesterol in human cells and antithrombotic action [75–77].

Beneficial effects are enhanced with resveratrol, a molecule considered effective in preventing different pathological conditions, for example, in the reduction of blood pressure and repression of lipid peroxidation, oxidative stress, and lipid levels [78, 79]. In vitro studies demonstrate that this stilbene has a beneficial effect as a coadjutant in the control of norovirus, a pathogenic agent involved in food diseases (at great risk in the elderly and in the immunocompromised). In addition, the resveratrol action has been demonstrated in the expression of genes that decrease insulin resistance, meaning possible beneficial effects for diabetics [80, 81].

Anthocyanins are natural pigments found in fruits and vegetables. These compounds provide blue, purple, and red colors and with proven activity as nutraceuticals. Grapes and their derivatives are sources of antioxidant substances and are rapidly absorbed, with various physiological activities in animal cells [82]. Cyanidin-3-O-β-glucoside is present in a large number of fruits and vegetables, and studies have been demonstrated its protective effect against lipopolysaccharide-induced lung injury by lowering cholesterol levels and suppressing the inflammatory response [83]. This anthocyanin has also shown to decrease the negative effects of free fatty acids, decreasing insulin resistance through the activation of cellular and cytoprotective antioxidant genes [84], as well as having preventive or complementary activity against inflammatory chronic diseases [85, 86]. It was demonstrated by in vitro study using *Morus alba* L. that the presence of cyanidin-3-O-β-glucoside, together with cyanidin 3-rutinoside, has an inhibitory effect on the migration and invasion of human lung carcinoma (A549) cells.

Malvidin-3-O-β-glycoside, the main anthocyanins in grapes, is a potent anti-inflammatory; it aids in the balance and inhibition of pro-inflammatory pathways, promoting cardiovascular health benefits. When malvidin-3-O-β-glycoside is in combination with peonidine, there is an increased beneficial effects activity. Studies in vitro and in vivo showed decreases of transcription of genes encoding proinflammatory cytokines, causing a decrease in anti-inflammatory activity in arthritic rats [87, 88].

Pelargonidine, another anthocyanin found in large quantities in grapes and derivatives, has a neuroprotective effect, improving memory deficit by mitigating oxidative stress, helping to reduce the symptoms of individuals with Alzheimer's disease. In addition, pelargonidine provides potential benefits against atherosclerosis in albino mice. This compound acts against stress generated by environmental toxicants (urethane and diepoxybutane), compounds classified as possible human carcinogens [89–91].

Delphinidin also has a beneficial activity and its anticancer action has been demonstrated. In vitro study has demonstrated inhibition of proliferation and migration of ovarian carcinoma cells. This effect may be an important health response, meaning a potential natural chemotherapeutic to prevent the development and progression of cancer cells [92, 93]. In addition, it has regenerative effect on cells exposed to UVB radiation (100 mJ/cm^2), demonstrating great potential in protecting skin damaged by the sun.

Several phenolic acids have been studied in reducing symptoms or diseases. It has been demonstrated that p-coumaric acid has the ability of eliminating free radicals and lowering LDL cholesterol [94]. Ferulic acid has been related to the protection of oxidative stress in rat renal tissue caused by cisplatin (anticancer agent). The use of this phenolic acid avoided renal damage and helped to recover the histological parameters of the kidney under normal conditions [95]. Chlorogenic acid found in sweet potatoes and coffee has antibacterial and anti-inflammatory activity and can inhibit tumors. Besides that, this phenolic acid has protective effects on the liver and antihypertensive function. In rats with the cerebral ischemia reperfusion injury, chlorogenic acid improves pathological lesions in brain tissue, reducing mortality. Studies demonstrated an effect against prostatic hyperplasia in mice, besides reducing the negative effects of high consumption of glucose and lipids, avoiding the occurrence of diabetes, obesity, cardiovascular diseases, and cancer in humans [96–98].

7 Interaction of Phenolic Compounds and Polyamines on Plants

Besides the effect of some polyphenols on the polyamines, the plants can form phenolamides, which are polyamines combined by amide bonding with some phenolic acids such as coumaric, caffeic, and ferulic acids, through the substitution of 1, 2, or 3 amino groups. This combination of the phenolic acids that occurs with the aliphatic polyamines (putrescine, cadaverine, spermidine, and spermine) forms the basic amides.

When occurs the bonding with the aromatics (tyramine, tryptamine, dopamine, and octopamine) or with aliphatics, forms the neutral amides [99, 100]. The phenolamides has been described as specific secondary metabolites and related to the development of plants defense mechanism, and the structure of these molecules are similar to the ones found in the insects toxins [101].

The phenolamides content can be affected in response to the pathogens attack, as during the hypersensitive reaction towards fungi or virus diseases. Some plant organs, as the leaves, can synthesize phenolamides as defense mechanism, decreasing the pathogens action [99]. Reference [102] describes an increase of phenolamides and decrease of the powdery mildew infection. Reference [103] suggests that the low incidence of virus diseases in flowers and seed can be related to the phenolamides content. However, in some cases of host-pathogen interactions, especially during the hypersensitive reaction towards fungi or viruses, leaves of

vegetative plants synthesize some phenolic amides at the same time as defense mechanisms, limit pathogen expansion.

Many foods can produce harmful substances from the oxidation of fatty acids [104] with mutagenic and carcinogenic activities during the processing, the storage, or by contaminants [105]. In order to fight the oxidation, synthetic or natural antioxidants can be used. Some species contain high phenolamides levels that can be used as antioxidants. Reference [106] described phenolamides in *Piper* and demonstrated a higher antioxidant capacity compared to alfa-tocopherol, and feruperine showed an antioxidant activity almost as high as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene).

8 Interaction of Phenolic Compounds and Polyamines on Human Health

Studies that related the beneficial effects of the interaction of the phenolic compounds with the polyamines indicate that phenolic compounds, as the chatequins, interact with the biosynthetic pathways of polyamines. This reaction occurs specifically reducing the production of histamine and putrescine due to the inhibition of the histidine decarboxylase (HDC; EC 4.1.1.22) and ODC activities and increase spermidine/spermine N1-acetyltransferase activity, which promotes the polyamines catabolism [107, 108]. High activity of ODC is characteristic of the tumor cells [109], and the inhibition of ODC expression may be one of several targets involved in the antiproliferative effects of resveratrol [42]. Resveratrol and piceatannol (Piceat) (derived from resveratrol) also were able to reduce the ODC levels in Caco-2 colorectal cancer cells [110]. This enzyme is directly related to the cellular proliferation and carcinogenesis. Similar results were observed by [42], where resveratrol induced a decrease in the ODC activity in Caco-2 cells and a significant decrease of intracellular putrescine content. However, it did not affect the SAMDC activity, not affecting the content of spermidine and spermine.

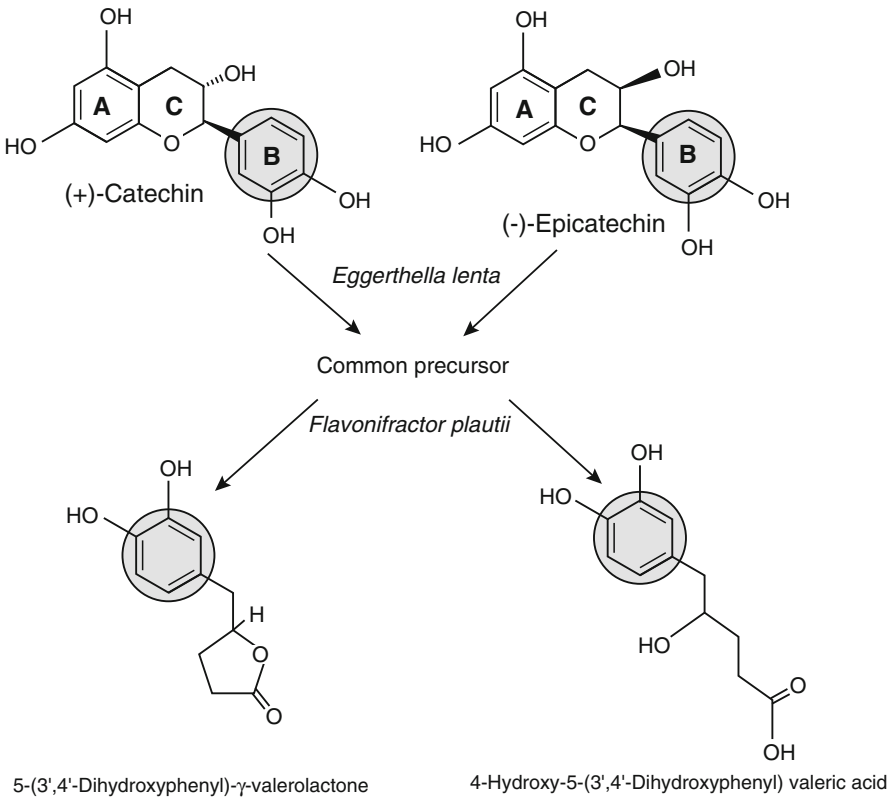
Polyphenols has the ability to targeting polyamines or biogenic amines. Besides the possible synergic activity of both compounds, some classes, as flavonoids, have shown the capacity of blockage key enzymes from biogenic amines biosynthetic pathways [108]. Dopamine and serotonin play critical role in neuropsychiatric disorders, such as depression, schizophrenia, and Parkinson's disease. Both biogenic amines are essential for serotonergic and dopaminergic systems to regulate the neurotransmission. Most of the psychotropic drugs used in the treatment of neuropsychiatric disorders, including antidepressants, antipsychotics, anxiolytics, and psychostimulants, exerted their pharmacological actions by interfering with serotonergic and dopaminergic transmission. Thus, deregulations in their levels (amines) are a key step in the control of neurological diseases. It is interesting to note that epigallocatechin gallate and epigallocatechin showed the ability to interact with and bind to the enzyme Dopa decarboxylase (converts L-Dopa and L-5-hydroxytryptophan into dopamine and serotonin, respectively), leading to its irreversible inhibition [111]. Histamine is other biogenic amine that can be regulated by

epigallocatechin gallate. To produce histamine neurons, mast cells, basophils, and monocytes/macrophages, among others, produce the enzyme HDC that catalyzes the conversion of histidine in histamine. A dual effect of epigallocatechin gallato was observed against histamine. The first was demonstrated in rat basophilic leukemia cells that epigallocatechin gallato prevented histamine release from mast cells [112]. The second effect was observed in mammalian cells, where epigallocatechin gallato demonstrated a potent inhibitory effect of HDC activity [113].

Interestingly it has also been reported that high concentrations of some phenolic compounds affect biogenic amine production by inhibiting lactic acid bacterial growth [114]. Low levels of biogenic amines in some types of wines can be related to the presence of large phenolic compounds quantities (e.g., anthocianins and stilbenes) that, by inhibiting the activity of naturally present bacteria, can reduce the formation of histamine, tyramine, and putrescine.

Usually the polyphenols are not absorbed by small intestine, and it has been estimated that only 10% is absorbed and the remaining 90% is metabolized in the large intestine by gut microbiota or excreted [115]. The polyphenols conversion in the human gut is a complex process and should be considered when assessing the bioavailability of plant compounds. For example, compounds as procyanidins are poorly absorbed in its original molecular structure, but the human gut microbiota might play an important role in the absorption process. This interaction was observed in the conversion of catechin and epicatechin [116]. Bacterial strains *Eggerthella lenta* and *Flavonifractor plautii*, isolated from human feces, showed the capacity to convert both compounds in active metabolites. The strain *Eg. lenta* was able to cleave the heterocyclic C-ring of both (+)-catechin and (−)-epicatechin to produce a common precursor, 1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propan-2-ol, that was subsequently converted in 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and 4-hydroxy-5-(3′,4′-dihydroxyphenyl) valeric acid by *F. plautii* strain (Scheme 1). To produce catechin metabolites, bacterial strains promote cleavage and/or dehydroxylation of C and A rings of catechins molecules and the B ring is preserved. Despite the well-known antioxidant capacity induced by polyphenols, several of these compounds act as redox-dependent poisons against type II enzymes [117]. This poisoning effect is the capacity of enhance DNA cleavage by topoisomerase II and depends on the number of -OH groups on the B and C-rings. Considering that many anticancer drugs target the type II enzyme, this feature can represent an interesting mechanism by which dietary polyphenols or its metabolites act preventing cancer [118].

Two of the most consumed beverages worldwide are coffee and tea, and both beverages are source or phenolic compounds. These beverages have shown several beneficial effects on health, in part due to the high content of phenolic substances. Tea is a rich source of catechins, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate, compounds which can promote diverse effects in humans. Catechins and its metabolites may be involved in the insulin signaling pathway, regulation of various transcription factors, and inhibition of pro-oxidant enzymes such as nitric oxide synthase, lipooxygenase, cyclooxygenase, and xanthine oxidase [119]. Similarly to tea, the coffee consumption may induce different



Scheme 1 Conversion of (+)-catechin and (−)-epicatechin by human intestinal bacteria. Both compounds are converted by *Eggerthella lenta* in a common precursor, which is further converted by *Flavonifractor plautii* to active metabolites that retain the polyphenol B-ring (gray background) (Adapted from: Kutschera et al. [116])

health benefits, according to the processing method used to obtain the beverage. Green coffee beans contain large amounts of chlorogenic acids, a family of water-soluble phenols formed by the esterification of (−)-quinic acid with one or more cinnamic acids. However, with the roasting process of coffee grains, the chlorogenic acid progressively decreases in relation to light to dark roasting degree. In addition to effects in phenolic compounds, the roasting process induces the formation of Maillard reaction products (high molecular weight products). Some of these high molecular weight compounds (such as melanoidins, which also have antioxidant capacity) increase in dark roasting compared to light roasting [120]. It is well known that polyphenols and melanoidins present in foods and beverages are involved in their antioxidant activity [121]. Therefore, the antioxidant capacity of coffee beverages is not only contributed by the components originally present in green grains, but also by the components generated during the roasting process.

Coffee is probably the richest dietary source of chlorogenic acid, with a single dose of espresso coffee supplying 24–423 mg [122]. Nonespresso beverages are also rich sources, and many regular coffee drinkers can easily consume 1–2 g per day of this phenolic compound, surpassing even the intake coming from fruit and vegetables. In addition to chlorogenic acid, other polyphenols, phenolic acids, and melanoidins have been linked to several health benefits, such as suppression of postprandial hyperglycemia and hyperinsulinemia (by inhibiting digestive enzymes) [123] and protection of DNA oxidative damage (by decreasing deoxyribose degradation, DNA scission, and inhibition of 8-hydroxydeoxyguanosine formation) [121]. Probably these effects are associated to direct interactions with membrane structures, enzymes, and receptors, besides the antioxidant capacity. The oxidative stress and the resulting oxidative damage are closely related to the development of inflammation and chronic diseases. In addition to the effects described above, the oxidative scavenger activity of coffee beverage has been shown in humans. The plasma antioxidant activity measured on subjects (1 and 2 h after the ingestion of 200 ml of coffee) exhibited a significantly higher antioxidant capacity than the controls that did not ingest coffee [124].Further, healthy subjects consuming instant coffee (800 mL/day, for 5 days), co-extracted from green and roasted grains, also showed a significant protection against oxidative damage [125]. In these subjects, urinary marker of lipid peroxidation (8-isoprostaglandin F2α) and plasma marker of functional protein modifications induced by nitric oxide (3-nitrotyrosine) were decreased by 15.3% and 16.1%, respectively [125].

The main drawback to using polyphenols as agents to prevent or treat diseases is their poor bioavailability and the variable bioaccessibility in the human body. Thus, to improve the desired effects in health promotion, consumption of several polyphenols or the association of polyphenols and drugs has been recommended [126–128]. In addition, it is necessary to consider that dietary fruits and vegetables provide other bioactive compounds, beside polyphenols, that help the human body to prevent chronic diseases as cancer, diabetes, hypertension, obesity, and cardiovascular disease [129–132]. An interesting mechanism of anticancer activity induced by polyphenols was showed in compounds coming from cocoa samples. The flavanols and procyanidins from cocoa were able to induce nonapoptotic cell death in cancer cell line Caco-2 [133]. This effect was mainly induced by decreasing ODC and SAMDC activities, two key enzymes of polyamine biosynthesis. High expression of ODC is an important characteristic of tumor cells and tumor development; therefore, the interaction of polyphenols with polyamines biosynthesis are interesting targets for cancer prevention.

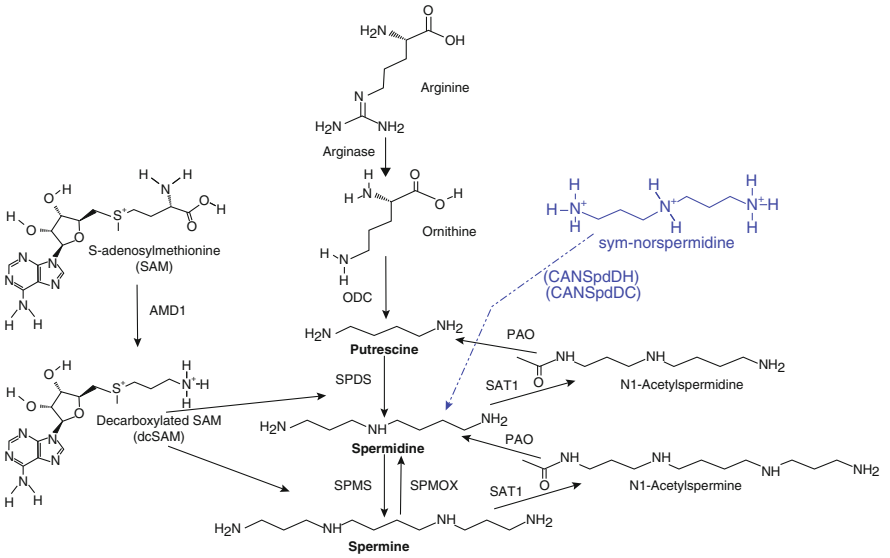
The human intestinal tract contains high levels of polyamines, which are derived from the diet and/or from de novo production by host and its microbiota. Besides that, humans cells have the ability to ubiquitously produce polyamines as putrescine, spermidine, and spermine, from amino acid arginine, which is converted in ornithine by ODC. These endogenous molecules play an essential role in the process of cell development, growth, and differentiation. However, deregulated levels of polyamines at low concentrations are linked to cell growth defects and at high concentrations to toxic effects and carcinogenesis. Diets based on foods and beverages rich

in arginine may induce increased synthesis of polyamines. Further, knowing that arginine is the amino acid precursor of polyamines and nitric oxide, it is expected that the enzymes arginase 1 and nitric oxide synthase compete for arginine and promote a bypass in metabolic pathways. The immune response, mainly induced by M1 macrophages, is dependent of nitric oxide to induce the production of pro-inflammatory cytokines. Spermine can inhibit M1 macrophage activation by suppressing the expression of ODC. However, even blocking nitric oxide pathway and M1 macrophage activation and the synthesis of anti-inflammatory transforming growth factor-β (TGFβ) and IL-10 were not altered [134].

In addition, polyamines have been associated to multiple effects in the central nervous system, acting as neurotransmitters or neuromodulators. Injuries in the central nervous system, such as ischemia and trauma, are associated to increased activities of ODC and spermidine/spermine-N(1)-acetyltransferase. The increased activity of these enzymes results in accumulation of putrescine, a candidate marker of brains injury. Increased amount of putrescine seems to be more associated to brain injury than spermine and spermidine, once levels of the former two were unaltered in three different models of forebrain ischemia and traumatic brain injury [135]. On the other hand, the administration of polyphenols and their supposed interaction with polyamines biosynthesis were demonstrated in animal model of ischemia-induced neuronal injury [136]. Neuronal damages and the polyamines content (in the ischemic tissue) were decreased after epigallocatechin gallato administration. In this study, authors speculated that although administration of epigallocatechin gallato did not show complete neuroprotection, it seems to be a promising strategy for attenuation of global ischemia-induced neuronal injury [136]. In this same study, the levels of spermine and spermidine were unaltered in the injured brain tissue, whereas the levels of putrescine were significantly decreased. It is well known that the spermidine may be synthesized from the putrescine, so is not unexpected the maintenance in its levels.

The spermidine biosynthesis is mediated SAMDC and aminopropyltransferase spermidine synthase (SPDS) through the addition of aminopropyl group (donated by decarboxylated S-adenosylmethionine (dcSAM)) to putrescine molecules (Scheme 2). The following steps are capable to convert spermidine to spermine through the spermine synthase (SPMS). When necessary, spermine and spermidine can be recycled to spermidine and putrescine, respectively, by spermidine/spermine-N1-acetyltransferase (SAT1) followed by oxidation by polyamine oxidase (PAO) [137]. In addition to dietary polyamines consumed by each individual, the gut microbiota of these individuals may be responsible for spermidine biosynthesis [138–140]. Several bacterial strains have the capacity to produce spermine via SAMDC and SPMS enzymes, or the orthologues enzymes carboxynorspermidine dehydrogenase (CANSpdDH) and carboxynorspermidine decarboxylase (CANSpdDC) that convert *sym*-norspermidine in spermidine [138].

The relevance of human microbiota in polyphenols and/or polyamines absorption is remarkable. In a human study, volunteers that consumed orange juice containing hesperetin-7-O-rutinoside showed increased concentrations of hesperetin-7- O-glucuronide in the plasma and urine [141]. Probably the disaccharide glycoside moiety of hesperetin-7-O-rutinoside was cleaved by colonic bacteria releasing hesperetin, which



Scheme 2 Polyamines biosynthetic pathway in human body. Arginine is the precursor amino acid converted to ornithine by arginase. Putrescine is synthesized through ornithine decarboxylase (ODC) or S-adenosylmethionine decarboxylase (SAMDC). S- adenosylmethionine (SAM) is decarboxylated (dcSAM) and the molecule provides aminopropyl groups to produce putrescine. After that, putrescine is converted in spermidine by spermidine synthase (SPDS) and/or spermine by spermine synthase (SPMS). Spermine can be recycled to spermidine directly by spermine oxidase (SPMOX). Spermine and spermidine can be recycled to spermidine and putrescine, respectively, by spermidine/spermine-N1-acetyltransferase (SAT1) followed by oxidation by polyamine oxidase (PAO). Alternatively, gut microbiota can synthesize spermidine (blue route) from *sym*-norspermidine by expressing orthologues enzymes carboxynorspermidine dehydrogenase (CANSpdDH) and carboxynorspermidine decarboxylase (CANSpdDC) (Adapted from: Brooks [137])

was acted upon by UDP-glucuronosyltransferases in the colonocytes, producing hesperetin-7-O-glucuronide. In addition, an unidentified hesperetin-O- glucuronide was found in plasma, showing that these metabolites passed into the portal vein to reach the blood stream [141]. Hesperetin and its conjugated metabolites are the major forms of hesperidin in human plasma. It occurs after hesperidin hydrolysis in the gastrointestinal tract and conjugation during absorption. In several situations, the metabolites have similar, or even, higher physiological effect than their precursors. An interesting example of this effect was observed in spontaneously hypertensive rats. The hesperetin-7-O-β-D-glucuronide exerted hypotensive, vasodilatory, and anti-inflammatory activities, similarly to hesperetin. On the other hand, the metabolite hesperetin-3′-O-β-D-glucuronide had little effect on these parameters [142].

The interaction of polyphenols and polyamines, as observed for catechins metabolites, was also described for hesperetin and naringerin [143]. Both compounds produced a remarkable reduction of B16-F10 melanoma cell, in vitro and in vivo, and the proposed mechanism was by lowering of the intracellular levels of polyamine, spermidine and spermine.

9 Concentrations of Phenolic Compounds and Biogenic Amines in Some Foods

The phenolic compounds and the polyamines are present in variable quantities in many foods and the knowledge of its content can influence positively or negatively in various diseases. Alterations in the content of these phytochemicals are verified depending on the food matrix used, on the method of cultivation, and on the treatment and/or on the processing the raw material is submitted [57, 61, 144]. In addition, following previous considerations, it should be useful to actively promote the production of functional beverages and foods, having a modified balance between amines and polyphenols, by using different agricultural management practices and processing methods.

10 Conclusion

Regular consumption of phenolic compounds and polyamines rich food has been shown to improve the human health. There is a need for detailed investigation of the complexities of effects on the absorption of these compounds, as well as their metabolism and de novo synthesis in the human organism. The gut microbiota produces metabolites, different from those that can be generated by human enzymes, and in most cases, reduces the activity of dietary compounds; however, sometimes the metabolites formed exhibit enhanced properties.

The gut microbiota and the dietary components are involved in a complex metabolic metabolism. Interactions between these components are essential for human responses to food-based interventions and to achieve desired health improvement.

Acknowledgment The authors are grateful to the National Council for Scientific and Technological Development (CNPq, Brazil) (305177/2015-0), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (02441/09-8) and the São Paulo Research Foundation (FAPESP) (2016/22665-2)

References

1. Du G, Li M, Ma F, Liang D (2009) Antioxidant capacity and the relationship with polyphenol and vitamin C in Actinidia fruits. Food Chem 113:557–562. https://doi.org/10.1016/j.foodchem.2008.08.025
2. Crozier A, Del Rio D, Clifford MN (2010) Bioavailability of dietary flavonoids and phenolic compounds. Mol Asp Med 31:446–467. https://doi.org/10.1016/j.mam.2010.09.007
3. Pan M-H, Lai C-S, Ho C-T (2010) Anti-inflammatory activity of natural dietary flavonoids. Food Funct 1:15. https://doi.org/10.1039/c0fo00103a
4. Heo HJ, Kim YJ, Chung D, Kim DO (2007) Antioxidant capacities of individual and combined phenolics in a model system. Food Chem 104:87–92. https://doi.org/10.1016/j.foodchem.2006.11.002

5. Tzin V, Galili G (2010) The biosynthetic pathways for shikimate and aromatic amino acids in *Arabidopsis thaliana*. Arabidopsis Book 8:e0132. https://doi.org/10.1199/tab.0132
6. Maeda H, Dudareva N (2012) The shikimate pathway and aromatic amino acid biosynthesis in plants. Annu Rev Plant Biol 63:73–105. https://doi.org/10.1146/annurev-arplant-042811-105439
7. Caretto S, Linsalata V, Colella G et al (2015) Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. Int J Mol Sci 16:26378–26394. https://doi.org/10.3390/ijms161125967
8. Saito K, Yonekura-Sakakibara K, Nakabayashi R et al (2013) The flavonoid biosynthetic pathway in Arabidopsis: structural and genetic diversity. Plant Physiol Biochem 72:21–34. https://doi.org/10.1016/j.plaphy.2013.02.001
9. Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell Online 7:1085–1097. https://doi.org/10.1105/tpc.7.7.1085
10. Zhang Z, Pang X, Xuewu D et al (2005) Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. Food Chem 90:47–52. https://doi.org/10.1016/j.foodchem.2004.03.023
11. Richard-Forget FC, Gauillard FA (1997) Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* Cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. J Agric Food Chem 45:2472–2476. https://doi.org/10.1021/jf970042f
12. Kumari P, Barman K, Patel VB et al (2015) Reducing postharvest pericarp browning and preserving health promoting compounds of litchi fruit by combination treatment of salicylic acid and chitosan. Sci Hortic 197:555–563. https://doi.org/10.1016/j.scienta.2015.10.017
13. Barman K, Siddiqui W, Patel VB, Prasad M (2014) Nitric oxide reduces pericarp browning and preserves bioactive antioxidants in litchi. Sci Hortic 171:71–77. https://doi.org/10.1016/j.scienta.2014.03.036
14. Ali S, Khan AS, Malik AU (2016) Postharvest l -cysteine application delayed pericarp browning, suppressed lipid peroxidation and maintained antioxidative activities of litchi fruit. Postharvest Biol Technol 121:135–142. https://doi.org/10.1016/j.postharvbio.2016.07.015
15. Haddouche L, Phalak A, Tikekar RV (2015) Inactivation of polyphenol oxidase using 254nm ultraviolet light in a model system. LWT Food Sci Technol 62:97–103. https://doi.org/10.1016/j.lwt.2015.01.039
16. Binh PNT, Soda K, Kawakami M (2010) Mediterranean diet and polyamine intake: possible contribution of increased polyamine intake to inhibition of age- associated disease. Nutr Diet Suppl:1. https://doi.org/10.2147/NDS.S15349
17. Shalaby AR (1996) Significance of biogenic amines to food safety and human health. Food Res Int 29:675–690. https://doi.org/10.1016/S0963-9969(96)00066-X
18. Maijala RL (1993) Formation of histamine and tyramine by some lactic acid bacteria in MRS-broth and modified decarboxylation agar. Lett Appl Microbiol 17:40–43. https://doi.org/10.1111/j.1472-765X.1993.tb01431.x
19. Santos MHS (1996) Biogenic amines: their importance in foods. Int J Food Microbiol 29:213–231. https://doi.org/10.1016/0168-1605(95)00032-1
20. Latorre-Moratalla ML, Veciana-Nogués T, Bover-Cid S et al (2008) Biogenic amines in traditional fermented sausages produced in selected European countries. Food Chem 107:912–921. https://doi.org/10.1016/j.foodchem.2007.08.046
21. Cooke JP, Singer AH, Tsao P et al (1992) Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. J Clin Invest 90:1168–1172. https://doi.org/10.1172/JCI115937
22. Cipolla BG, Havouis R, Moulinoux JP (2007) Polyamine contents in current foods: a basis for polyamine reduced diet and a study of its long term observance and tolerance in prostate carcinoma patients. Amino Acids 33:203–212. https://doi.org/10.1007/s00726-007-0524-1
23. Thomas T, Thomas TJ (2003) Polyamine metabolism and cancer. J Cell Mol Med 7:113–126. https://doi.org/10.1111/j.1582-4934.2003.tb00210.x
24. Alcázar R, Altabella T, Marco F et al (2010) Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. Planta 231:1237–1249. https://doi.org/10.1007/s00425-010-1130-0

25. Li Y, Sun H, Chen Z et al (2016) Implications of GABAergic neurotransmission in Alzheimer's disease. Front Aging Neurosci 8:1–12. https://doi.org/10.3389/fnagi.2016.00031
26. Mody I, De Koninck Y, Otis TS, Soltesz I (1994) Bridging the cleft at GABA synapses in the brain. Trends Neurosci 17:517–525. https://doi.org/10.1016/0166-2236(94)90155-4
27. Bouchereau A, Guénot P, Larher F (2000) Analysis of amines in plant materials. J Chromatogr B Biomed Sci Appl 747:49–67. https://doi.org/10.1016/S0378-4347(00)00286-3
28. Minois N, Carmona-Gutierrez D, Madeo F (2011) Polyamines in aging and disease. Aging (Albany NY) 3:716–732. https://doi.org/10.1016/S0044-8486(03)00351-X
29. Kusano T, Yamaguchi K, Berberich T, Takahashi Y (2007) Advances in polyamine research in 2007. J Plant Res 120:345–350. https://doi.org/10.1007/s10265-007-0074-3
30. Lima GPP, Vianello F (2011) Review on the main differences between organic and conventional plant-based foods. Int J Food Sci Technol 46:1–13. https://doi.org/10.1111/j.1365-2621.2010.02436.x
31. Wallace HM, Fraser AV (2004) Inhibitors of polyamine metabolism: review article. Amino Acids 26:353–365. https://doi.org/10.1007/s00726-004-0092-6
32. Wallace H, Caslake R (2001) Polyamines and colon cancer. Eur J Gastroenterol Hepatol 13:1033. https://doi.org/10.1097/00042737-200109000-00006
33. Narisawa T, Takahashi M, Niwa M et al (1989) Increased mucosal ornithine decarboxylase activity in large bowel with multiple tumors, adenocarcinoma, and adenoma. Cancer 63:1572–1576. https://doi.org/10.1002/1097-0142(19890415)63:8<1572::AID-CNCR2820630821>3.0.CO;2-U
34. Gill SS, Tuteja N (2010) Polyamines and abiotic stress tolerance in plants. Plant Signal Behav 5:26–33. https://doi.org/10.4161/psb.5.1.10291
35. Pottosin I, Shabala S (2014) Polyamines control of cation transport across plant membranes: implications for ion homeostasis and abiotic stress signaling. Front Plant Sci 5:1–16. https://doi.org/10.3389/fpls.2014.00154
36. Edison TNJI, Atchudan R, Sethuraman MG, Lee YR (2016) Reductive-degradation of carcinogenic azo dyes using Anacardium occidentale testa derived silver nanoparticles. J Photochem Photobiol B Biol 162:604–610. https://doi.org/10.1016/j.jphotobiol.2016.07.040
37. Rau DC, Parsegian VA (1992) Direct measurments of the intermolecular forces between counterion-condensed DNA double helices. Biophys J 61:246–259
38. Schellman JA, Parthasarathy N (1984) X-ray diffraction studies on cation-collapsed DNA. J Mol Biol 175:313–329. https://doi.org/10.1016/0022-2836(84)90351-6
39. Iacomino G, Picariello G, Stillitano I, D'Agostino L (2014) Nuclear aggregates of polyamines in a radiation-induced DNA damage model. Int J Biochem Cell Biol 47:11–19. https://doi.org/10.1016/j.biocel.2013.11.007
40. Fujisawa S, Kadoma Y (2005) Kinetic evaluation of polyamines as radical scavengers. Anticancer Res 25:965–969
41. González-Montelongo R, Gloria Lobo M, González M (2010) Antioxidant activity in banana peel extracts: testing extraction conditions and related bioactive compounds. Food Chem 119:1030–1039. https://doi.org/10.1016/j.foodchem.2009.08.012
42. Schneider Y, Vincent F, Duranton B et al (2000) Anti-proliferative effect of resveratrol, a natural component of grapes and wine, on human colonic cancer cells. Cancer Lett 158:85–91. https://doi.org/10.1016/S0304-3835(00)00511-5
43. Moinard C, Cynober L, Debandt J (2005) Polyamines: metabolism and implications in human diseases. Clin Nutr 24:184–197. https://doi.org/10.1016/j.clnu.2004.11.001
44. Cipolla B, Guillí F, Moulinoux J-P (2003) Polyamine-reduced diet in metastatic hormone-refractory prostate cancer (HRPC) patients. Biochem Soc Trans 31:384–387. https://doi.org/10.1042/BST0310384
45. Murador DC, Mercadante AZ, De Rosso VV (2015) Cooking techniques improve the levels of bioactive compounds and antioxidant activity in kale and red cabbage. Food Chem 196:1101–1107. https://doi.org/10.1016/j.foodchem.2015.10.037
46. Lima GPP, Costa SM, de Monaco KA et al (2017) Cooking processes increase bioactive compounds in organic and conventional green beans. Int J Food Sci Nutr 7486:1–12. https://doi.org/10.1080/09637486.2017.1324563

47. Drinkwater JM, Tsao R, Liu R et al (2015) Effects of cooking on rutin and glutathione concentrations and antioxidant activity of green asparagus (Asparagus officinalis) spears. J Funct Foods 12:342–353. https://doi.org/10.1016/j.jff.2014.11.013
48. Gliszczyńska-Świgło A, Ciska E, Pawlak-Lemańska K et al (2006) Changes in the content of health-promoting compounds and antioxidant activity of broccoli after domestic processing. Food Addit Contam 23:1088–1098. https://doi.org/10.1080/02652030600887594
49. Minatel IO, Borges CV, Ferreira MI et al (2017) Phenolic compounds: functional properties, impact of processing and bioavailability. Phenolic Compd-Biol Act. https://doi.org/10.5772/66368
50. Bardocz S, Grant G, Brown DS et al (1993) Polyamines in food – implications for growth and health. J Nutr Biochem 4:66–71
51. Kalač P (2014) Health effects and occurrence of dietary polyamines: a review for the period 2005–mid 2013. Food Chem 161:27–39. https://doi.org/10.1016/j.foodchem.2014.03.102
52. Tassoni A, Tango N, Ferri M (2013) Comparison of biogenic amine and polyphenol profiles of grape berries and wines obtained following conventional, organic and biodynamic agricultural and oenological practices. Food Chem 139:405–413. https://doi.org/10.1016/j.foodchem.2013.01.041
53. Leong SY, Oey I (2012) Effects of processing on anthocyanins, carotenoids and vitamin C in summer fruits and vegetables. Food Chem 133:1577–1587. https://doi.org/10.1016/j.foodchem.2012.02.052
54. Gupta K, Sengupta A, Chakraborty M, Gupta B (2016) Hydrogen peroxide and polyamines act as double edged swords in plant abiotic stress responses. Front Plant Sci 7:1343. https://doi.org/10.3389/fpls.2016.01343
55. Faller ALK, Fialho E (2010) Polyphenol content and antioxidant capacity in organic and conventional plant foods. J Food Compos Anal 23:561–568. https://doi.org/10.1016/j.jfca.2010.01.003
56. Lima GPP, da Rocha SA, Takaki M et al (2008) Comparison of polyamine, phenol and flavonoid contents in plants grown under conventional and organic methods. Int J Food Sci Technol 43:1838–1843. https://doi.org/10.1111/j.1365-2621.2008.01725.x
57. Rossetto MRM, Vianello F, Saeki MJ, Lima GPP (2015) Polyamines in conventional and organic vegetables exposed to exogenous ethylene. Food Chem 188:218–224. https://doi.org/10.1016/j.foodchem.2015.04.125
58. Winter CK, Davis SF (2006) Organic foods. J Food Sci 71:R117–R124. https://doi.org/10.1111/j.1750-3841.2006.00196.x
59. Monaco KA, Costa SM, Minatel IO et al (2016) Influence of ozonated water sanitation on postharvest quality of conventionally and organically cultivated mangoes after post-harvest storage. Postharvest Biol Technol 120:69–75. https://doi.org/10.1016/j.postharvbio.2016.05.003
60. Cao YY, Chen YH, Chen MX et al (2016) Growth characteristics and endosperm structure of superior and inferior spikelets of indica rice under high-temperature stress. Biol Plant 60:532–542. https://doi.org/10.1007/s10535-016-0606-6
61. da Silva Borges L, de Souza Vieira MC, Vianello F et al (2016) Antioxidant compounds of organically and conventionally fertilized jambu (Acmella oleracea). Biol Agric Hortic 32:149–158. https://doi.org/10.1080/01448765.2015.1103304
62. Selmar D, Kleinwächter M (2013) Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation of natural products. Plant Cell Physiol 54:817–826. https://doi.org/10.1093/pcp/pct054
63. Kong J-Q (2015) Phenylalanine ammonia-lyase, a key component used for phenylpropanoids production by metabolic engineering. RSC Adv 5:62587–62603. https://doi.org/10.1039/C5RA08196C
64. Oliveira AB, Moura CFH, Gomes-Filho E et al (2013) The impact of organic farming on quality of tomatoes is associated to increased oxidative stress during fruit development. PLoS One 8:e56354. https://doi.org/10.1371/journal.pone.0056354

65. Vallverdú-Queralt A, Medina-Remón A, Casals-Ribes I, Lamuela-Raventos RM (2012) Is there any difference between the phenolic content of organic and conventional tomato juices? Food Chem 130:222–227. https://doi.org/10.1016/j.foodchem.2011.07.017
66. Alasalvar C, Al-Farsi M, Quantick PC et al (2005) Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. Food Chem 89:69–76. https://doi.org/10.1016/j.foodchem.2004.02.013
67. McRae JM, Schulkin A, Kassara S et al (2013) Sensory properties of wine tannin fractions: implications for in-mouth sensory properties. J Agric Food Chem 61:719–727. https://doi.org/10.1021/jf304239n
68. Corredor Z, Rodriguez-Ribera L, Coll E et al (2016) Unfermented grape juice reduce genomic damage on patients undergoing hemodialysis. Food Chem Toxicol 92:1–7. https://doi.org/10.1016/j.fct.2016.03.016
69. Leong SY, Burritt DJ, Oey I (2016) Evaluation of the anthocyanin release and health-promoting properties of Pinot Noir grape juices after pulsed electric fields. Food Chem 196:833–841. https://doi.org/10.1016/j.foodchem.2015.10.025
70. Vanzo A, Terdoslavich M, Brandoni A et al (2008) Uptake of grape anthocyanins into the rat kidney and the involvement of bilitranslocase. Mol Nutr Food Res 52:1106–1116. https://doi.org/10.1002/mnfr.200700505
71. Bisht K, Wagner KH, Bulmer AC (2010) Curcumin, resveratrol and flavonoids as anti-inflammatory, cyto- and DNA-protective dietary compounds. Toxicology 278:88–100. https://doi.org/10.1016/j.tox.2009.11.008
72. Mukhopadhyay P, Prajapati AK (2015) Quercetin in anti-diabetic research and strategies for improved quercetin bioavailability using polymer-based carriers – a review. RSC Adv 5:97547–97562. https://doi.org/10.1039/C5RA18896B
73. Chen AY, Chen YC (2013) A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. Food Chem 138:2099–2107. https://doi.org/10.1016/j.foodchem.2012.11.139
74. Schlachterman A, Valle F, Wall KM et al (2008) Combined resveratrol, quercetin, and catechin treatment reduces breast tumor growth in a nude mouse model. Transl Oncol 1:19–27. https://doi.org/10.1593/tlo.07100
75. Che J, Liang B, Zhang Y et al (2017) Kaempferol alleviates ox-LDL-induced apoptosis by up-regulation of autophagy via inhibiting PI3K/Akt/mTOR pathway in human endothelial cells. Cardiovasc Pathol 31:57. https://doi.org/10.1016/j.carpath.2017.08.001
76. Choi JH, Park SE, Kim SJ, Kim S (2015) Kaempferol inhibits thrombosis and platelet activation. Biochimie 115:177–186. https://doi.org/10.1016/j.biochi.2015.06.001
77. Nam S-Y, Jeong H-J, Kim H-M (2017) Kaempferol impedes IL-32-induced monocyte-macrophage differentiation. Chem Biol Interact 274:107–115. https://doi.org/10.1016/j.cbi.2017.07.010
78. Iacopini P, Baldi M, Storchi P, Sebastiani L (2008) Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: content, in vitro antioxidant activity and interactions. J Food Compos Anal 21:589–598. https://doi.org/10.1016/j.jfca.2008.03.011
79. Grujic-Milanovic J, Miloradovic Z, Jovovic D et al (2017) The red wine polyphenol, resveratrol improves hemodynamics, oxidative defence and aortal structure in essential and malignant hypertension. J Funct Foods 34:266–276. https://doi.org/10.1016/j.jff.2017.04.035
80. Oh M, Lee JH, Bae SY et al (2015) Protective effects of red wine and resveratrol for foodborne virus surrogates. Food Control 47:502–509. https://doi.org/10.1016/j.foodcont.2014.07.056
81. Norouzzadeh M, Amiri F, Saboor-Yaraghi AA et al (2017) Does resveratrol improve insulin signalling in HepG2 cells? Can J Diabetes 41:211–216. https://doi.org/10.1016/j.jcjd.2016.09.015
82. Fernandes I, Faria A, Calhau C et al (2014) Bioavailability of anthocyanins and derivatives. J Funct Foods 7:54–66. https://doi.org/10.1016/j.jff.2013.05.010

83. Fu Y, Zhou E, Wei Z et al (2014) Cyanidin-3-O-β-glucoside ameliorates lipopolysaccharide-induced acute lung injury by reducing TLR4 recruitment into lipid rafts. Biochem Pharmacol 90:126–134. https://doi.org/10.1016/j.bcp.2014.05.004
84. Fratantonio D, Cimino F, Molonia MS et al (2017) Cyanidin-3-O-glucoside ameliorates palmitate-induced insulin resistance by modulating IRS-1 phosphorylation and release of endothelial derived vasoactive factors. Biochim Biophys Acta Mol Cell Biol Lipids 1862:351–357. https://doi.org/10.1016/j.bbalip.2016.12.008
85. Ferrari D, Speciale A, Cristani M et al (2016) Cyanidin-3-O-glucoside inhibits NF-kB signalling in intestinal epithelial cells exposed to TNF-α and exerts protective effects via Nrf2 pathway activation. Toxicol Lett 264:51–58. https://doi.org/10.1016/j.toxlet.2016.10.014
86. Chen P-N, Chu S-C, Chiou H-L et al (2006) Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. Cancer Lett 235:248–259. https://doi.org/10.1016/j.canlet.2005.04.033
87. Decendit A, Mamani-Matsuda M, Aumont V et al (2013) Malvidin-3-O-β glucoside, major grape anthocyanin, inhibits human macrophage-derived inflammatory mediators and decreases clinical scores in arthritic rats. Biochem Pharmacol 86:1461–1467. https://doi.org/10.1016/j.bcp.2013.06.010
88. Mackert JD, McIntosh MK (2016) Combination of the anthocyanidins malvidin and peonidin attenuates lipopolysaccharide-mediated inflammatory gene expression in primary human adipocytes. Nutr Res 36:1353–1360. https://doi.org/10.1016/j.nutres.2016.11.003
89. Khandelwal N, Abraham SK (2014) Intake of anthocyanidins pelargonidin and cyanidin reduces genotoxic stress in mice induced by diepoxybutane, urethane and endogenous nitrosation. Environ Toxicol Pharmacol 37:837–843. https://doi.org/10.1016/j.etap.2014.02.012
90. Sohanaki H, Baluchnejadmojarad T, Nikbakht F, Roghani M (2016) Pelargonidin improves memory deficit in amyloid β25-35 rat model of Alzheimer's disease by inhibition of glial activation, cholinesterase, and oxidative stress. Biomed Pharmacother 83:85–91. https://doi.org/10.1016/j.biopha.2016.06.021
91. Son JE, Jeong H, Kim H et al (2014) Pelargonidin attenuates PDGF-BB-induced aortic smooth muscle cell proliferation and migration by direct inhibition of focal adhesion kinase. Biochem Pharmacol 89:236–245. https://doi.org/10.1016/j.bcp.2014.02.015
92. Lim W, Jeong W, Song G (2016) Delphinidin suppresses proliferation and migration of human ovarian clear cell carcinoma cells through blocking AKT and ERK1/2 MAPK signaling pathways. Mol Cell Endocrinol 422:172–181. https://doi.org/10.1016/j.mce.2015.12.013
93. Sobiepanek A, Milner-Krawczyk M, Bobecka-Wesołowska K, Kobiela T (2016) The effect of delphinidin on the mechanical properties of keratinocytes exposed to UVB radiation. J Photochem Photobiol B Biol 164:264–270. https://doi.org/10.1016/j.jphotobiol.2016.09.038
94. Zang LY, Cosma G, Gardner H et al (2000) Effect of antioxidant protection by p-coumaric acid on low-density lipoprotein cholesterol oxidation. Am J Phys Cell Phys 279:C954–C960
95. Bami E, Ozakpınar OB, Ozdemir-Kumral ZN et al (2017) Protective effect of ferulic acid on cisplatin induced nephrotoxicity in rats. Environ Toxicol Pharmacol 54:105–111. https://doi.org/10.1016/j.etap.2017.06.026
96. Miao M, Cao L, Li R et al (2017) Protective effect of chlorogenic acid on the focal cerebral ischemia reperfusion rat models. Saudi Pharm J 25:556–563. https://doi.org/10.1016/j.jsps.2017.04.023
97. Meng S, Cao J, Feng Q et al (2013) Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. Evid-Based Complement Alternat Med 2013:1–11. https://doi.org/10.1155/2013/801457
98. Huang Y, Chen H, Zhou X et al (2017) Inhibition effects of chlorogenic acid on benign prostatic hyperplasia in mice. Eur J Pharmacol 809:191–195. https://doi.org/10.1016/j.ejphar.2017.04.017
99. Martin-Tanguy J (1985) The occurrence and possible function of hydroxycinnamoyl acid amides in plants. Plant Growth Regul 3:381–399. https://doi.org/10.1007/BF00117595

100. Bassard JE, Ullmann P, Bernier F, Werck-Reichhart D (2010) Phenolamides: bridging polyamines to the phenolic metabolism. Phytochemistry 71:1808–1824. https://doi.org/10.1016/j.phytochem.2010.08.003
101. Blagbrough IS, Moya E, Taylor S (1994) Polyamines and polyamine amides from wasps and spiders. Biochem Soc Trans 22:888–893
102. Walters D, Cowley T, Mitchell A (2002) Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings. J Exp Bot 53:747–756. https://doi.org/10.1093/jexbot/53.369.747
103. Edreva AM, Velikova VB, Tsonev TD (2007) Phenylamides in plants. Russ J Plant Physiol 54:287–301. https://doi.org/10.1134/S1021443707030016
104. Yonei S, Furui H (1981) Lethal and mutagenic effects of malondialdehyde, a decomposition product of peroxidized lipids, on *Escherichia coli* with different DNA-repair capacities. Mutat Res 88:23–32
105. Pariza MW (1982) Mutagens in heated foods. Food Technol 36:53
106. Nakatani N, Inatani R, Ohta H, Nishioka A (1986) Chemical constituents of peppers (Piper spp.) and application to food preservation: naturally occurring antioxidative compounds. Environ Health Perspect 67:135–142. https://doi.org/10.1289/ehp.8667135
107. Kusano T, Berberich T, Tateda C, Takahashi Y (2008) Polyamines: essential factors for growth and survival. Planta 228:367–381. https://doi.org/10.1007/s00425-008-0772-7
108. Melgarejo E, Urdiales JL, Sánchez-Jiménez F, Medina MÁ (2010) Targeting polyamines and biogenic amines by green tea epigallocatechin-3-gallate. Amino Acids 38:519–523. https://doi.org/10.1007/s00726-009-0411-z
109. Pegg AE (1988) Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res 48:759–774
110. Wolter F, Ulrich S, Stein J (2004) Molecular mechanisms of the chemopreventive effects of resveratrol and its abalogs in colorectal cancer. Key role of polyamines. J Nutr 134:3219–3222
111. Bertoldi M, Gonsalvi M, Borri Voltattorni C (2001) Green tea polyphenols: novel irreversible inhibitors of dopa decarboxylase. Biochem Biophys Res Commun 284:90–93. https://doi.org/10.1006/bbrc.2001.4945
112. Yamashita K, Suzuki Y, Matsui T et al (2000) Epigallocatechin gallate inhibits histamine release from rat basophilic leukemia (RBL-2H3) cells: role of tyrosine phosphorylation pathway. Biochem Biophys Res Commun 274:603–608. https://doi.org/10.1006/bbrc.2000.3200
113. Rodríguez-Caso C, Rodríguez-Agudo D, Sánchez-Jiménez F, Medina MA (2003) Green tea epigallocatechin-3-gallate is an inhibitor of mammalian histidine decarboxylase. Cell Mol Life Sci 60:1760–1763. https://doi.org/10.1007/s00018-003-3135-3
114. Alberto MR, Arena ME, Manca de Nadra MC (2007) Putrescine production from agmatine by Lactobacillus hilgardii: effect of phenolic compounds. Food Control 18:898–903. https://doi.org/10.1016/j.foodcont.2006.05.006
115. Cardona F, Andrés-Lacueva C, Tulipani S et al (2013) Benefits of polyphenols on gut microbiota and implications in human health. J Nutr Biochem 24:1415–1422. https://doi.org/10.1016/j.jnutbio.2013.05.001
116. Kutschera M, Engst W, Blaut M, Braune A (2011) Isolation of catechin-converting human intestinal bacteria. J Appl Microbiol 111:165–175. https://doi.org/10.1111/j.1365-2672.2011.05025.x
117. Bandele OJ, Clawson SJ, Osheroff N (2008) Dietary polyphenols as topoisomerase II poisons: B ring and C ring substituents determine the mechanism of enzyme-mediated DNA cleavage enhancement. Chem Res Toxicol 21:1253–1260. https://doi.org/10.1021/tx8000785
118. Pommier Y, Leo E, Zhang H, Marchand C (2010) DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem Biol 17:421–433. https://doi.org/10.1016/j.chembiol.2010.04.012
119. Pastoriza S, Mesías M, Cabrera C, Rufián-Henares JA (2017) Healthy properties of green and white teas: an update. Food Funct 8:2650–2662. https://doi.org/10.1039/c7fo00611j

120. Opitz SEW, Goodman BA, Keller M et al (2017) Understanding the effects of roasting on antioxidant components of coffee brews by coupling on-line ABTS assay to high performance size exclusion chromatography. Phytochem Anal 28:106–114. https://doi.org/10.1002/pca.2661
121. Rivero D, Pérez-Magariño S, González-Sanjosé ML et al (2005) Inhibition of induced DNA oxidative damage by beers: correlation with the content of polyphenols and Melanoidins. J Agric Food Chem 53:3637–3642. https://doi.org/10.1021/jf048146v
122. Crozier TWM, Stalmach A, Lean MEJ, Crozier A (2012) Espresso coffees, caffeine and chlorogenic acid intake: potential health implications. Food Funct 3:30–33. https://doi.org/10.1039/C1FO10240K
123. Murase T, Yokoi Y, Misawa K et al (2012) Coffee polyphenols modulate whole-body substrate oxidation and suppress postprandial hyperglycaemia, hyperinsulinaemia and hyperlipidaemia. Br J Nutr 107:1757–1765. https://doi.org/10.1017/S0007114511005083
124. Natella F, Nardini M, Giannetti I et al (2002) Coffee drinking influences plasma antioxidant capacity in humans. J Agric Food Chem 50:6211–6216. https://doi.org/10.1021/jf025768c
125. Hoelzl C, Knasmüller S, Wagner K-H et al (2010) Instant coffee with high chlorogenic acid levels protects humans against oxidative damage of macromolecules. Mol Nutr Food Res 54:1722–1733. https://doi.org/10.1002/mnfr.201000048
126. Lecumberri E, Dupertuis YM, Miralbell R, Pichard C (2013) Green tea polyphenol epigallocatechin-3-gallate (EGCG) as adjuvant in cancer therapy. Clin Nutr 32:894–903. https://doi.org/10.1016/j.clnu.2013.03.008
127. Fantini M, Benvenuto M, Masuelli L et al (2015) In vitro and in vivo antitumoral effects of combinations of polyphenols, or polyphenols and anticancer drugs: perspectives on cancer treatment. Int J Mol Sci 16:9236–9282. https://doi.org/10.3390/ijms16059236
128. Ho JWS, Cheung MWM (2014) Combination of phytochemicals as adjuvants for cancer therapy. Recent Pat Anticancer Drug Discov 9:297–302
129. Okarter N, Liu RH (2010) Health benefits of whole grain phytochemicals. Crit Rev Food Sci Nutr 50:193–208. https://doi.org/10.1080/10408390802248734
130. Zhang Y-J, Gan R-Y, Li S et al (2015) Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules 20:21138–21156. https://doi.org/10.3390/molecules201219753
131. Kaulmann A, Bohn T (2014) Carotenoids, inflammation, and oxidative stress – implications of cellular signaling pathways and relation to chronic disease prevention. Nutr Res 34:907–929. https://doi.org/10.1016/j.nutres.2014.07.010
132. Minatel IO, Francisqueti FV, Corrêa CR, Lima GPP (2016) Antioxidant activity of γ-Oryzanol: a complex network of interactions. Int J Mol Sci 17:1107. https://doi.org/10.3390/ijms17081107
133. Carnésecchi S, Schneider Y, Lazarus SA et al (2002) Flavanols and procyanidins of cocoa and chocolate inhibit growth and polyamine biosynthesis of human colonic cancer cells. Cancer Lett 175:147–155. https://doi.org/10.1016/S0304-3835(01)00731-5
134. Zhang M, Wang H, Tracey KJ (2000) Regulation of macrophage activation and inflammation by spermine: a new chapter in an old story. Crit Care Med 28:N60–N66
135. Adibhatla RM, Hatcher JF, Sailor K, Dempsey RJ (2002) Polyamines and central nervous system injury: spermine and spermidine decrease following transient focal cerebral ischemia in spontaneously hypertensive rats. Brain Res 938:81–86. https://doi.org/10.1016/S0006-8993(02)02447-2
136. Lee S-Y, Kim C-Y, Lee J-J et al (2003) Effects of delayed administration of (−)-epigallocatechin gallate, a green tea polyphenol on the changes in polyamine levels and neuronal damage after transient forebrain ischemia in gerbils. Brain Res Bull 61:399–406. https://doi.org/10.1016/S0361-9230(03)00139-4
137. Brooks WH (2013) Increased polyamines alter chromatin and stabilize autoantigens in autoimmune diseases. Front Immunol 4:91. https://doi.org/10.3389/fimmu.2013.00091
138. Hanfrey CC, Pearson BM, Hazeldine S et al (2011) Alternative spermidine biosynthetic route is critical for growth of campylobacter jejuni and is the dominant polyamine pathway in human gut microbiota. J Biol Chem 286:43301–43312. https://doi.org/10.1074/jbc.M111.307835

139. Di Martino ML, Campilongo R, Casalino M et al (2013) Polyamines: emerging players in bacteria–host interactions. Int J Med Microbiol 303:484–491. https://doi.org/10.1016/j.ijmm.2013.06.008
140. Tabor H, Tabor CW, Rosenthal SM (1961) The biochemistry of the polyamines: spermidine and spermine. Annu Rev Biochem 30:579–604. https://doi.org/10.1146/annurev.bi.30.070161.003051
141. Mullen W, Archeveque M-A, Edwards CA et al (2008) Bioavailability and metabolism of orange juice flavanones in humans: impact of a full-fat yogurt. J Agric Food Chem 56:11157–11164. https://doi.org/10.1021/jf801974v
142. Yamamoto M, Jokura H, Hashizume K et al (2013) Hesperidin metabolite hesperetin-7-O-glucuronide, but not hesperetin-3′-O-glucuronide, exerts hypotensive, vasodilatory, and anti-inflammatory activities. Food Funct 4:1346. https://doi.org/10.1039/c3fo60030k
143. Lentini A, Forni C, Provenzano B, Beninati S (2007) Enhancement of transglutaminase activity and polyamine depletion in B16-F10 melanoma cells by flavonoids naringenin and hesperitin correlate to reduction of the in vivo metastatic potential. Amino Acids 32:95–100. https://doi.org/10.1007/s00726-006-0304-3
144. Young JE, Zhao X, Carey EE et al (2005) Phytochemical phenolics in organically grown vegetables. Mol Nutr Food Res 49:1136–1142. https://doi.org/10.1002/mnfr.200500080

Intake of Mediterranean Foods

2

Charalampos Siotos, Marco Vinceti, and Androniki Naska

Contents

Abstract

The traditional Mediterranean diet is characterized by: (a) high consumption of cereals, vegetables, fruit, nuts, legumes, fish, and seafood; (b) the use of olive oil as the main, if not the only, added lipid; (c) moderate consumption of milk and dairy products; (d) moderate intake of alcohol, in the form of wine and preferably during meals; and (e) low consumption of meat and meat products. The prevalent consumption of olive oil and the low consumption of animal products are reflected in the high ratio of monounsaturated to saturated fat intake, typical of the dietary pattern in the region. There is increasing evidence from observational and experimental

C. Siotos · A. Naska (✉)
Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
e-mail: Siotos-harrys@outlook.com; anaska@med.uoa.gr

M. Vinceti
Research Center for Environmental, Genetic and Nutritional Epidemiology (CREAGEN), University of Modena and Reggio Emilia, Modena, Italy

Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA
e-mail: marco.vinceti@unimore.it

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_26

epidemiological studies, further enriched by the conclusions of their systematic reviews and meta-analyses, that adherence to the Mediterranean dietary pattern promotes health and reduces the risk of premature death from chronic degenerative diseases. Mediterranean countries and especially the European ones have experienced a "westernization" process of their food habits, and have increased the per capita supply of non-Mediterranean foods (animal fats, vegetable oils other than olive oil, sugar, and meat) and decreased the supply of legumes and alcoholic beverages, including wine. The evidence that Mediterraneans are gradually departing from their traditional eating habits does not only refer to the adult population in the region, but it has also been reproduced in large-scale nutritional surveys among children, adolescents, and young adults – the trend-setters for future generations. Next to the effect on people's health, the gradual abandoning of the traditional Mediterranean diet cannot support sustainable development in the way the Mediterranean diet does. Being adjusted to the cultural, climatic, and other environmental characteristics of the region, the Mediterranean diet is protective and helpful to biodiversity, accessible and economically affordable, and contributes to food and nutrition security.

Keywords
Mediterranean diet · Score · Food intake · Health

1 Introduction

The Mediterranean basin has historically been a crossroad of civilizations and this is reflected in the culture, the scenery, the flora, and the food resources. Some plants, like the olive tree, wheat, and the grapevine, have been in this area for millennia, whereas citrus fruit, tomatoes, eggplants, corn, rice, beans, and potatoes were imported at different time periods. Over the years, this large variety of plant foods has been integrated in people's culinary habits paving the way to what has become in the twentieth century the worldwide famous Mediterranean diet [1].

The countries bordering the Mediterranean Sea have their own dietary traditions, and olive oil occupies a central position in all. Without the presumption of a scientific definition, the Mediterranean diet could be considered as the dietary pattern found in the olive oil growing areas of the Mediterranean region in the late 1950s and early 1960s [2]. Traditionally, in addition to olive oil, the daily intake included a high intake of cereals, fruit, nuts, vegetables, and legumes combined with a low intake of dairy products, meat, and meat products. In the traditional Mediterranean diet, fish and seafood intake depended on the vicinity to the sea and ethanol intake was moderate and mainly in the form of wine during meals [3]. This combination of intakes is rich in components with well-established cardioprotective functions: the liberal use of olive oil guarantees an increased intake of monounsaturated fatty acids and tocopherols; fish, vegetables, and nuts are good sources of n-3 and n-6 polyunsaturated fatty acids; the high consumption of vegetables, fresh fruit, and cereals provide fiber, a variety of vitamins (such as vitamin C, vitamin E, and beta-carotene), important minerals (potassium, for instance), and other beneficial substances (such as polyphenols and anthocyanins) [4, 5]. The limited

consumption of meat and dairies provided few saturated fatty acids and the consumption of locally grown products, such as the wild green leafy vegetables, conveys additional benefits due to their high flavonoid content [6].

Contrary to other also highly cited dietary regimes, the Mediterranean dietary pattern has not been developed by health professionals on the basis of analytical evidence for health-promoting dietary choices. It is rather a natural experiment, a holistic lifestyle that existed in the region for years and was discovered in the second half of the twentieth century after ecological observations that people in this region experienced mortality rates which were far lower than those of more developed and affluent countries [7]. The first systematic attempt to investigate dietary patterns in the Mediterranean region dates back to 1948 on the island of Crete. At that time, the Greek government was worried about the post-war conditions and invited the Rockefeller Foundation to undertake an epidemiological study in order to provide advice on how to raise the population's standard of living. The study assembled data collected from 128 Cretan households. The final report was published in 1953 and provides a succinct but vivid description of people's diet. In particular, the investigators wrote: "olives, cereal grains, legumes, fruits, wild greens and herbs, together with limited quantities of goat meat and milk, game and fish, consist the basic Cretan food. . .no meal was complete without bread. Olives and olive oil contributed heavily to the energy intake. Food seemed literally to be "swimming" in oil. . ." and concluded that the food consumption levels ". . .were surprisingly good. On the whole, their food patterns and food habits were extremely well adapted to their natural and economic resources, as well as their needs" [8].

2 Mediterranean Diet: Definition and Assessment of Adherence

Overall, the traditional Mediterranean diet has the following characteristics:

- High consumption of cereals, vegetables, fruit, nuts, and legumes
- High consumption of fish and seafood
- Use of olive oil as the main, if not the only, added lipid
- Moderate consumption of milk and dairy products
- Low consumption of meat and meat products
- Moderate intake of alcohol, in the form of wine

The traditional Mediterranean diet has predominantly been a plant-based diet with olive oil as the principal added lipid, allowing a high intake of monounsaturated and a low intake of saturated fatty acids – the latter contributed 7% to 8% of total energy intake [2].

The operationalization of the Mediterranean dietary pattern has been achieved through various computational scores, which are used to reflect the aggregated dietary exposure particularly when diet-disease associations are evaluated. The first and more often cited index to assess adherence to the Mediterranean diet among adults and elderly is the Mediterranean diet score calculated on the basis of the aforementioned

characteristics of the Mediterranean diet [9]. According to this score, individuals receive a value of +1 if their daily intake of foods frequently included in the traditional Mediterranean dietary pattern (i.e., vegetables, legumes, fruit and nuts, cereals, fish and seafood, and monounsaturated to saturated fat ratio to reflect the high olive oil intake) is above the gender-specific median value of the study sample. Correspondingly, individuals are assigned a value of 0 if their daily intake of the aforementioned foods is below the gender-specific median. Hence, an individual receives +1 value if his daily intake of vegetables is above the median vegetable consumption in this study sample. Similarly, the individual will receive an additional +1 value if his intake of fruit and nut is also above the sample median. However, if the individual's daily intake of fish and seafood is below the sample median, he will receive the value of 0. In addition, individuals receive values of +1 if their daily intake of milk and dairies, meat and meat products is equal to or lower than the gender-specific median and are assigned values of 0 otherwise. Thus, an individual with daily meat intake below the sample median will receive an additional +1 value, but if his daily intake of milk and dairies is above the sample median, he will receive a 0 value. Lastly, a value of +1 is further given to individuals consuming a moderate amount of alcohol (i.e., between 5 and 25 g per day for women and between 10 and 50 g per day for men) and a value of 0 otherwise. Finally, all values received are added and the total score ranges between 0 (minimal adherence to the traditional Mediterranean diet) and 9 (maximal adherence to the traditional Mediterranean diet) (Fig. 1).

Variations of this score, as well as other indices to assess conformity to Mediterranean dietary traditions are also available in the literature [10–15]. Although these employ various scoring techniques, they are all based on the dietary characteristics described above, which capture the essence of the Mediterranean dietary profile.

Among children and adolescents, adherence to the Mediterranean diet is usually assessed through the KIDMED index [16], which comprises of 16 questions combining principles of a Mediterranean dietary pattern (i.e., eating fruit, vegetables, and legumes regularly; using olive oil at home), together with general dietary guidelines for children (for instance, always to have breakfast). Respondents receive positive scores if they conform to principles of the Mediterranean diet and follow nutritional guidelines and negative scores otherwise. The total score of the KIDMED index for children and adolescents is usually classified into three levels: equal or higher than 8 indicates an optimal adherence to Mediterranean diet; scores between 4 and 7 denote a necessity for improvement in order to adjust intake to the Mediterranean diet; and scores below or equal to 3 point to a diet of very low quality. Next to the KIDMED index, other scores have also been proposed in the literature to assess adherence to the Mediterranean diet among children and adolescents [17].

3 Mediterranean Diet and Health

The ecological observation that adults living in the Mediterranean area have displayed favorable health statistics induced the classic Seven Countries Study which has prospectively investigated long-term incidence and mortality from coronary heart

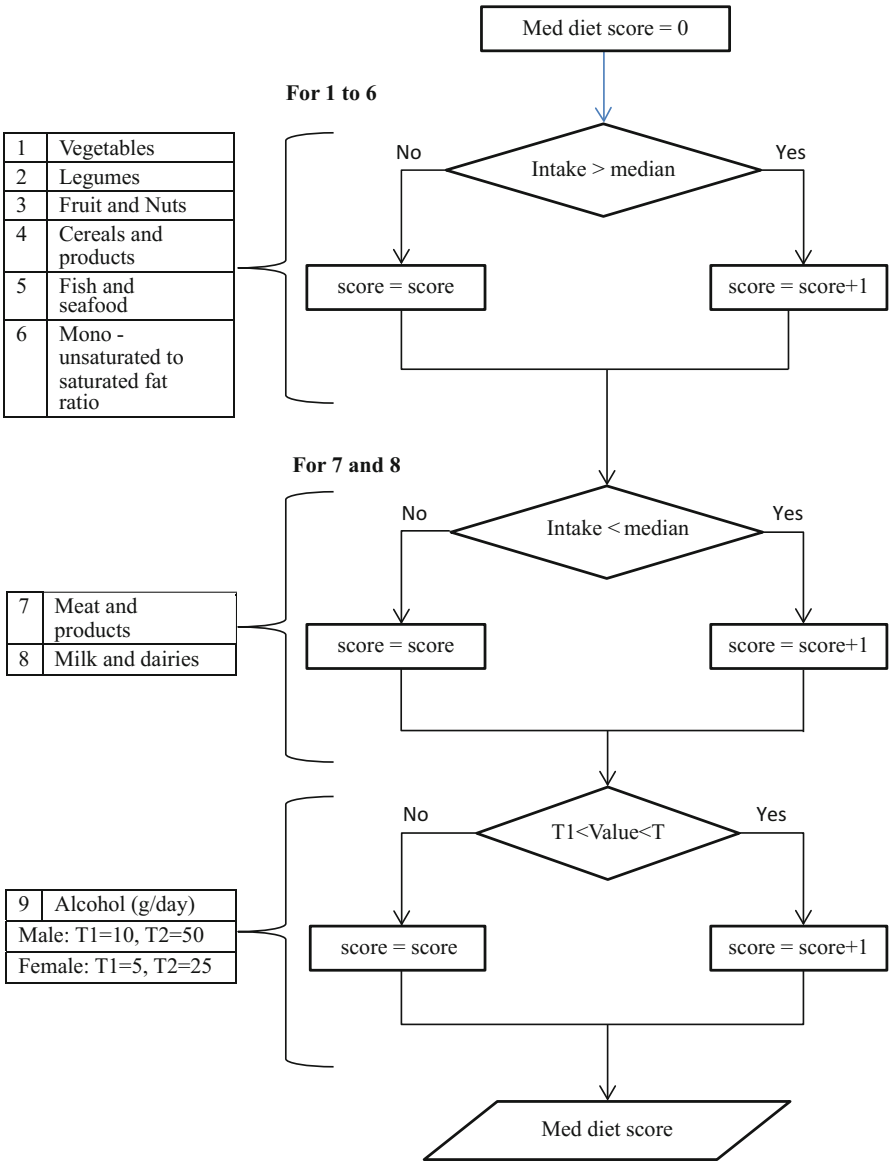


Fig. 1 Schematic presentation for the estimation of the Mediterranean diet score (Med diet score) [9]

disease in cohorts of men aged 40–59 years in seven countries, including three Mediterranean ones: Italy, Greece, and Croatia [18]. The baseline survey was carried out between 1958 and 1964 and information on diet was collected through food diaries in random subsamples of the 16 study cohorts. The study primarily provided evidence

for a strong positive association between the average saturated fat intake and overall mortality or mortality from coronary heart disease [19]. Although the Seven Countries Study mainly contributed to the understanding of the importance of the type of fat consumed on coronary heart disease risk, it certainly assisted in putting the whole diet of populations around the Mediterranean into the limelight. Indeed, the health indices observed in the region could not be explained by differences in education levels, financial status, or health care systems, simply because in this area in the 1960s, socioeconomic indicators were much lower than those in more developed areas of the world. Therefore, attention has focused on the overall diet of Mediterranean populations through a holistic approach, as one of the key explanatory factors [19].

The interest of the scientific community in the traditional diet of populations in the Mediterranean region and its health promoting effects thrived after mid 1990s and the publication of a prospective study conducted among elderly Greeks residing in rural areas [20]. The researchers recorded the usual dietary intakes of about 180 males and females aged 70 years and over and observed that higher adherence to Mediterranean diet (as assessed by the score described above) was significantly associated with a reduced risk of death from any cause by 17% per one unit increase in the score (95%CI: 1–31%). Authors additionally evaluated the association of each one of the components of the Mediterranean diet with overall mortality and noted that the individual components of the diet score had weak and generally nonsignificant associations with survival, whereas the overall score had a substantial and statistically significant effect.

In several case-control and prospective cohort studies, as well as experimental studies, mostly conducted among Mediterranean populations in Europe, the Mediterranean diet has been associated with a reduced incidence of coronary heart disease, stroke, and cancer, and with beneficial effects on cardiovascular disease-related markers [21–23]. Dietary intakes following the principles of Mediterranean diet have also been associated with self-perceived mental and physical health, global cognitive function, as well as an overall higher quality of life [24–26]. The consistent evidence from large-scale observational studies for its protective effects lead a group of investigators in Spain to the undertaking of the "Prevención con Dieta Mediterránea" (PREDIMED) study, a large nutritional trial assessing the long-term effects of Mediterranean diet on the primary prevention of cardiovascular disease. Men (55–80 years of age) and women (60–80 years of age) with no cardiovascular disease at enrolment were eligible for inclusion in the study if they had either type II diabetes mellitus or at least three of the following risk factors: smoking, hypertension, elevated low-density lipoprotein cholesterol levels, low high-density lipoprotein cholesterol levels, overweight or obesity, or a family history of premature coronary heart disease. About 7500 individuals at high cardiovascular risk were randomized to follow: (a) a Mediterranean diet supplemented with freely provided extra virgin olive oil, (b) a Mediterranean diet supplemented with mixed nuts again provided to participants at no cost, or (c) the control diet (advice to reduce all dietary fats). The main findings of the trial showed that a Mediterranean diet supplemented with extra virgin olive oil reduced the incidence of major cardiovascular events by approximately 30% [hazard ratio (HR) 0.70; 95% CI: 0.54, 0.92) and by 28%

[HR 0.72; 95% CI: 0.54, 0.96] when supplemented with nuts, in comparison to the control diet [27]. Although PREDIMED was planned as a 6-year trial, it was stopped after a median follow-up time of 4.8 years (interquartile range, 2.8–5.8 years) on the basis of early statistically significant results on the beneficial effect of following a Mediterranean diet on premature mortality. Subsequent analyses identified further benefits on blood pressure, insulin sensitivity, lipid profiles, lipoprotein particles, inflammation, oxidative stress, and carotid atherosclerosis [4, 28], providing strong experimental support for the advantages of consuming a Mediterranean diet.

The bulk of publications around the health-promoting effects of the Mediterranean diet have been systematically reviewed through several meta-analyses. In a review of prospective cohort studies that investigated possible associations of adherence to the Mediterranean diet and overall or disease-specific mortality, Sofi et al. [29] observed that a two-point increase of adherence to the Mediterranean diet was associated with a significant protection against premature death and the incidence of cardiovascular and other chronic degenerative diseases. These findings were further confirmed in 2010 in a meta-analysis which considered the studies of the earlier analysis, together with seven prospective studies published in 2009 and 2010 (one study for overall mortality; three studies for cardiovascular incidence and mortality; one study for cancer incidence and mortality; and two studies for neurodegenerative diseases) [21]. The meta-analysis included 50,000 incident cases or deaths from any cause and/or cause-specific. Authors observed that a two-point increase in adherence to the Mediterranean diet was associated with a significant reduction of overall mortality (pooled relative risk (RR): 0.92; 95% CI: 0.90, 0.94, cardiovascular incidence and mortality (pooled RR: 0.90; 95% CI: 0.87, 0.93), cancer incidence and mortality (pooled RR: 0.94; 95% CI: 0.92, 0.96), and occurrence of neurodegenerative disease (pooled RR: 0.87; 95% CI: 0.81, 0.94). There was no evidence for publication bias in the included results [21].

In their systematic review of randomized controlled trials assessing the effect of diets with unrestricted fat intake and following the principles of the Mediterranean diet to CVD risk compared to any control diet, Liyanage et al. [30] retrieved six studies among adults with follow-up periods longer than 3 months. The studies reported effects on major vascular events ($n = 477$), death from any cause ($n = 693$), and vascular deaths ($n = 315$). When data from all studies were combined, a Mediterranean-type diet protected against major vascular events (pooled RR: 0.63, 95% CI: 0.53–0.75), coronary events (pooled RR: 0.65, 95% CI: 0.50–0.85), stroke (pooled RR: 0.65, 95% CI: 0.48–0.88), and heart failure (pooled RR: 0.30, 95% CI: 0.17–0.56), but not for all-cause mortality (pooled RR: 1.00, 95% CI: 0.86–1.15) or cardiovascular mortality (pooled RR: 0.90, 95% CI: 0.72–1.11). Schwingshackl and Hoffmann [31] reviewed observational (case-control and cohort) studies to assess the effect of adherence to Mediterranean Diet on overall cancer mortality, incidence of different types of cancer, as well as fatality among cancer survivors. An overall population of 1,784,404 individuals from 56 studies was included in this analysis. The highest score of adherence to Mediterranean diet was associated with a lower risk of death from cancer at any site (pooled RR: 0.87, 95% CI 0.81–0.93), colorectal cancer (pooled RR: 0.83, 95% CI

0.76–0.89), breast cancer (pooled RR: 0.93, 95% CI 0.87–0.99), gastric cancer (pooled RR: 0.73, 95% CI 0.55–0.97), prostate cancer (pooled RR: 0.96, 95% CI 0.92–1.00), liver cancer (pooled RR: 0.58, 95% CI 0.46–0.73), head and neck cancer (pooled RR: 0.40, 95% CI 0.24–0.66), pancreatic cancer (pooled RR: 0.48, 95% CI 0.35–0.66), and respiratory cancer (pooled RR: 0.10, 95% CI 0.01–0.70). Based on a small number of studies among cancer survivors, there was no association between the highest adherence to Mediterranean diet and risk of death (three cohort studies), or cancer recurrence (one cohort study). In particular, the pooled RR for death from cancer among patients was 1.01 when individuals with highest adherence to Mediterranean diet were compared to those with lower adherence (95% CI: 0.81–1.26).

In order to summarize the evidence and evaluate the validity of the association between adherence to the Mediterranean diet and multiple health outcomes reported by several systematic reviews, Dinu et al. [32] reviewed evidence across meta-analyses of observational studies and randomized clinical trials. Thirteen meta-analyses of observational studies and 16 meta-analyses of randomized trials investigating the association between adherence to Mediterranean diet and 37 different health outcomes were analyzed. The evidence for a beneficial effect of Mediterranean diet was robust and supported by a strong statistically significant finding, large simple sizes, and not considerable heterogeneity among studies for overall mortality, cardiovascular diseases, overall cancer incidence, neurodegenerative diseases, and diabetes. For most of the site-specific cancers, as well as for inflammatory and metabolic parameters, the evidence was only suggestive or weak. No evidence was reported for bladder, endometrial and ovarian cancers, as well as for low density lipoprotein-cholesterol levels.

Furthermore, investigations among individuals residing in non-Mediterranean regions such as Denmark and the Netherlands in Europe, Melbourne in Australia, China, and the US provided additional evidence that adherence to the principles of the Mediterranean diet affects the survival of elderly people [33–37]. In these studies, an increase in a diet score derived from the key features of the traditional Mediterranean diet was associated with a significant reduction in overall and cause-specific mortality. The evidence that the beneficial effect of adherence to Mediterranean diet on the risk of premature death prevails also among individuals in areas distant to the Mediterranean basin rejected the possibility of an association confounded by nondiet-related factors, as well as the probability of effect modifiers shaping the health-promoting effects of the Mediterranean diet. Thus, one could safely argue that it is not the climatic, social, and cultural conditions that shaped this association, that the evidence for an independent effect of diet on overall survival is reinforced.

The findings of epidemiological studies are additionally supported by accumulating evidence from laboratory studies, which point to the converging effects of Mediterranean diet and olive oil (particularly the virgin or extra virgin olive oil that has not undergone excessive process) on the homeostatic control of genes implicated in cardiovascular risk, such as lipid metabolism, vessel protection, and blood pressure control, immune-inflammatory pathways, metabolic regulation, and detoxification of reactive species [5]. The impressive growth of the omic technologies provides new insight in the molecular and metabolic effects of functional components

of olive oil and other Mediterranean foods. Transcriptomic fingerprints revealed molecular targets in the area of primary prevention and clinical management of cardiovascular disease, as well as other degenerative age-related disorders [38].

4 Spatial and Time Differences in Food Intake

4.1 Sources of Dietary Data

In spite of the catholic recognition of Mediterranean diet as a health-conducive pattern, several populations in the region seem to be gradually abandoning it. Data to monitor food consumption over time and among different regions can be derived from three main sources: (a) the Food and Agriculture Organization assembled Food Balance Sheets (FBS), (b) the Household Budget Surveys (HBS), and (c) Nutrition Surveys, the latter collecting detailed, quantitative data on the usual food and beverage intake of a large – and often representative – sample of the target population.

The FBS provide information on food quantities assumed to be available for human consumption in the country, estimated on the basis of the annual food production, imports and exports, changes in stocks, agricultural and industrial uses within a country, as well as losses during storage and transportation (http://www.fao.org/faostat/en/#data/FBS). The data refer to the early stages of the food chain and the per capita supply is obtained by dividing the respective food quantity by the population partaking of it. The total population estimates, however, refer as a rule to resident population only, while nonresident population, such as tourists, illegal immigrants, refugees, etc., are generally not included. This omission may therefore result in an underestimation of the total partaker population and an overestimation of the various per-person food supplies. The accuracy of FBS data are further dependent on the reliability of the underlying basic statistics, which vary in terms of coverage and accuracy. Although import and export data are generally accurate, in some cases there may be some trade across national boundaries that goes unrecorded. Data on own food production are collected in some countries, but this information can be substantially under-recorded where there is a thriving economy in home production. Waste and food given to pets may also be sources of error. Hence, given the nature of the FBS and their inherent limitations, the data are interpreted to provide information on annual per capita supply of foods to the population.

Household budget surveys are systematically conducted by National Statistical Offices and aim at collecting, among others, data on food availability at household level. Household budget surveys provide information on products available for consumption to a nationally representative sample. The members of the participating households are asked to record information on all foods and beverages available in the household during a reference period, including purchases, contributions from own production, and food items offered to members as gifts. The survey is implemented over a period of a year, with due attention to capture seasonal variation in food intake. Information on the demographic and socioeconomic characteristics of the household

members is also recorded, allowing analyses on the effect of socioeconomic determinants on food choices (http://www.hhf-greece.gr/DafnesoftWebV2/). The HBS are not primarily designed to collect nutritional information and the food data bear limitations, which need to be considered when they are used for the purpose of nutritional surveillance. In most cases, no records are collected on the type and quantity of food items and beverages consumed outside the home (at restaurants, canteens, and similar establishments, for example); food losses and waste, foods given to pets, as well as meals offered to guests are not consistently collected; and gender- and age-specific estimations of food consumption require the application of statistical modeling [39]. The data collected though the HBS provide information on the daily availability of foods and beverages to a nationally representative sample of each country's households.

The individual-based nutrition surveys constitute the optimal method for assessing dietary patterns, quantifying determinants of food choices, and evaluating diet-disease associations. Being expensive and labor intensive, however, representative individual-based surveys are undertaken regularly in only a limited number of countries, usually those with robust economies and years of experience in this field. Furthermore, the implementation of various protocols and data collection methods limits the potential to undertake comparisons between countries.

Notwithstanding their caveats, countries with no routine information on the food consumption of large samples of their population, as well as those interested in comparing their national dietary patterns over time or with those of other populations, have traditionally used the FBS or the HBS data [40, 41]. They both provide a resource for conducting comparative nutritional analyses and could help highlighting issues such as (a) the dietary patterns prevailing in different countries and their sociodemographic determinants; (b) time trends in the food habits of populations; and (c) the evaluation of nutrition action plans, interventions, and related strategies implemented at national or international level [42].

5 Monitoring the Intake of Mediterranean Foods

Making use of FBS data referring to the periods between 1961 and 1963 and between 1998 and 2000, Balanza et al. [43] calculated changes in total energy, energy provided from macronutrients, and specific food groups for three European regions: Southern or Mediterranean (including Spain, Portugal, Italy, Greece, France, Cyprus, and Albania), Northern (combining the UK, Sweden, Norway, Finland, Germany, Ireland, Denmark, and Iceland), and Eastern Europe (comprising the Czech Republic, Slovakia, Poland, Bulgaria, Hungary, and Romania). Over the study period, the supply of total energy and energy from lipids increased considerably in all European areas, while the percentage of energy from carbohydrates decreased. The greatest changes have occurred in Mediterranean Europe where, contrary to traditional eating habits, a significant reduction in the energy supplied by cereals (30%) and wine (55%) and an increase in the supply of milk (78%) and dairy products (24%) was noted. Overall, such changes led to an increase of 20% in

the total supply of calories, comprising an increase of 48% in calories from lipids, mostly of animal origin and a fall of 21% in calories from carbohydrates.

In another comparison of FBS data referring to the periods 1961–1965 and 2000–2004 and covering Mediterranean countries in Europe, Africa and the Middle-East, as well as countries in other regions (North and Central Europe, North and South America, Asia, and Australia), Vareiro et al. [41] confirmed previous findings that the greatest changes in food supplies took place in Mediterranean Europe, with an increase in the per capita supply of non-Mediterranean foods (animal fats, vegetable oils other than olive oil, sugar, and meat) and a decrease in the supply of legumes and alcoholic beverages, including wine. However, although gradually abandoning some of its typical characteristics, the Mediterranean countries of Europe still recorded higher per capita supplies of olive oil, vegetables, fruit, and fish than other areas. Interestingly, during the same periods, northern European countries tended to increase the consumption of Mediterranean foods such as olive oil and fruit.

According to an analysis of HBS data collected in the 1980s and early 1990s including nationally representative samples of households from several European countries [44], in Greece and Spain the mean daily population intake exceeded the WHO recommendations of at least 400 g of combined fruit and vegetable intake per day. However, the correlation between the proportion of low fruit and low vegetable consumers was unexpectedly weak (Spearman's correlation coefficient: +0.18), suggesting differential preferences toward the consumption of fruit and vegetables among the surveyed populations. Moreover, more than 50% of the households in the surveyed populations were likely to consume less than the recommended daily vegetable intake of three portions, and this applied even to the two Mediterranean populations included in this analysis. In particular, 56% of the households in Greece and 76% of the households in Spain failed to report three portions of vegetables per day.

Using FBS data collected from 1961 up to 2013 (http://www.fao.org/faostat/en/#data/FBS), Tables 1 and 2 present the annual supplies as well as the average change in the annual supply of food groups, components of the Mediterranean diet. Table 1 presents the supply of foods of livestock production and milk (animal foods); vegetables, fruit, cereals and cereal products, beans, peas, and other legumes (plant foods); and all types of fish, crustaceans, cephalopods, and mollusks (fish and seafood) in 15 countries surrounding the Mediterranean basin. Table 2 presents data for the supply of olive oil and wine. In general, annual supplies reflect local production and food preferences shaped by cultural norms. For instance, the supply of olive oil is highest in the three main olive oil producing countries (Greece, Italy, and Spain), while the supply of wine is low in countries with a high percentage of Muslims in the population (Algeria, Egypt, and Turkey). In the 50-year period between 1961 and 2013, all Mediterranean countries recorded an increase in the supply of animal foods, fish, and seafood. The supply of plant foods increased in the majority of countries; a decrease between 1961 and 2013 was however recorded in Cyprus (approximately 80 kg/capita) and in Spain (about 65 kg/capita) and to a lesser extent in Israel (7 kg/capita). On average, all countries recorded a small – and

Table 1 Supply of animal and plant foods, fish and seafood (kg/capita/year) in Mediterranean countries in 1961 and 2003 and average changes per year

	Animal foods			Plant foods			Fish and seafood		
	1961	2013	Average annual change[a]	1961	2013	Average annual change	1961	2013	Average annual change[a]
Albania	112.7	365.65	4.86	325.76	541.75	4.15	1.92	4.54	0.05
Algeria	56.19	162.24	2.04	194.64	505.87	5.99	3.31	3.91	0.01
Bosnia and Herzegovina	120.52[b]	206.17	4.08	335.8[b]	491.74	7.43	0.16[b]	4.25	0.19
Croatia	197.12[b]	296.45	4.73	213.76[b]	310.86	4.62	2.6[b]	18.22	0.74
Cyprus	91.51	186.98	1.84	340.16	256.94	−1.60	5.73	20.78	0.29
Egypt	40.35	88.37	0.92	305.46	554.01	4.78	3.74	22.05	0.35
Greece	121.12	327.88	3.98	422.77	486.04	1.22	16.21	18.34	0.04
Israel	185.19	288.74	1.99	454.88	447.73	−0.14	18.83	22.98	0.08
Italy	175.63	325.29	2.88	415.76	432.41	0.32	11.56	20.58	0.17
Lebanon	87.17	154.14	1.29	419.02	440.34	0.41	3.95	10.26	0.12
Malta	175.63	271.11	1.84	300.25	435.2	2.60	10.7	27.79	0.33
Montenegro	412.63[c]	433.7	3.01	476.9[‡]	513.12	5.17	7.44[c]	11.2	0.54
Spain	103.59	256.15	2.93	367.51	302.61	−1.25	24.87	35.28	0.20
Tunisia	54.6	143.84	1.72	300.09	555.45	4.91	4.09	13.54	0.18
Turkey	196.01	229.05	0.64	503.27	589.44	1.66	2.4	5.7	0.06

Source: Food Balance Sheet data available at http://www.fao.org/faostat/en/#data/FBS (accessed on September 2017)
Animal foods: All types of meat and milk
Plant foods: Vegetables, Fruit, Cereals and their products, Beas, Peas and other Pulses
Fish and seafood: All types of fish, Crustaceans, Cephalopods, and Mollusks
[a]Estimated as: (Supply in 2013) – (Supply in 1961)/number of years in between
[b]1992
[c]2006

Table 2 Supply of olive oil and wine (g/capita/year) in Mediterranean countries in 1961 and 2003 and average changes per year

	Olive oil			Wine		
	1961	2013	Average annual change[a]	1961	2013	Average annual change[a]
Albania	1,110	570	−10.4	1,660	7,970	121.3
Algeria	870	1,730	16.5	20	10	−0.2
Bosnia and Herzegovina	0[b]	220	10.5	5,100[b]	1,880	−153.3
Croatia	610[b]	710	4.8	40,410[b]	13,800	−1,267.1
Cyprus	2390	2,320	−1.3	12,150	15,700	68.3
Egypt	40	80	0.8	70	50	−0.4
Greece	14,550	13,550	−19.2	33,710	18,690	−288.8
Israel	110	2,150	39.2	4,460	860	−69.2
Italy	9,120	9,960	16.2	109,590	30,550	−1,520.0
Lebanon	2,840	3,180	6.5	2,110	2,700	11.3
Malta	90	1170	20.8	930	15,370	277.7
Montenegro	600[c]	510	12.9	17,980[c]	20,770	398.6
Spain	8,200	10,930	52.5	59,210	20,980	−735.2
Tunisia	6,720	3,150	−68.7	2,490	2,680	3.7
Turkey	3,800	1,280	−48.5	960	390	−11.0

Source: Food Balance Sheet data available at http://www.fao.org/faostat/en/#data/FBS (accessed on September 2017)
[a]Estimated as: (Supply in 2013) – (Supply in 1961)/number of years in between
[b]1992
[c]2006

in many cases negligible – increase in the supply of fish and seafood. Regarding olive oil, some countries recorded remarkable reductions in the per capita supply between 1961 and 2013. The per capita annual supply of olive oil reduced by 3.6 kg in Tunisia, 2.5 kg in Turkey, and 1 kg in Greece. The changes in the per capita annual supply of wine were more variable – some countries recorded an increase (such as Malta and Montenegro), while others recorded a substantial decrease (Italy and Croatia).

Yearly trends in food supply are graphically presented in Figs. 2–6 for six countries (Algeria, Greece, Italy, Spain, Tunisia, and Turkey) selected on the basis of geographical coverage and observations from Tables 1 and 2. In the years between 1961 (when the Mediterranean diet was registered through the Seven Countries Study) and 2013 (the latest year available in the database), the daily supply of animal foods (livestock products and milk) was steadily increasing (Fig. 2). A slight decrease in the daily supply of animal foods was recorded in Italy in the period between 1983 and 1993, but in 2013, per capita supplies were higher than the ones in early 1960s. The pattern of changes over time is more variable in the case of foods of plant origin (Fig. 3). Overall, the daily supply increased in Algeria and Tunisia, remained rather stable in Italy and Greece and since late 1980s decreased in Spain. The consistency in the daily supply of fish and seafood present in Table 1 is also

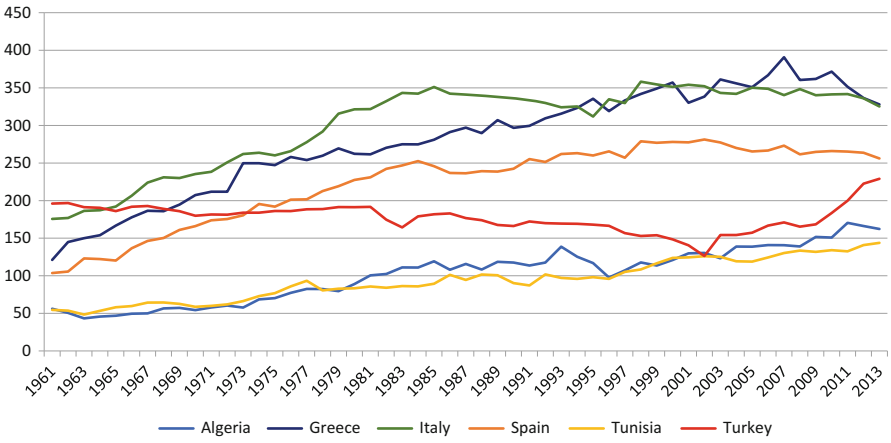


Fig. 2 Trends over time in the supply of animal foods (kg/capita/year) in Mediterranean countries

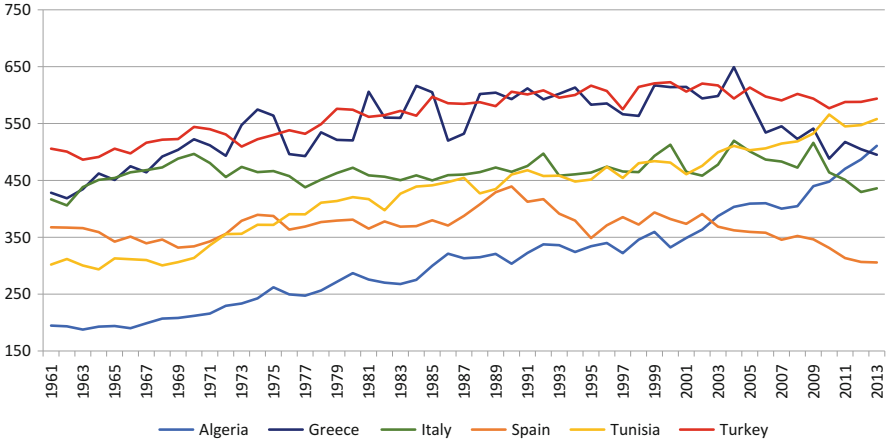


Fig. 3 Trends over time in the supply of plant foods (kg/capita/year) in Mediterranean countries

evident through Fig. 4, where changes per year are presented. Fish intake in Spain is popular, since not only the daily supply constantly outweighs that of the other Mediterranean countries, but it increased steadily leading to 10 kg/capita higher daily supply in 2013 in comparison to 1961. Greece has been leading the daily supply of olive oil since the early 1960s (Fig. 5), followed only by Italy and Spain after the late 1970s. However, the daily supply of olive oil has been constantly decreasing in Greece since mid-1970s, and although it remains the highest, the difference with the other Mediterranean countries in Europe is narrowing. Over time, changes in the daily supply of wine are presented in Fig. 6. Values are very low among countries with large percentage of Muslims in their population and they probably reflect the consumption of wine by tourists visiting these countries. Differences over the years were not remarkable in Greece and Spain, while in Italy the

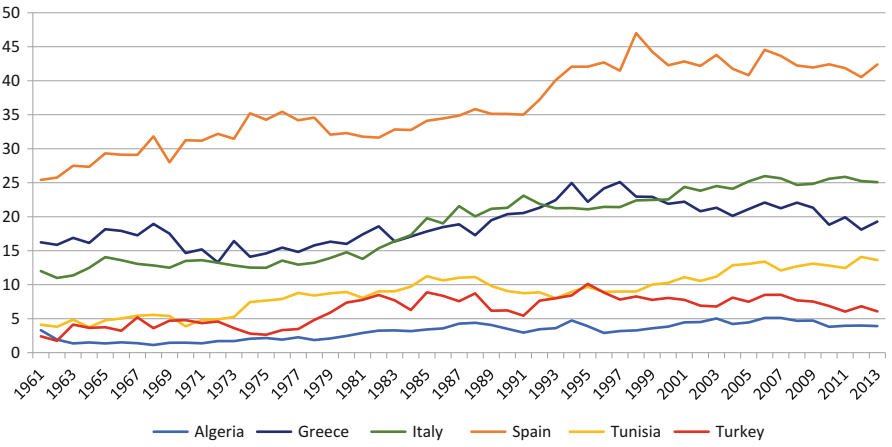


Fig. 4 Trends over time in the supply of fish and other seafood (kg/capita/year) in Mediterranean countries

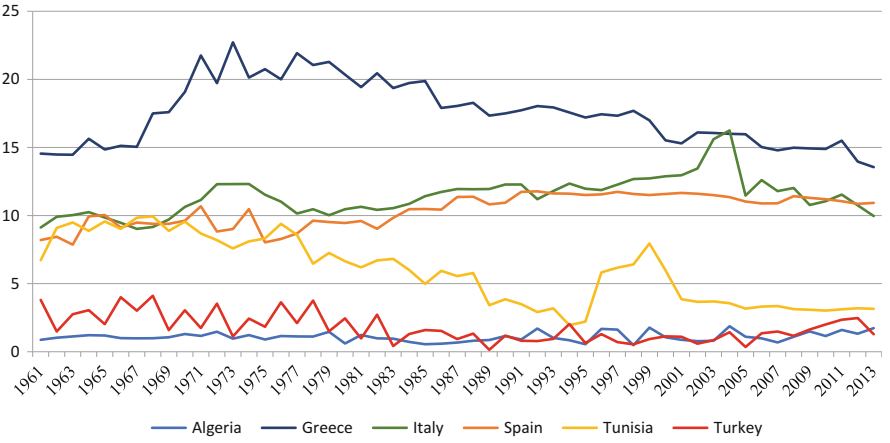


Fig. 5 Trends over time in the supply of olive oil (kg/capita/year) in Mediterranean countries

supply of wine continuously decreased since mid-1970s and is now closer to that recorded in other European Mediterranean countries.

6 Monitoring Adherence to the Overall Mediterranean Dietary Pattern

The ecological evidence that Mediterranean populations are gradually departing from their traditional eating habits has been reproduced in large-scale nutritional surveys among children, adolescents, and adults [45, 46]. In a report presenting harmonized data for nutrition and health indices in Europe based on studies with

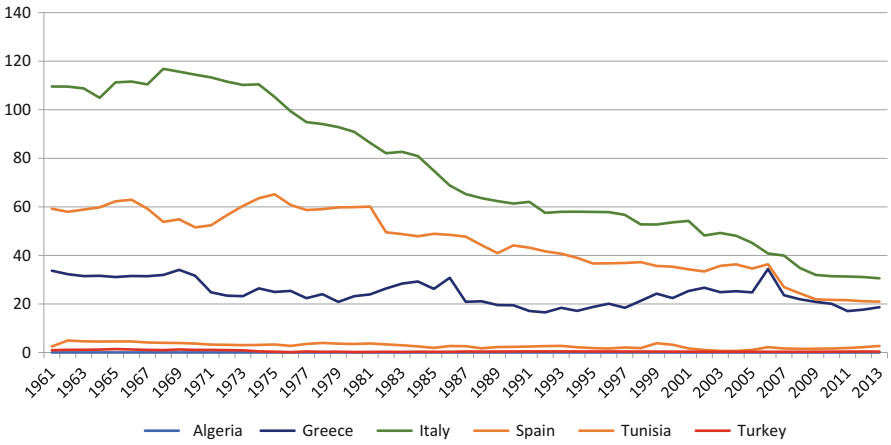


Fig. 6 Trends over time in the supply of wine (kg/capita/year) in Mediterranean countries

wide population coverage, individuals in the Mediterranean region reported higher intakes of red meat and saturated fatty acids and lower intakes of plant products in comparison to their European counterparts [47]. Publications presenting changes over time in the dietary habits of populations participating in the Seven Countries Study further support that the Mediterraneans are abandoning their traditional eating choices. In early 1990s, Kafatos and colleagues conducted a follow-up study of 245 surviving men in Crete aged 70–89 years, who had participated in the Seven Countries Study in the 1960s [48]. A representative subsample of 21 among them further provided 3-day weighed food records, which indicated increases in the intake of saturated and decreases in monounsaturated fatty acids [48]. De Lorenzo and colleagues [49] studied differences in food habits in Nicotera, one of the south Italian rural areas of the Seven Countries Study, between 1960 and 1996. In the second assessment, 80 inhabitants of Nicotera (43 males, 37 females, aged 40–59 years) with no indication of chronic disease and not under regular medication or on a special diet were selected at random. Based on dietary data collected through a food frequency questionnaire, authors estimated the participants' Mediterranean Adequacy Index and noted that the mean index decreased from 9.4 for males and 11.4 for females in 1960 to 2.8 and 2.5, respectively, in 1996. Regarding specific food items, authors observed an increase in the consumption of animal foods, cakes, pies, cookies, and sweet beverages among both men and women. Not disregarding methodological characteristics of the 1996 study that may limit direct comparisons with the 1960 data, results indicate a significant reduction to the extent through which the population adhered to the Mediterranean diet. Similar results were also observed in Crevalcore (northern Italy) and Montegiorgio (central Italy), the other two rural Italian cohorts participating to the Seven Countries Study [49], as well as to other studies among adult Mediterranean populations [45].

Lower adherence to the traditional Mediterranean diet has also been consistently reported among children, adolescents, and young adults, the trend-setters of dietary

habits and lifestyle choices of future generations. In their review of studies assessing the adherence to Mediterranean diet among children and adolescents, Iaccarino Idelson et al. [46] analyzed the results of studies which applied the KIDMED index and were mainly conducted in European Mediterranean countries (49 out of the 55 eligible studies). Adherence varied widely both within and between Mediterranean populations. Based on studies covering large samples, poor adherence to Mediterranean diet varied from 1.6% in Spanish children to 62.8% in Greek adolescents; average adherence from 28.0% in Greek adolescents to 73.8% in Italian adolescents residing in rural areas; and good adherence from 4.3% in Greek 10–12 year olds to 53.9% in Spanish children. According to most of the studies, adherence to Mediterranean diet was directly associated with physical activity (and possibly with diet adequacy) and inversely with sedentary behavior. Gender, age, the family's socioeconomic status, and the children's body weight were possible determinants of the level of adherence.

Low consumption of plant foods and a prevalent intake of commercial sweet and savory cereal products have been the main components explaining poor to average adherence. In their review of the diet of preschool children in the Mediterranean countries of the European Union, Pereira-da-Silva et al. [50] reported that in the majority of countries, young children consumed frequently fruit and vegetables, but also sugared beverages and snacks. The majority of children reported high energy and high protein intakes mainly from dairy products, together with excessive sodium intake. The consumption of energy-dense foods and overweight were recorded as early as in toddler and preschool ages. Furthermore, most children reported low adherence to the Mediterranean diet, which was associated with being overweight or obese, lower maternal educational level, and parental unemployment.

In a study among 1740 Italian children 8–9 years old who were recruited from regions all over the country, a low consumption of fruit, vegetables, and legumes, and a high intake of commercially baked goods for breakfast and sweets were identified as contributors to the low adherence. About one-third of the sample reported that hindrances to the consumption of fruit, vegetables, and legumes were affecting their ability to follow a Mediterranean diet. In this study, only 5% of the children were identified as high adherers based on the KIDMED index [51].

In the cross-sectional analysis of the IDEFICS study, Tognon and colleagues [17] assessed adherence to the Mediterranean diet of 16,220 children aged 2–9 years old from eight European countries (Sweden, Germany, Hungary, Italy, Cyprus, Spain, Belgium, and Estonia), using a Mediterranean Diet Score based on the relative frequency of food group consumption. One point was given for intakes higher than the median relative frequency for (a) potatoes, vegetables, and legumes; (2) fruits and nuts; (3) cereals; or (4) fish; and one point if intakes were below the median for: (5) dairy and (6) meat products. The final score added up to a maximum of 6 points and a score equal or higher to 3 was interpreted as high adherence to a Mediterranean-like dietary pattern. In this study, the largest proportion of children with high intake frequencies of cereals, vegetables, potatoes, fruits, and nuts was recorded in Sweden. More than half of the Swedish children (56.7%) reported high adherence to components of the Mediterranean diet, followed by the Italians (37.5%) and the Germans (35.1%). The lowest levels were observed in Cyprus, where 75.8% of children had score values below 3 [17]. The

highest prevalence of children with a score higher than 3, which could be interpreted as average to high adherence, was recorded among the Italian preschool boys (55.9%) and the lowest among the Spanish school-aged girls (26.0%). Apparently, higher adherence to a Mediterranean-like dietary pattern was generally not associated with living in a Mediterranean country or in a highly educated or high-income family. Differences in adherence between boys and girls or age groups varied between countries without any general pattern. It should be noted, however, that the results on the level of adherence also varied according to the index used: for example, the proportion of children with high adherence ranged from 24.2% in Cyprus to 56.7% in Sweden using the score described above, and from 29.4% in Germany to 49.3% in Italy using a different score, formulated on the basis of an adaptation of the Mediterranean diet score [46].

In the multi-center IDEFICS study, different food groups contributed to high adherence levels in each country. Hence, among Italian children, the low adherence could be attributed to a low consumption of vegetables and legumes (prevalence of high consumers: 36.2%), while in Sweden high adherence was achieved through a low consumption of dairy and meat products (prevalence of high consumers of dairies: 24.6%; and prevalence of high consumers of meat: 30.9%). The consumption of olive oil and vegetable oils is still more prevalent among children in the Mediterranean region than in other European countries – the prevalence of children above the median for the unsaturated to saturated fatty acid ratio was equal to 72.6% in Italy and to 58.7% in Cyprus. The children in Spain have consistently been identified among the highest consumers of fish and other seafood.

Some alarming findings have been reported from studies conducted in Greece and Cyprus. In a representative sample of 554 Greek children and 358 adolescents, Kontogianni and colleagues assessed adherence to the Mediterranean diet using the KIDMED index [52]. Only 11.3% of children and 8.3% of adolescents had an optimal score ($\geq$8). Among children, KIDMED scores did not differ between boys (mean $\pm$ SD: 5.4 $\pm$ 1.9) and girls (5.4 $\pm$ 1.7), but in adolescents, the index tended to be higher among females (4.9 $\pm$ 2.1) than among males (4.6 $\pm$ 2.0) (p-value: 0.07). In a study of 1140 Cypriot children aged 9–13 years old using the KIDMED score too, only 8.7% of the boys and 5.3% of the girls were classified as high adherers (score $\geq$ 8) and 38.5% of boys and 36.3% of girls had a KIDMED score ranging from 0 to 3 [53]. In the context of the GRECO study, including a representative sample of Greek children aged 10–12 years old, Farajian et al. [54] reported that only 4.3% of the children had an optimal KIDMED score ($\geq$8) and about half of the children (46.8%) were classified as poor adherers ($\leq$3). The score values did not differ between boys and girls, normal weight, overweight, or obese children. Children with higher KIDMED scores resided in semiurban or rural regions and reported higher physical activity levels.

7 Conclusion

The Mediterranean diet is characterized by a high intake of plant foods (fruit, vegetables, cereals, nuts, legumes) and fish, together with a low intake of products of animal origin (meat, milk and diaries). Olive oil is the most frequent – if not the

only – lipid added to food, and alcohol intake is moderate and mostly in the form of wine, preferably during meals. There is increasing evidence from observational and experimental epidemiological studies that adherence to the Mediterranean dietary pattern promotes health and reduces the risk of premature death from chronic degenerative diseases. These conclusions are further supported by large systematic reviews and meta-analyses. Nonetheless, Mediterranean countries and especially the European ones have experienced a "westernization" process of their food habits, and have increasingly been exhibiting patterns of food choices similar to non-Mediterranean ones. In a period when other western countries experience a nutrition transition that favors the Mediterranean dietary pattern [47], traditional food choices in Italy, Greece, and other Mediterranean regions are abandoned. This could be attributed to the globalization of the food supply, the overall improvement in socioeconomic conditions in Europe that made food (especially of animal origin) more affordable, and the urbanization of life, which primarily affects the younger generations.

The westernized diets – particularly followed by the youth in the region – have been associated with higher disease and mortality rates in North Europe and USA [55, 56]. Unfavorable changes in disease rates may eventually appear and may lead to an increase of the demand and the cost of health services, an issue of concern in era of limited resources both at the individual and governmental levels. Moreover, these food choices cannot support sustainable development, in the way the Mediterranean diet does. Being adjusted to the cultural, climatic, and other environmental characteristics of the region, the Mediterranean diet is protective and helpful to biodiversity; accessible and economically affordable; can lead to a cut down of country's expenses for food imports; and contributes to food and nutrition security, as well as to the health of present and future generations [57, 58].

References

1. Trichopoulou A, Lagiou P, Kuper H, Trichopoulos D (2000) Cancer and Mediterranean dietary traditions. Cancer Epidemiol Biomark Prev 9(9):869–873
2. Trichopoulou A, Lagiou P (1997) Healthy traditional Mediterranean diet – an expression of culture, history and lifestyle. Nutr Rev 55:383–389
3. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, Medina FX, Battino M, Belahsen R, Miranda G, Serra-Majem L, Mediterranean Diet Foundation Expert Group (2011) Mediterranean diet pyramid today Science and cultural updates. Public Health Nutr 14:2274–2284
4. Martínez-González MA, Salas-Salvadó J, Estruch R, Corella DD, Fitó M, Ros E (2015) Benefits of the Mediterranean diet: insights from the PREDIMED study. Prog Cardiovasc Dis 58:50–60
5. Piroddi M, Albini A, Fabiani R, Giovannelli L, Luceri C, Natella F, Rosignoli P, Rossi T, Taticchi A, Servili M, Galli F (2017) Nutrigenomics of extra-virgin olive oil: A review. Biofactors 43(1):17–41
6. Trichopoulou A, Vasilopoulou E (2000) Mediterranean diet and longevity. Br J Nutr 84(Suppl 2):S205–S209
7. WHO Europe. The European health for all database http://data.euro.who.int/hfadb/ (July 2016).
8. Nestle M (1995) Mediterranean diets: historical and research overview. Am J Clin Nutr 61(Suppl 6):1313S–1320S

9. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D (2003) Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 348(26):2599–2608
10. Alberti-Fidanza A, Fidanza F (2004) Mediterranean adequacy index of Italian diets. Public Health Nutr 7:937e41
11. Trichopoulou A, Orfanos P, Norat T, Bueno-de-Mesquita B, Ocké MC, Peeters PH, van der Schouw YT, Boeing H, Hoffmann K, Boffetta P, Nagel G, Masala G, Krogh V, Panico S, Tumino R, Vineis P, Bamia C, Naska A, Benetou V, Ferrari P, Slimani N, Pera G, Martinez-Garcia C, Navarro C, Rodriguez-Barranco M, Dorronsoro M, Spencer EA, Key TJ, Bingham S, Khaw KT, Kesse E, Clavel-Chapelon F, Boutron-Ruault MC, Berglund G, Wirfalt E, Hallmans G, Johansson I, Tjonneland A, Olsen A, Overvad K, Hundborg HH, Riboli E, Trichopoulos D (2005) Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. BMJ 330(7498):991
12. Buckland G, Agudo A, Travier N, Huerta JM, Cirera L, Tormo MJ, Navarro C, Chirlaque MD, Moreno-Iribas C, Ardanaz E, Barricarte A, Etxeberria J, Marin P, Quirós JR, Redondo ML, Larrañaga N, Amiano P, Dorronsoro M, Arriola L, Basterretxea M, Sanchez MJ, Molina E, González CA (2011) Adherence to the Mediterranean diet reduces mortality in the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). Br J Nutr 106(10):1581–1591
13. Agnoli C, Krogh V, Grioni S, Sieri S, Palli D, Masala G, Sacerdote C, Vineis P, Tumino R, Frasca G, Pala V, Berrino F, Chiodini P, Mattiello A, Panico S (2011) A priori-defined dietary patterns are associated with reduced risk of stroke in a large Italian cohort. J Nutr 141(8):1552–1558
14. Milà-Villarroel R, Bach-Faig A, Puig J, Puchal A, Farran A, Serra-Majem L, Carrasco JL (2011) Comparison and evaluation of the reliability of indexes of adherence to the Mediterranean diet. Public Health Nutr 14(12A):2338–2345
15. Malagoli C, Malavolti M, Agnoli C, Crespi CM, Fiorentini C, Farnetani F, Longo C, Ricci C, Albertini G, Lanzoni A, Veneziano L, Virgili A, Pagliarello C, Santini M, Fanti PA, Dika E, Sieri S, Krogh V, Pellacani G, Vinceti M (2015) Diet Quality and Risk of Melanoma in an Italian Population. J Nutr 145(8):1800–1807
16. Serra-Majem L, Ribas L, Ngo J, Ortega R, Garcia A, Perez C, Aranceta J (2004) Food, youth and the Mediterranean Diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. Public Health Nutr 7:931–935
17. Tognon G, Hebestreit A, Lanfer A, Moreno LA, Pala V, Siani A, Tornaritis M, De Henauw S, Veidebaum T, Molnár D, Ahrens W, Lissner L (2013) Mediterranean diet, overweight and body composition in children from eight European countries: Cross-sectional and prospective results from the IDEFICS study. Nutr Metab Cardiovasc Dis 24(2):205–213
18. Menotti A, Kromhout D, Blackburn H, Fidanza F, Buzina R, Nissinen A (1999) Food intake patterns and 25-year mortality from coronary heart disease: cross-cultural correlations in the Seven Countries Study. The Seven Countries Study Research Group. Eur J Epidemiol 15(6):507–515
19. Menotti A, Puddu PE (2015) How the Seven Countries Study contributed to the definition and development of the Mediterranean diet concept: a 50-year journey. Nutr Metab Cardiovasc Dis 25 (3):245–252
20. Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Polychronopoulos E, Vassilakou T, Lipworth L, Trichopoulos D (1995) Diet and overall survival in elderly people. BMJ 311(7018):1457–1460
21. Sofi F, Abbate R, Gensini GF, Casini A (2010) Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr 92(5):1189–1196
22. Verberne L, Bach-Faig A, Buckland G, Serra-Majem L (2010) Association between the Mediterranean diet and cancer risk: a review of observational studies. Nutr Cancer 62(7):860–870
23. Nordmann AJ, Suter-Zimmermann K, Bucher HC, Shai I, Tuttle KR, Estruch R, Briel M (2011) Meta-analysis comparing Mediterranean to low-fat diets for modification of cardiovascular risk factors. Am J Med 124(9):841–851

24. Martinez-Gonzalez MA, Bes-Rastrollo M, Serra-Majem L, Lairon D, Estruch R, Trichopoulou A (2009) Mediterranean food pattern and the primary prevention of chronic disease: recent developments. Nutr Rev 67(Suppl 1):S111–S116
25. Muñoz MA, Fíto M, Marrugat J, Covas MI, Schröder H, REGICOR and HERMES Investigators (2009) Adherence to the Mediterranean diet is associated with better mental and physical health. Br J Nutr 101(12):1821–1827
26. Loughrey DG, Lavecchia S, Brennan S, Lawlor BA, Kelly ME (2017) The impact of the Mediterranean diet on the cognitive functioning of healthy older adults: A systematic review and meta-analysis. Adv Nutr 8(4):571–586
27. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez JA, Martínez-González MA, PREDIMED Study Investigators (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med 368(14):1279–1290
28. Guasch-Ferré M, Salas-Salvadó J, Ros E, Estruch R, Corella D, Fitó M, Martínez-González MA, PREDIMED Investigators (2017) The PREDIMED trial, Mediterranean diet and health outcomes: How strong is the evidence? Nutr Metab Cardiovasc Dis 27(7):624–632
29. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A (2008) Adherence to Mediterranean diet and health status: meta-analysis. BMJ 337:a1344
30. Liyanage T, Ninomiya T, Wang A, Neal B, Jun M, Wong MG, Jardine M, Hillis GS, Perkovic V (2016) Effects of the Mediterranean Diet on Cardiovascular Outcomes-A Systematic Review and Meta-Analysis. PLoS One 11(8):e0159252
31. Schwingshackl L, Hoffmann G (2015) Adherence to Mediterranean diet and risk of cancer: An updated systematic review and meta-analysis of observational studies. Cancer Med 4(12):1933–1947
32. Dinu M, Pagliai G, Casini A, Sofi F (2017) Mediterranean diet and multiple health outcomes: an umbrella review of meta-analyses of observational studies and randomised trials. Eur J Clin Nutr. https://doi.org/10.1038/ejcn.2017.58. [Epub ahead of print]
33. Kouris-Blazos A, Gnardellis C, Wahlqvist ML, Trichopoulos D, Lukito W, Trichopoulou A (1999) Are the advantages of the Mediterranean diet transferable to other populations? A cohort study in Melbourne, Australia. Brit J Nutr 82:57–61
34. Osler M, Schroll M (1999) Diet and mortality in a cohort of elderly people in a north European community. Int J Epidemiology 26:155–159
35. Woo j WKS, Leung SS, Chook P, Liu B, Ho SC, Chan SW, Feng JZ, Celermajer DS (2001) The Mediterranean score of dietary habits in Chinese populations in four different geographical areas. Eur J Clin Nutr 55(3):215–220
36. Knoops KT, de Groot LC, Kromhout D, Perrin AE, Moreiras-Varela O, Menotti A, van Staveren WA (2004) Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. JAMA 292(12):1433–1439
37. Mitrou PN, Kipnis V, Thiébaut AC, Reedy J, Subar AF, Wirfält E, Flood A, Mouw T, Hollenbeck AR, Leitzmann MF, Schatzkin A (2007) Mediterranean dietary pattern and prediction of all-cause mortality in a US population: results from the NIH-AARP Diet and Health Study. Arch Intern Med 167(22):2461–2468
38. Konstantinidou V, Covas MI, Sola R, Fitó M (2013) Up-to date knowledge on the in vivo transcriptomic effect of the Mediterranean diet in humans. Mol Nutr Food Res 57(5):772–783
39. Trichopoulou A, Naska A, Costacou T, on behalf of the DAFNE III Group (2002) Disparities in food habits across Europe. Proc Nutr Soc 61:553–558
40. Naska A, Fouskakis D, Oikonomou E, Almeida MD, Berg MA, Gedrich K, Moreiras O, Nelson M, Trygg K, Turrini A, Remaut AM, Volatier JL, Trichopoulou A, DAFNE participants (2006) Dietary patterns and their socio-demographic determinants in ten European countries. Data from the DAFNE databank. Eur J Clin Nutr 60(2):181–190
41. Vareiro D, Bach-Faig A, Raidó Quintana B, Bertomeu I, Buckland G, Vaz de Almeida MD, Serra-Majem L (2009) Availability of Mediterranean and non-Mediterranean foods during

the last four decades: comparison of several geographical areas. Public Health Nutr 12(9A):1667–1675
42. Naska A, Berg MA, Cuadrado C, Freisling H, Gedrich K, Gregoric M, Kelleher C, Leskova E, Nelson M, Pace L, Remaut AM, Rodrigues S, Sekula W, Sjöstrom M, Trygg K, Turrini A, Volatier JL, Zajkas G, Trichopoulou A, Data Food Networking (DAFNE) participants (2009) Food balance sheet and household budget survey dietary data and mortality patterns in Europe. Br J Nutr 102(1):166–171
43. Balanza R, García-Lorda P, Pérez-Rodrigo C, Aranceta J, Bonet MB, Salas-Salvadó J (2007) Trends in food availability determined by the Food and Agriculture Organization's food balance sheets in Mediterranean Europe in comparison with other European areas. Public Health Nutr 10(2):168–176
44. Naska A, Vasdekis VG, Trichopoulou A, Friel S, Leonhäuser IU, Moreiras O, Nelson M, Remaut AM, Schmitt A, Sekula W, Trygg KU, Zajkás G (2000) Fruit and vegetable availability among ten European countries: how does it compare with the 'five-a-day' recommendation? DAFNE I and II projects of the European Commission. Br J Nutr 84(4):549–556
45. Benhammou S, Heras-González L, Ibáñez-Peinado D, Barceló C, Hamdan M, Rivas A, Mariscal-Arcas M, Olea-Serrano F, Monteagudo C (2016) Comparison of Mediterranean diet compliance between European and non-European populations in the Mediterranean basin. Appetite 107:521–526
46. Iaccarino Idelson P, Scalfi L, Valerio G (2017) Adherence to the Mediterranean Diet in children and adolescents: A systematic review. Nutr Metab Cardiovasc Dis 27(4):283–299
47. Elmadfa I, Meyer A, Nowak V, Hasenegger V, Putz P, Verstraeten R, Remaut-DeWinter AM, Kolsteren P, Dostálová J, Dlouhý P, Trolle E, Fagt S, Biltoft-Jensen A, Mathiessen J, Velsing Groth M, Kambek L, Gluskova N, Voutilainen S, Erkkilä A, Vernay M, Krems C, Strassburg A, Vasquez-Caicedo AL, Urban C, Naska A, Efstathopoulou E, Oikonomou E, Tsiotas K, Bountziouka V, Benetou V, Trichopoulou A, Zajkás G, Kovács V, Martos E, Heavey P, Kelleher C, Kennedy J, Turrini A, Selga G, Sauka M, Petkeviciene J, Klumbiene J, Holm Totland T, Andersen LF, Halicka E, Rejman K, Kowrygo B, Rodrigues S, Pinhão S, Ferreira LS, Lopes C, Ramos E, Vaz Almeida MD, Vlad M, Simcic M, Podgrajsek K, Serra Majem L, Román Viñas B, Ngo J, Ribas Barba L, Becker W, Fransen H, Van Rossum B, Ocké M, Margetts B, Rütten A, Abu-Omar K, Gelius P, Cattaneo A (2009) European Nutrition and Health Report 2009. Ann Nutr Metab 55(Suppl 2):1–40
48. Kafatos A, Diacatou A, Voukiklaris G, Nikolakakis N, Vlachonikolis J, Kounali D, Mamalakis G, Dontas AS (1997) Heart disease risk-factor status and dietary changes in the Cretan population over the past 30 y: the Seven Countries Study. Am J Clin Nutr 65(6):1882–1886
49. De Lorenzo A, Alberti A, Andreoli A, Iacopino L, Serranò P, Perriello G (2001) Food habits in a southern Italian town (Nicotera) in 1960 and 1996: still a reference Italian Mediterranean diet? Diabetes Nutr Metab 14(3):121–125
50. Pereira-da-Silva L, Rêgo C, Pietrobelli A (2016) The diet of preschool children in the Mediterranean countries of the European Union: A systematic review. Int J Environ Res Public Health 13(6):E572
51. Roccaldo R, Censi L, D'Addezio L, Toti E, Martone D, D'Addezio D, Cernigliaro A, ZOOM8 Study Group (2014) Adherence to the Mediterranean diet in Italian school children. Int J Food Sci Nutr 65(5):621–628
52. Kontogianni MD, Vidra N, Farmaki AE, Koinaki S, Belogianni K, Sofrona S, Magkanari F, Yannakoulia M (2008) Adherence rates to the Mediterranean diet are low in a representative sample of Greek children and adolescents. J Nutr 138(10):1951–1956
53. Lazarou C, Panagiotakos DB, Matalas AL (2009) Level of adherence to the Mediterranean diet among children from Cyprus: the CYKIDS study. Public Health Nutr 12(7):991–1000
54. Farajian P, Risvas G, Karasouli K, Pounis GD, Kastorini CM, Panagiotakos DB, Zampelas A (2011) Very high childhood obesity prevalence and low adherence rates to the Mediterranean diet in Greek children: The GRECO study. Atherosclerosis 217(2):525–530

55. Sinha R, Cross AJ, Graubard BI, Leitzmann MF, Schatzkin A (2009) Meat intake and mortality: A prospective study of over half a million people. Arch Intern Med 169(6):562–571
56. Akbaraly T, Sabia S, Hagger-Johnson G, Tabak AG, Shipley MJ, Jokela M, Brunner EJ, Hamer M, Batty GD, Singh-Manoux A, Kivimaki M (2013) Does overall diet in midlife predict future aging phenotypes? A cohort study. Am J Med 126(5):411–419
57. Burlingame B, Dernini S (2011) Sustainable diets: the Mediterranean diet as an example. Public Health Nutr 14(12A):2285–2287
58. Trichopoulou A (2012) Diversity v. globalization: traditional foods at the epicentre. Public Health Nutr 15(6):951–954

Flavonoids – Food Sources, Health Benefits, and Mechanisms Involved

3

Aleksandra Kozłowska and Dorota Szostak-Węgierek

Contents

Abstract

In recent years, growing attention has been focused on the use of natural sources of antioxidants in the prevention of chronic diseases. Flavonoids are the examples of such substances. It is a group of bioactive compounds that are widely distributed in many plant-based foods and beverages. Flavonoid-rich products include,

A. Kozłowska (✉)
First Faculty of Medicine, Department of Social Medicine and Public Health, Medical University of Warsaw, Warsaw, Poland
e-mail: aleksandra.kozlowska@wum.edu.pl; akozlowska0@gmail.com

D. Szostak-Węgierek
Faculty of Health Science, Department of Clinical Dietetics, Medical University of Warsaw, Warsaw, Poland
e-mail: dorota.szostak-wegierek@wum.edu.pl

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_54

among others, berries, citrus fruits, grapes, cherries, dock, arugula, onions, artichokes, soybeans, cowpeas, black beans, parsley, oregano, and tea. Flavonoids exhibit a wide range of positive effects, such as strong antioxidant, anti-inflammatory, and antiplatelet activities. They may contribute to the prevention of chronic diseases, including metabolic disorders, diabetes, and cardiovascular disease, because of their beneficial effect on blood lipids, blood pressure, plasma glucose levels, and also stabilization of athetosclerotic plaque. Furthermore, evidence from epidemiological, animal, and in vitro studies support protective effects of foods and dietary supplements rich in flavonoids against some types of cancer, Alzheimer's disease, Parkinson's disease, some viral infections, cataract, erectile dysfunction, and inflammatory bowel disease. Consumption of flavonoids with diet appears to be safe. There is a growing body of evidence that a diet rich in these substances is beneficial for health and its promotion is thus justifiable.

Keywords
Flavonoids · Antioxidants · Bioactive compounds · Chronic diseases · Prevention · Neurodegenerative diseases · Cataract · Erectile dysfunction · Inflammatory bowel disease · Type 2 diabetes

1 Introduction

This chapter provides the current state of knowledge about the role of flavonoids in prevention and treatment of chronic diseases (CD). It is a comprehensive review of literature on both flavonoids as the whole group and also flavonoid subclasses. We believe that the presented data will be useful for both researchers and practitioners in medicine and dietetics.

The history of research on flavonoids goes back several decades. In 1930, a new substance was isolated from lemon juice by Hungarian Nobel laureate Albert Szent-Gyorgyi, who also discovered vitamin C. At that time, it was believed to be a member of a new class of vitamins and was referred to as vitamin P. Later, it became clear that this substance was a flavonoid (rutin) and till now at least 6000 different types of flavonoids have been identified [1, 2].

Flavonoids are secondary metabolites of higher plants and represent a large class of phenolic compounds found in fruits, vegetables, herbs, cocoa, and some beverage products [3]. They have several important functions in plants, such as providing protection against harmful UV radiation or plant pigmentation. In addition, they regulate gene expression and modulate enzymatic action. They also have antiviral and antibacterial properties, as well as high antioxidant capacity. These compounds are primarily synthesized for the plants' own defense against oxidative stress. However, they maintain antioxidant properties also ex planta and hence contribute to the pharmaceutical and dietary attributes of plant foods. Therefore, antioxidant properties of flavonoids are all-important for both plant and human biology [4, 5].

2 Flavonoids: Classification, Sources, and Dietary Intake

Flavonoids constitute a class of polyphenols. All naturally occurring flavonoids possess three hydroxyl groups, two of which are on the ring A at positions five and seven and one is located on the ring B, position three. They can be subdivided into different subgroups depending on the number of the carbon atom of the C ring to which the B ring is attached and also the degree of unsaturation and oxidation of the C ring. Flavonoids in which the B ring is linked in position 3 of the C ring are called isoflavones. Those in which the B ring is linked in position 2 can be further subdivided into several subgroups on the basis of the structural features of the C ring. These following subgroups are: flavones, flavonols, flavanones, flavanols or catechins, and anthocyanins. Some authors have additionally distinguished flavanonols, chalcone, and neoflavonoids subclasses [6]. Table 1 presents the basic structure of flavonoid and examples of specific substances in each flavonoid subclass.

Important dietary sources of flavonoids are vegetables, fruits, seeds, and some cereals, together with wine, tea, and certain spices (Table 2). The most abundant sources are berries, cowpeas, dock, kale, dark chocolate, parsley, oregano, capres, green and black tea. It is important to note that the presence of flavonoids in vegetables and fruits may greatly vary depending on the crop variety, processing, climate, seasonality, plant species, manufacture, and storage. Differences in flavonoid contents between plant species are usually moderate, although in a few cases very high amounts have been observed, e.g., in certain berries and tea prepared from leaves of the Quingmao tree [7].

Table 1 Basic structure of flavonoid and subclasses of flavonoids (Authors' selection based on Refs. [5–8])

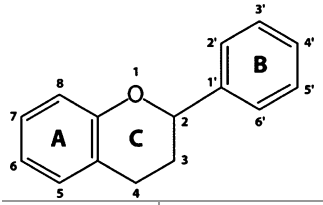
Basic structure of flavonoid	
	
Subclass	Examples of compounds
Anthocyanins	Pelargonidin, cyanidin, delphinidin, peonidin, petunidin, malvidin
Flavanols	Epicatechin, catechin, epicatechin gallate (ECG), gallocatechin, epigallocatechin (EGC), epigallocatechin gallate (EGCG)
Flavanones	Naringenin, naringin, hesperetin, eriodicytol
Flavones	Sinensetin, isosinensetin, nobiletin, tangeretin, luteolin, apigenin, chrysin, galangin
Flavonols	Kaempferol, quercetin, fisetin, isorhamnetin, myricetin
Isoflavones	Daidzein, genistein, daidzin

Table 2 Content of flavonoids in chosen foodstuffs (mg/100 g foodstuff) (Authors' selection based on Ref. [7])

Range of flavonoids' content	Products (flavonoids mg/100 g)
>1500 mg/100 g	Parsley, dried (4854.49), oregano, Mexican, dried (1545.79)
300 mg–1500 mg/100 g	Elderberries (518.13), Capres, raw (493.03), chokeberry (368.66)
100 mg–300 mg/100 g	Cowpeas (277.41), parsley, fresh (233.16), currants, black (167.47), blueberries (158.51), blackberries (137.66), green tea, brewed (121.27), black tea, brewed (119.32), cranberries (113.58), dark chocolate (108.60), cocoa, dry powder (106.68), dock (102.20)
70 mg–100 mg/100 g	Kale (92.98), fennel (84.50), currants, red (79.49), kumquats (79.26), black currant juice (78.04) white tea, brewed (74.60)
40 mg–70 mg/100 g	Arugula (69.27), cabbage, red (64.34), peppermint, fresh (60.48), grapefruit (55.40), lemons (53.38), oolong tea, brewed (52.37), limes (48.60), grapes, red (48.35), thyme, fresh (47.75), raspberries (47.58), onions, red (44.87), oranges (43.49), wine, table red (40.84), cherries (40.00)
10 mg–40 mg/100 g	Strawberries (34.31), pecans (34.01) beans, black (28.00), radishes (26.52), wheat, purple (25.85), Orange juice (24.13), celery hearts, green (22.60), artichokes (22.20), chives (21.67), pears (21.53), peppers, hot chili (21.17), broadbeans, cooked (20.63), lettuce, red (19.40), pink grapefruit juice (17.97), buckwheat (15.38), asparagus, cooked (15.16), milk chocolate (15.04), pistachio (14.37), plums (14.23), cress, fresh (14.00), apple (13.73), bananas (13.69), hazelnuts (11.96), chicory, green (11.79), spinach (11.44), almonds (11.00), beans, kidney, red (10.87), apricots (10.67), chard (10.43), endive (10.10)
<10 mg/100 g	Sorghum, red (8.43), Brussels sprouts, cooked (7.68), peppers, green (6.98), beer (3.34)

Extent of biological effects of flavonoids depend not only on their content in food, but also on bioavailability that is influenced by the source type (food or pharmaceutical formulation), consumers' characteristics, such as gender and age, their interindividual variations, including physiological and molecular factors, composition and activity of gastrointestinal microflora, and also differences in mechanisms of absorption and biotransformation of different compounds [2]. The main food sources of substances from particular subgroups are shown in Table 3.

There are several databases of flavonoids contents in foods which can be used to assess their consumption with human diet. Most studies estimate flavonoids intake using food composition tables such as the USDA databases and the online Phenol-Explorer database (www.phenol-explorer.eu). However, these tables might be of limited use because only a restricted number of foods have been analysed for their polyphenol content using different analytical techniques. For this reason, the results of nutritional assessments may vary depending on the sort of database used. It should be emphasized that many surveys performed in 1990s could underestimate the actual intake of flavonoids as the databases of flavonoid content of foodstuffs were incomplete at that time. For

Table 3 Content of flavonoid subclasess in chosen foodstuffs (mg/100 g foodstuff) (Authors' selection based on Ref. [7])

Anthocyanins (mg/100 g)					
Elderberry juice concentrate	411.40	Blackberries	90.64	Pecan nuts	25.02
Chokeberry	349.79	Red currants	75.02	Red table wine	23.18
Bilberries	285.21	Red cabbage	63.50	Black grapes	21.63
Chickpeas	262.49	Raspberries	40.63	Pears	12.18
Black currants	154.77	Red bilberries	40.15	Morello cherries	7.45
American bilberries	141.03	Strawberries	27.76	Hazel nuts	6.71
Flavanols (mg/100 g)					
Green tea, brewed	116.15	Blackberries	42.50	Apples	9.17
Black tea, brewed	115.57	Cooked broad beans	20.63	Peaches	8.60
Dark chocolate	108.60	Pecan nuts	15.99	Apricots	8.41
Cocoa, dry powder	52.73	Red table wine	11.05	Apple juice	5.96
Flavanones (mg/100 g)					
Dried Mexican oregano	412.13	Limes	46.40	Grapefruit juice	18.98
Grapefruit	54.50	Oranges	42.57	Artichokes	12.51
Lemons	49.81	Orange juice	18.99		
Flavones (mg/100 g)					
Dried parsley	4523.25	Artichokes	9.69	Chicory	2.85
Dried oregano	1046.46	Green pepper	4.71	Lemons	1.90
Fresh parsley	216.15	Celeriac	3.90	Red grapes	1.30
Flavonols (mg/100 g)					
Fresh capers	493.03	Goji berries	31.20	Chicory	8.94
Dried parsley	331.24	Fresh cranberries	21.59	Buckwheat	7.09
Elderberry juice concentrate	108.16	Cooked asparagus	15.16	Dried and sweetened cranberries	6.91
Sorrel	102.20	Blackcurrants	11.53	Fresh figs	5.47
Rocket lettuce	69.27	American bilberries	10.59	Cooked brussel sprouts	5.24
Red onions	38.34	Morello cherries	9.41	Apples	3.40
Isoflavones (mg/100 g)					
Soy flour	166.66	Soybeans, raw	48.95	Clover, red	21.0
Soybeans mature seeds (Europe)	103.56	Miso	41.45	Soybeans, cooked	17.92
Natto	82.29	Soy yoghurt	33.17	Sufu	13.75
Tempeh	60.61	Tofu, different types	28.91	Pistachio nuts	3.63

proper interpretation of the results of assessment of flavonoids intake with diet, a kind and year of release of used database should be considered.

Recent evidence suggests that the total daily intake of flavonoids varies among nations and cultures. Habitual dietary intake of flavonoids is variable and in USA, estimated daily intake was on average 200.1 ± 8.9 mg/d, while in Europe, mean intakes among European adults ranged from 506 mg/d (the Central region) to 348 mg/d (the Northern region) and the 301 mg/d (the Southern region) [9, 10]. The mean total flavonoid intake in the Polish population was equal to 403.5 mg/day, when calculated with the use of the Phenol-Explorer database, and 525 mg/day, when the USDA databases were used [11]. These results contrast with our own results that demonstrated that the average daily flavonoid consumption among Polish students was equal to 801 mg [12].

3 Flavonoids Action and Current Research and Trends

It seems that a diet rich in flavonoids may be beneficial for human health [3]. Emerging evidence from epidemiological and randomized controlled trials support protective effects of foods and dietary supplements rich in flavonoids against chronic diseases, including coronary heart disease, type 2 diabetes mellitus, cancer, Alzheimer's disease, Parkinson's disease (PD), cataract, erectile dysfunction, and inflammatory bowel disease. In this subsection, we summarized results of epidemiological and animal studies, human clinical trials, and also in vitro studies on both flavonoids and flavonoids subclasses, that showed the main effects and mechanisms of flavonoid action.

3.1 Antioxidant and Anti-Inflammatory Action of Flavonoids

Flavonoids possess many biochemical properties, but the best investigated effect of almost all subclasses of flavonoids is their antioxidants activity. Almost every group of flavonoids has a capability to act as antioxidants. It was reported that flavones and catechins seem to be the most powerful flavonoids for protecting the body against reactive oxygen species [13, 14].

Antioxidant activity of flavonoids depends on their functional hydroxyl groups that can mediate antioxidant effects by scavenging free radicals and/or by chelating metal ions. Mechanisms of antioxidant action include suppression of reactive oxygen species (ROS) formation, either by inhibition of enzymes, or by chelating trace elements involved in free radical generation, scavenging ROS, and upregulation or protection of antioxidant defenses [2]. The chelation of metals seems to be crucial in the prevention of radical generation which damage target biomolecules. Some flavonoids are capable of inhibiting the enzymes involved in ROS generation, for example, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, NADH oxidase [15]. Antioxidant action of flavonoids may also

result from activation of antioxidant enzymes, such as catalase, glutathione peroxidase, and heme oxygenase-1 (HO-1), which have radical scavenging ability.

Flavonoids also may exhibit also anti-inflammatory action what can be mediated by inhibition of both the activity and production of various proinflammatory substances and enzymes, such as tumor necrosis factor α (TNF-α), cyclooxygenase (COX), and lipoxygenase (LOX) [16]. Moreover, flavonoids may play a role in resolution of inflammation also by inhibition of activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which is responsible for control of transcription of DNA, cytokine production, and cell survival, and also by suppression of prostaglandin E2 (PGE2), which impairs T cell receptor signaling [17].

3.2 Cardioprotecive Action of Flavonoids

The well-recognised antioxidant properties of flavonoids resulted in the interest in their potential role in prevention of cardiovascular disease. Interestingly, the direct mechanisms are not well understood. It seems that the action of flavonoids is multifaceted and depends on parallel processes. Among the main mechanisms, there are: antioxidant and anti-inflammatory activities, regulation of blood pressure, decreases in cholesterol levels, protection of LDL against oxidative modification, and antiplatelet effects.

Lipid peroxidation is a common consequence of oxidative stress. High blood concentration of oxidatively modified low-density lipoproteins (ox-LDL) is one of the most important factors wich accelerates development of atherosclerosis. Flavonoids protect lipids against oxidative damage by various mechanisms. Because of their antioxidant and chelating properties, flavonoids inactivate reactive oxygen species (ROS) and this way counteract plasma LDL oxidation and ameliorate inflammation of the blood vessel endothelium [18].

Antiarteriosclerotic action of flavonoids is related also to the reduction of inflammation in the blood vessel wall through inhibition of the influx of leukocytes. It has been reported that some flavonoids exert also antiplatelet aggregation effects through various mechanisms, among which the inhibition of the arachidonic acid-based pathway seems to be the most representative [19]. They decrease activity of enzymes that participate in the formation of prostaglandins leukotrienes and thromboxane A2, substances that mediate inflammation and aggregation, from arachidonic acid. This results in decrease in inflammation and platelet aggregation. Inhibition of these enzymes results also in protection of LDL against oxidation and regulates capillary pressure back to normal [20].

Beyond of protection of blood vessels against ox-LDL, antiatheromatous action of flavonoids results also from suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (hHMGR) activity. This enzyme plays a key role in the synthesis of cholesterol in the human body and thereby influences its plasma levels. Inhibition of its activity lowers intracellular cholesterol concentrations and results in the following increase in expression of LDL receptors. This in turn raises the cellular lipoprotein uptake and removal of cholesterol from the circulation. Based on the

criteria of inhibition of hHMGR activity, many cholesterol-lowering drugs, such as statins, have been developed, and their efficacies in controlling blood cholesterol levels have been well recognized [20].

Anthocyanins, found in berries, blackcurrants, red grapes, plums, and cherries, are a good example of a flavonoids which reduce blood cholesterol level in patietns with high CVD risk. In a 6-month study, low-density lipoprotein cholesterol (LDL-C) concentrations were reduced following anthocyanins supplementation (320 mg/d) in 122 hypercholesterolemic patients, while the similar result was observed in another study performed among 58 diabetic patients who received purified anthocyanin supplementation (320 mg/d). This intervention significantly decreased serum LDL cholesterol (by 7.9%; $P < 0.05$), triglycerides (by 23.0%; $P < 0.01$), and increased HDL cholesterol (by 19.4%; $P < 0.05$) compared with placebo after the 24 weeks [21, 22]. Another randomized, double-blind, placebo-controlled clinical trial (80 hyperlipidemic patients) showed that 2 months supplementation with extract from fruit rich in anthocyanins, whortleberry fruit (350 mg capsule every 8 h) reduced serum levels of total cholesterol, triglyceride, and LDL-C by 27.6%, 19.2%, and 26.3%, respectively and increased that of HDL-C by 37.5% [23]. In turn, a 8-weeks dose-response study (50 g freeze-dried blueberries, which is egual to approximately 350 g fresh blueberries), performed in 48 participants with metabolic syndrome, demonstrated beneficial effects on plasma oxidized LDL (ox-LDL) and combined serum malondialdehyde (MDA) and hydroxynonenal (HNE) concentrations, which are biomarkers of lipid and lipoprotein oxidation and have also been associated with coronary artery disease. The decreases in ox-LDL and combined MDA and HNE were greater in the blueberry supplemented group (−28 and −27%, respectively) than in controls (−9 and −9%) ($P = 0.009$ and $P = 0.005$) (Basu et al. 2010).

It is also important to emphasize the remarkable role of anthocyanins in reduction of biomarkers of CVD risk. Results of four out of five analyzed recent studies suggested that increased habitual anthocyanins intake is significantly associated with a reduction in risk of coronary heart disease (CHD) by 12–32% in multivariate analyses [24–28].

Catechins, next to anthocyanins, play an important role in amelioration of cardiovascular disease risk factors. Catechins are the generic terms of flavanols, as well as the most important tea flavonoids. It was found that the intake of catechins at a rate of 576 mg per day for 24 weeks can significantly decrease body fat mass among 40 obese or near-obese children. Catechins could regulate the RNA and protein expression of fatty acid metabolism enzymes in the liver. They may affect the activity of metabolic enzymes, which can improve the oxidation of fatty acids and the inhibit fatty acid synthesis. This specific action of catechins may result in reduction of lipid levels in the blood and liver, as well as may decrease body fat deposition, thus reducing early onset of cardiovascular disease in children [29].

Quercetin, a flavonoid from the flavonol subclass, may be also effective as a cardioprotective agent. It was shown that its anti-inflammatory, antioxidant, and antiapoptotic effects can effectively protect against myocardial damage in rats. Compared with the control group, mRNA and protein levels of TNF-alpha and

IL-1beta and also malondialdehyde (MDA) content in myocardial tissue of rats in both the low-dose (100 mg quercetin/kg/daily) and high-dose (400 mg quercetin/kg/daily) groups decreased significantly. Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) activities increased significantly. The cell apoptosis index was significantly reduced [30]. In turn, human intervention studies demonstrated positive effects of 6 week treatment with quercetin-rich onion skin extract (162 mg/d quercetin) on blood pressure and endothelial function in 70 overweight-to-obese patients with (pre-) hypertension [31]. The mechanisms responsible for the BP-lowering effect remain unclear. The putative pathways include improvement of vascular function in an endothelium-dependent or endothelium-independent manner, decrease in oxidative stress, and/or interference with the renin–angiotensin–aldosterone system. Importantly, flavonoids can improve endothelial function by stimulating the nitric oxide (NO) bioavailability, a key regulator of vascular function, leading to improvements in vascular tone and blood pressure regulation [31, 32].

However, while many studies demonstrated significant inverse associations between flavonoid intake and CVD incidence, the Framingham Offspring Cohort failed to support such observation [28]. The results showed that there was no significant association between flavonoid intake and CVD incidence after multivariable adjustment. This finding, based on a long-term follow-up of middle-aged and elderly Americans, suggests that the relationship between flavonoid intake and CVD risk is not clear and requires further investigation.

Data from intervention studies suggest that there is a wide interindividual variability in flavonoid metabolism as 15–99% of their ingested dose was recovered as a wide range of flavonoid metabolites. It implies that flavonoids' metabolism may play a critical role in explaining the differential responses in CVD risk biomarkers observed in clinical trials (responders vs nonresponders) [33–35].

This diversity in responsiveness to flavonoids intake may relate to a number of factors, where the microbiome is likely to be critical and it plays a key role in flavonoids metabolism. Furthermore, it is likely that intake alters the composition and function of the gut microbiome and conversely, that the microbiota may enhance the metabolism of flavonoids, but this bidirectional relationship has not been studied enough yet [34]. To understand the importance of metabolism, particularly microbiome-mediated biotransformation, in explaining the CV health effects of flavonoids, a combination of epidemiological studies and dietary intervention trials are needed.

3.3 Antidiabetic Action of Flavonoids

Flavonoids may exert beneficial effects in diabetes by various, parallel intracellular signaling pathways in pancreatic β-cells, liver, adipose tissue, and skeletal muscles. These compounds are able to influence β-cell mass and function, as well as energy metabolism and insulin sensitivity in peripheral tissues. Flavonoids can increase glucose uptake through translocation of GLUT4 vesicles to the cell membrane. They

may also enhance glucose uptake in response to insulin through the stimulation of adenosine monophosphate-activated protein kinase (AMPK) and other kinases such as ERK1/2 and p38 mitogen-activated protein kinase (p38MAPK) [36].

Epidemiological studies and meta-analyses suggested an inverse relationship between the consumption of flavonoid-rich diets and development of diabetes. A recent study evaluated the relationship between dietary intake of total flavonoid intake and type 2 diabetes (T2DM). This analysis, consisting of six prospective cohort studies including 18,146 cases and 284,806 participants, reported that the highest intake of total flavonoids could reduce risk of diabetes by 9%, when compared with the lowest. Moreover, an increase in the total flavonoids intake of 500 mg/d was associated with a significant risk reduction of 5% [37]. Furthermore, in another epidemiological study, it was shown that high consumption of flavonoids from flavanones subclass was also associated with a reduced risk of diabetes. After multivariable adjustment, the authors observed a 31% reduction in new-onset diabetes in the highest compared with the lowest tertile of flavanones intake in near 6 years of follow-up (18,900 person-years) [38]. Population-based studies have also suggested that flavonoids-rich foods, such as soy (isoflavones, genistein), tea (flavanols), citrus fruit (flavanones), and blueberry (anthocyans), exerted beneficial effects on blood pressure, lipid metabolism, insulin resistance, and glucose uptake, which are related to the development of T2DM [39–42].

Numerous in vitro and animal studies also support a beneficial effect of dietary flavonoids on glucose homeostasis. The supplementation of a dietary apple/kale extract (AKE), which is rich in flavonoids, significantly improved both blood glucose levels and oral glucose tolerance test (OGTT) in mice. Furthermore, in situ uptake of glucose was significantly inhibited by AKE. Authors concluded that AKE exhibits antidiabetic properties by a dual mechanism, including the inhibition of α-glucosidase and sodium-dependent glucose transporter 1 (SGLT1) [43]. Some studies support the concept that naringenin is a potent insulin sensitizer in vivo. Fibroblast growth factor 21 (FGF21) regulates energy homeostasis and the metabolic adaptation to fasting. After naringenin supplementation of diet of both genotypes of wild-type mice, FGF21-positive and FGF21-negative, improvement of glucose tolerance was observed [44]. Moreover, in streptozotocin-induced diabetic rats fed high-fat diet, naringenin improved postprandial hyperglycemia. Oral intake of naringenin (25 mg/kg body mass) exerted significant inhibition of intestinal α-glucosidase activity in vivo and thereby delayed the absorption of carbohydrates in T2DM rats, thus resulting in significant lowering of postprandial blood glucose levels [45].

In a study performed in women (n = 1997), it was shown that the highest intakes of both flavones and anthocyanins, when compared with the lowest, were linked to reduced risk of diabetes. These flavonoids consumption was also inversely associated with biomarkers of inflammation, insulin concentration, and also value of HOMA-IR (homeostasis model assessment of insulin resistance) index. Participants with high intakes of flavones had lower HOMA-IR and insulin concentrations and higher adiponectin concentrations, with respective differences of −0.1 (P-trend = 0.04), −0.5 μU/mL (P-trend = 0.02), and 0.6 μg/L (P-trend = 0.01)

comparing extreme quintiles of intake [46].Thus, the described results suggest that total flavonoids, their particular subclasses, and specific substances have the potential to serve as natural plant bioactive compounds for dietary prevention strategies against T2DM.

3.4 Anticancer Action of Flavonoids

Flavonoid compounds display a remarkable spectrum of biological activities, including those that may affect such stages of carcinogenesis as initiation, promotion, and progression. In the initiation and promotion stages, they include: inactivation of carcinogens, inhibition of cell proliferation, enhancement of DNA repair processes, and reduction of oxidative stress. In the progression phase, flavonoids may induce apoptosis, inhibit angiogenesis, exhibit antioxidant activity, and also induce cytotoxic or cytostatic action against cancer cells [47].

Preventing carcinogen metabolic activation is one of the most important mechanism by which flavonoids can exert their chemoprevention effects. Flavonoids may interact with phase I metabolizing enzymes (e.g., cytochrome P450), which metabolically activate a large number of procarcinogens. Flavonoids inhibit certain cytochrome P450 isozymes, such as CYP1A1 and CYP1A2, and thus protect against cellular damage by carcinogens. Another important mechanism of action is possibility induction of phase II metabolizing antioxidant enzymes such as glutathione S-transferase (GST) and UDP-glucuronyltransferase (UDP-GT).

Stimulation of apoptosis is the next important mechanism by which flavonoids may be recognized as anticancer substances. Dysregulation of apoptosis plays a critical role in oncogenesis. Flavonoids were shown to induce apoptosis in some cancer cell lines, while sparing normal cells. It was recently reported that flavonoids possess antiproliferative and apoptosis-inducing activities in several cancer human cell lines, i.e., gastric, colon, breast, lung liver, head, and neck cancers [48–55]. The molecular mechanisms by which flavonoids induce apoptosis have not been clarified.

The anticancer effects of flavonoids may be also explained by the cell cycle inhibition. Cyclin-dependent kinases (CDKs) are recognized as key regulators of cell cycle progression. Various types of cancers exhibit hyperactivation of CDKs due to mutation of CDK genes or CDK inhibitor genes. These disturbances may be constrained by flavonoids [47]. Moreover, such flavonoid as quercetin stimulates the inhibition of HMGB1-induced TNF-α and IL-1β mRNA expression, which suggests that quercetin modulates cell signaling that in turn regulates the action of proinflammatory cytokines. The HMGB1 protein, which is present outside the cell, can increase the release of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and other inflammatory mediators from monocytes (Khan et al. 2016). All of the described mechanisms help to explain the chemopreventive effects of flavonoids against carcinogenesis.

Increasing evidence from both epidemiological and laboratory studies suggests that the high dietary intake of flavonoids reduces the risk of developing certain types

of cancers. It concerns, among others, the breast cancer. In general, the prevalence of the breast cancer is lower in Asian than in North American and European countries [56]. It may be explained by higher intake of soy in Asian populations, that is an abundant source of isoflavones. A meta-analysis of 22 observational studies showed that high isoflavone intake was linked to reduced breast cancer risk rather in Asian than in Western populations. The protective effect of isoflavones in Asian subjects concerned both pre- and postmenopausal women [57]. On the contrary, a population-based prospective cohort study performed in Japan (15,607 women) showed a beneficial effect only in postmenopausal females [58]. These results suggest that soy and isoflavone intakes may have a protective effect mainly against postmenopausal breast cancer in Asian populations, but their beneficial action is possible also in premenopausal women.

High consumption of total flavonoids was also reported to be associated with a significant reduction of ovarian cancer risk by 18% [59], as well as of the risk of smoking-related cancers, such as aerodigestive tract cancer (33% risk reduction) and lung cancer (16% risk reduction) [60]. Guo et al. demonstrated that higher green tea consumption (above 7 cups/day) was linearly linked to reduced prostate cancer risk. It was concluded that green tea catechins were effective for preventing prostate cancer [61].

The epidemiological data presented above concern only prevention and the results of the studies are not always unambiguous and apply only to some types of tumors. The present review of literature suggests that results may be promising but are inconclusive. Further, prospective cohorts assessing dietary flavonoid intake and studies using other methods to evaluate exposure (i.e., markers of consumption, metabolism, excretion, bioavailability, drug–drug interactions) are needed to overcome the limitations emphasized in observational studies testing dietary intakes and determine consumption levels required to achieve health benefits [62, 63].

Results of laboratory studies which consider flavonoids as anticancer agents are very promising. Recently, epigallocatechin-3-gallate (EGCG) – a major flavonoid constituent in green tea – has received much attention as a potential cancer chemopreventive agent with DNA topoisomerase I (Topo I) inhibitory activity [64]. Topo I is a crucial enzyme that works to relax supercoiled DNA during replication, transcription, and mitosis. In a number of human solid tumors, the intracellular level of Topo I is higher than that in normal tissues. It seems that controlling the Topo I level is essential in treating cancers and a large number of Topo-directed agents (e.g., camptothecin, topotecan, and irinotecan) are currently in clinical use. Topo I inhibitors exert their antitumor activities by stabilizing the cleavable Topo I–DNA ternary complex, blocking rejoining of the DNA breaks, and inhibiting enzyme binding to DNA. It should be emphasised that EGCG possessed low cytotoxicity with much higher half maximal inhibitory concentration (IC50) to human tumor cell lines than the traditional Topo-directed agents [64].

Another interesting substance is kaempferol, flavonoid from flavonols subclass. It was recently reported that this flavonoid possesses antiproliferative and apoptosis-inducing activities in several cancer cell lines, i.e., human cervical cancer cells (SiHa and Hela), human osteosarcoma cells, cholangiocarcinoma cells, human breast

carcinoma (MCF-7) cells, human bladder cancer cells (5637, T24), human stomach carcinomacells (SGC-7901), and human lung carcinoma cells (A549) [48–54]. It was also found that kaempferol may play an important role as a potential therapeutic agent for cancer metastasis [54]. The antimetastatic action of kaempferol is related to inhibition of matrix metalloproteinases (MMPs) that are regarded as major critical molecules assisting tumor cells during metastasis, because of excessive degradation of the extracellular matrix, and thus facilitation of cancer cells invasion [65].

These findings demonstrate that kaempferol and epigallocatechin-3-gallate may be novel anticancer agents but more detailed studies should be performed to confirm their anticancer effects for clinical use.

The biological properties of citrus flavonoids and their effectiveness in cancer prevention have been widely studied. Recenty, Wang et al. have reported on anti-cancer ability of flavonoids composition of citrus fruits varieties [13]. In vitro tumor cytotoxicity of the citrus extracts were measured on three gastric cancer cell lines, i. e., SGC-7901, BGC-823, and AGS. For the cytotoxicity of individual compounds, the highest correlation coefficient with cytotoxicity of extracts on all three gastric cancer cell lines was shown for nobiletin, a flavonoid from flavones subclass [13].

Not only citrus exctract but also apple exctrat was shown to have a positive effect in vivo. George et al. demonstrated that preexposure with apple flavonoids protected from various carcinogen-induced toxicity in normal human bronchial epithelial cells significantly reduced toxic effects of various carcinogens, especially nicotine-derived nitrosamine ketones, by inhibiting DNA damage response signaling and initiated DNA repair mechanisms [66].

Other promising phytochemicals are Grape Seed Proanthocyanidins (GSPs). GSPs have been shown to have cytotoxic activity against various cancer cell lines with no significant toxic effects on normal cells. Treatment with GSPs significantly reduced the viability and induced apoptosis of human head and neck squamous cell carcinoma (HNSCC) cells, including HNSCC cell lines derived from the oral cavity, larynx, pharynx, and tongue [55].

A number of human, animal, and cell-culture studies reported the potential effects of genistein on different types of cancer, including breast, prostate, and ovarian cancers. Genistein belongs to isoflavone subclasses. The mechanism of its influence on cancer is complex and requires detailed investigations. Depending on the cancer type, the same molecule may exert adverse or beneficial effects. Among breast cancers, there are three subtypes: Triple Negative BC (TNBC, which do not express estrogen, progesterone, or HER2 receptors), HER2 (that possess HER2 receptors), and ER (that possess estrogen receptors). It was demonstrated that genistein plays different roles in this triple scenario [67]. Some studies showed favorable effects of both high and low doses of genistein as they decreased proliferation of ERα-β and HER2 breast cancer cells [68–70]. However, it was reported by other authors that high doses of genistein may enhance markers of proliferation, such as FGFR2, E2F5, BUB1, CCNB2, MYBL2, CDK1, and CDC20 [71]. On the other hand, genistein and daidzein and their metabolites may interact with ATP-binding cassette (ABC) G2 efflux transporter, which is also called the breast cancer resistance protein (BCRP) and mediates the disposition and excretion of numerous endogenous chemicals and xenobiotics. These

isoflavones may serve the BCRP as substrates, inhibitors, and/or modulators of gene expression. When acting as BCRP inhibitors, these substances may induce reversal of multidrug resistance and may enhance effectiveness of chemotherapy. On the other hand, they may bring about also negative outcomes, such as increase in the toxicity or a decrease in the efficacy of specific medications [72].

3.5 Flavonoids and the Nervous System

Enhancing cognitive abilities has become an important scientific challenge, recently driven by the interest in preventing age-related cognitive decline and sustaining normal cognitive performance in response to cognitively demanding environments. Effectiveness of flavonoids in prevention of age-related neurodegenerative diseases has been much investigated in the recent years. It concerns particularly dementia, Parkinson's (PD), and Alzheimer's diseases (AD). It seems that flavonoids can modulate neuronal function. Diets rich in these substances were shown to beneficially affect maintenance of human cognitive functions, probably through protection of neurons, enhancement of their function and regeneration [73].

The main pathological mechanisms of neurodegenerative diseases, including AD, are both increased oxidative stress and inflammation [74–76]. It seems that flavonoids may protect effectively against oxidative neuronal damage and may also decrease inflammation and thus have positive effects on the nervous system.

The multiplicity of beneficial effects of flavonoids exerted on the nervous system seems to result from variety of mechanisms. One of them is regulation of the neuronal signal cascade what brings about inhibition of cell apoptosis caused by neurotoxic substances, such as amyloid β (Aβ) or 6-hydroxydopamine, (6-OHDA). This promotes neuronal survival and differentiation. Another mechanism is improvement of the cerebral blood flow, what can induce angiogenesis and growth of new neurons in the hippocampus. Furthermore, flavonoids may increase the amount of the brain-derived neurotrophic factor (BDNF) which is crucial to adult neurogenesis, synaptic growth, and neuronal survival. All of these processes are crucial for maintenance of neuronal and cognitive brain functions [77, 78].

The neurodegenerative process in Alzheimer's diseases is characterized by the presence of cerebral extracellular deposition of amyloid-β plaques, intraneuronal neurofibrillary tangles, and cerebral atrophy [79]. The enzyme β-Secretase 1 (BACE1) controls the rate-limiting step of Aβ generation. The newly synthetized Aβ rapidly aggregates to form neurotoxic amyloid fibrils. Shimmyo et al. demonstrated that four flavonols (myricetin, quercetin, kaempherol, and andmorin) and one flavone (apigenin) could inhibit BACE-1 activity in both the cell-free system and neuronal cells. Each flavonoid significantly decreased concentrations of amyloid β peptide-40 (Aβ1-40) and amyloid β peptide-42 (Aβ1-42) in neurons. These results showed that flavonoids may reduce neuronal Aβ level and that this phenomenon is related to BACE-1 inhibition [80].

Some studies also show that catechins, which are found in tea, may prevent the formation of amyloid-β plaques, enhance cognitive functions, and may slow down

the onset and progression of AD. These substances (or their metabolites) can pass the blood-brain barrier (BBB) and exert neuroprotective effects. Several studies demonstrated that EGCG and other flavonoids, such as luteolin, could reduce toxic levels of Aβ in the brain, and also mitochondrial dysfunction in AD brains [81]. EGCG also prevented Aβ peptides-induced neurotoxicity, elongation of the fibrils, and stabilization of the formed fibrils in cell cultures, and also enhanced clearance of phosphorylated tau protein in primary neurons in rats [82–84].

Parkinson's disease (PD) is a progressive neurodegenerative disease, characterized by selective loss of dopaminergic neurons in the human midbrain region known as the substantia nigra pars compacta (SNpc). The factors that trigger the onset of this disease still remain unknown. Some evidence supports the role of oxidative stress and imbalance in the natural antioxidant defense system. It is presumed that these factors may contribute to the formation and/or accumulation of abnormal or "toxic" proteins in the neuronal cells, which can lead to neuronal death [76].

Flavonoids such as quercetin glycosides, rutin, and isoquercitrin have distinct features in upregulating the production of intracellular antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) and glutathione in a 6-hydroxydopamine (6-OHDA-) induced in PC-12, rat pheochromocytoma cells [85, 86]. It can protect neuronal cells from oxidative stress through elevation of intracellular antioxidant enzymes. Apart from that, the neuroprotective effects of flavonoids were further demonstrated in animal studies using tangeretin [87]. Tangeretin, which is found mainly in citrus fruit, given to mice passes the BBB and protects the nigrostriatal pathway against adverse effects of 6-hydroxydopamine.

Not only research performed on animals but also on humans confirm an important role of flavonoids (or their metabolites) in PD's. In a prospective study, it was observed in men that in the highest quintile of total intake of flavonoids there was a 40% lower PD risk than in the lowest quintile. However, the authors admitted that a protective effect of other constituents of plant foods should be taken into account, and that it seems that the whole dietary pattern is of a great importance [88].

Effectiveness of flavonoids in prevention of age-related cognitive impairment, particularly among patients at risk, has been much investigated in the recent years. Research investigating the relations between cocoa flavanols and cognition shows dose-dependent improvements in general cognition, attention, processing speed, and working memory. Moreover, cocoa and cocoa-derived food could also improve normal cognitive functioning and exert a protective role on delaying or preventing age-related deterioration of cognitive decline [89].

In another study, compared to the consuming a low cocoa flavanol (48 mg) drink, administration of intermediate (520 mg) and high (993 mg) cocoa flavanols drinks over an 8-week period was associated with reduction of some measures of age-related cognitive dysfunction in healthy aged participants. Interestingly, such cognitive beneficial effects were paralleled by improvements in insulin resistance, suggesting a role of glucose sensitivity in modulating cognitive function in these patients. These data suggest that the habitual intake of flavanols can support healthy cognitive function with age [90]. These results were consistent with the findings of the study performed by Samieri et al. who showed that high flavonoid intake in

midlife helped to maintain good health and well-being during aging among women. In this large prospective study (13,818 women from the Nurses' Health Study), higher intake of several flavonoid subclasses (i.e., flavonols, flavones, flavanones, flavan-3-ol polymers, and anthocyanins), as well as total flavonoids in the age-adjusted analyses were associated with better odds of healthy aging. Healthy aging was defined as survival to more than 70 years with maintenance of four health domains (no major chronic diseases or major impairments in cognitive or physical function or mental health). In analyses of each component of healthy aging, such flavonoid subclasses as flavones and flavanones were significantly associated with two of four domains in authors' definition of healthy aging, although associations were generally weaker than for overall healthy aging. For example, compared with women in the lowest quintile, women in the highest quintiles of flavone and flavanone intakes had 32% and 24%, better odds of no mental health limitations, respectively, and 23% and 15%, better odds of no physical function limitations, respectively [91].

Collectively, it seems that regular consumption of foods rich in flavonoids reduces the risk of neurodegenerative diseases and counteracts or delays the onset of age-related cognitive disorders. Flavonoid compounds have been found to activate the endogenous antioxidant status in neuronal cells and hence protect them against neurodegeneration. Studies also show that catechins may prevent the formation of amyloid-β plaques and enhance cognitive functions, and thus may be useful in treating patients with Alzheimer's disease or dementia.

3.6 Flavonoids: Promising Natural Compounds Against Erectile Dysfunction

It should be emphasized that such disorders as Parkinson's and Alzheimer's diseases, stroke, and cerebral trauma often cause erectile dysfunction (ED) by decreasing libido or causing inability to initiate the erectile process [92]. Besides of neurological diseases also, endothelial dysfunction may result in erectile disorder. Pathophysiology of ED is multifactorial and and is usually triggered by impaired release of NO. Although NO-mediated vessel relaxation plays a central role in erection, other mediators such as cyclic-guanine monophosphate (cGMP), acetylcholine (ACh), prostaglandins, and bradykinins are also important. Angiotensin-I-converting enzyme (ACE) deactivates bradykinin, a vasodilator which is involved in erectile function via the release of NO and relaxation of corpus cavernosum. The conversion of angiotensin I to angiotensin II and deactivation of bradykinin can also induce high blood pressure which in turn additionally impairs erectile function [92, 93].

Oboh et al. have shown a relationship between ED and oxidative stress due to excessive generation of free radicals [94]. In an animal study, flavonoid and other polyphenols-rich extract inhibited angiotensin-I-converting enzyme (ACE) and arginase activities – key enzymes linked to erectile dysfunction – and oxidative stress markers in rats' penile tissues [94].

Furthermore, in a prospective study performed among 25,096 men from the Health Professionals Follow-Up Study, it was shown that higher intakes of flavonoids from such subclasses as flavanones, anthocyanins, and flavones were significantly associated with a reduction in the risk of erectile dysfunction in men below 70 years of age but not in older [95].

3.7 Flavonoids: Promising Natural Compounds Against Cataract

Cataract is the major ophthalmic dysfunction of lens in which the clear lens gradually develops into a cloud opaque and impairs vision. Cataract is one of the secondary complications of diabetes affecting more than 20 million people and accounts 51% of all blindness globally [96, 97]. It appears that flavones apigenin,and chrysin play an important role in attenuation of sugar-induced cataractogenesis. Aldose reductase (AR) is a key enzyme of polyol pathway that is responsible for the reduction of glucose and galactose. Under normoglycemic condition, less than 3% of glucose is metabolized through polyol pathway. However, in the case of hyperglycemia more than 30% of glucose enters polyol pathway that leads to excessive accumulation of sorbitol in the lens, what in turn results in several adverse pathological changes which ultimately accelerate the process of cataractogenesis and other diabetic complications. The mechanism of anticataract action of apigenin and chrysin includes inhibition of AR, what was shown in sugar-induced lens cataract animal model studies [98, 99].

A panel of ten dietary flavonoids from different subclasses was evaluated as possible inhibitors of sugar-induced cataractogenesis using bovine lens organ culture studies. The results of lens organ studies clearly indicate that addition of rutin and silibinin in the cultured lenses containing glucose significantly prevented the progression of lens opacity and maintained the lens transparency as compared with other tested flavonoids. It clearly demonstrated the efficacy of rutin and silibinin as promising agents that lead to inhibition of glycation reaction and amelioration of sugar-induced cataractogenesis. The findings of this study may be useful for designing and development of the novel safe molecules for the management of diabetic cataract [96].

Anthocyanins also seem to protect against cataract. It is commonly regarded that in the pathogenesis of this disease apoptosis of the lens epithelial cells plays an important role, similarly as oxidative stress seems to be the major cause of lens opacity. It was demonstrated that anthocyanin from black soybean may protect human lens epithelial cell line (HLE-B3) from oxidative stress. This substance was shown to reduce HLE-B3 cell death in oxidative stress circumstances [100].

3.8 Flavonoids: Promising Natural Compounds Against Viral Infections

Research focused on antiviral agents isolated from plants started in 1950s, when the activity of 142 plants against influenza A virus was evaluated in embryonated eggs. Twelve of them suppressed virus multiplication [101]. Nowadays, the main

directions of the research include the efficacy of flavonoids in the treatment of hepatitis B virus (HBV), herpes simplex virus (HSV), and human immunodeficiency virus (HIV) infections.

One of the examples is amelioration of chronic hepatitis by flavones. Luteolin-6-C-β-D-boivinopyranosyl-3′-O-β-D-glucopyranoside and chrysoeriol-6-C-β-D-boivinopyranosyl-4′-O-β-D-glucopyranoside, flavones isolated from Alternanthera philoxeroides, an aquatic plant originated from South America, were shown to have significant anti-HBV activities. These substances inhibited the secretion of HBsAg from in the HepG2.2.15 cells [102]. Similarly, in HepG2.117 cells, EGCG inhibited HBV replication through impairing HBV replicative intermediates of DNA synthesis, thereby reducing the production of HBV [103]. Furthermore, in the culture of hepatoma HepG2 cells, it was shown that EGCG treatment raised lysosomal acidification, and thus enhanced autophagy, which appeared to inhibit HBV replication [104]. Thus, botanical agents may be attractive sources of a new anti-HBV drugs.

Behbahani et al. found that kaempferol and kaempferol-7-O-glucoside have strong HIV-1 reverse transcriptase inhibitory activity. These compounds exerted their potent inhibitory effects, at a concentration of 100 μg/ml, on the early stage of HIV replication in target cells [105]. Furthermore, quercetin exerted a significant effect on the pharmacokinetics of ritonavir in rats. Liang et al. have suggested a new strategy of treatment that might result in higher brain distributions, reduced toxicity, and improved efficacy of HIV-1 protease inhibitors (HPIs) which are key components of the highly effective antiretroviral therapy (HAART) currently used to treat HIV-1 infection. Among the eight investigated flavonoids, quercetin was shown to exert the strongest effect on the accumulation of ritonavir in human brain-microvascular endothelial cells (HBMECs), by improving the intracellular accumulation of ritonavir by 76.9% [106].

Numerous studies reported anti HSV-1 and HSV-2 effects of flavonoids, especially of EGCG and quercetin, in animal and cell-culture studies [8, 104, 107–110]. Oral infection with herpes simplex virus type 1 (HSV-1) or genital infection with HSV type 2 (HSV-2) represent the most common infectious diseases in humans. Isaacs et al. found that EGCG can inactivate HSV virions by binding to the envelope glycoproteins gB and gD, which are essential for HSV infectivity. Moreover, the EGCG digallate dimers theasinensin A, P2, and theaflavin-3,3′-digallate inactivated HSV-1 and HSV-2 more effectively than did monomeric forms. These dimers are stable at vaginal pH, indicating their potential to be antiviral agents against HSV infections [111]. In addition, modification of green tea flavanol EGCG with palmitate increased the effectiveness of EGCG as a potent inhibitor of herpes simplex virus 1 [108]. Hung et al. have suggested possible mechanisms whereby quercetin may exert its anti-HSV activity. They revealed that quercetin inhibits the infection of HSV-1, HSV-2 and acyclovir-resistant HSV-1 mainly by blocking viral binding and penetration in the beginning of infection. They also reported that quercetin suppressed NF-κB activation, which is essential for HSV gene expression [112]. Flavonoids may also influence autophagy that plays a crucial role in the regulation of viral replication. Enhancement of autophagy is therefore regarded to be a potential therapeutic strategy for viral infection [113].

3.9 Flavonoids and Their Therapeutic Benefits in Inflammatory Bowel Disease

Ulcerative colitis (UC) and Crohn's disease are two major manifestations of inflammatory bowel disease (IBD). An increasing number of studies have associated the intake of flavonoids-rich foods, especially catechin-rich food, with the prevention and treatment of IBD. Mechanisms by which flavonoids benefit IBD have not yet been entirely recognized, while human experiments demonstrating that catechins can improve IBD are still scarce so far. However, there is abundant evidence showing that flavonoids may work through a combination of parallel processes such as oxidation inhibition, alteration of cellular signaling, and regulation of intestinal flora [114, 115]. Such flavonoids as catechins epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and its stereoisomer gallocatechin (GC), EGCG and its stereoisomer gallocatechin gallate (GCG) could significantly reduce the oxidative damages of the colon by promoting the activation of the antioxidative agents, such as glutathione peroxidases (GPO) and glutathione (GSH). In addition, reduction of the inflammation by regulation of the infiltration and proliferation of immune related-cells, such as neutrophils, colonic epithelial cells, macrophages, and T lymphocytes by catechins can provide benefits to IBD. Such anti-inflammatory properties of catechins as regulating the activation or deactivation of inflammation-related oxidative stress-related cell signaling pathways, such as NF-κB, MAPKs, transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2), signal transducer and the activator of transcription 1/3 (STAT1/3) pathways can be also important for IBD treatment. Finally, catechins can also stabilize the structure of the gastrointestinal microecological environment via promoting the proliferation of beneficial intestinal bacteria and regulating the balance of intestinal flora, which seems be beneficial for IBD [115].

Catechins may be effective agents to relieve IBD without obvious side effects in animal and in vitro studies [114]. It was demonstrated that EGCG applied orally at the dose of 6.9 mg/kg body weight, in combination with piperine which was used to enhance the bioavailability of EGCG, inhibited colon inflammation in colitis in murine model of colitis. Additionally, it significantly reduced the loss of body weight, improved the clinical course, and increased overall survival in comparison with untreated groups [116]. Reduction of tissue concentrations of malondialdehyde and increase in activity of SOD as well as GPO have been shown. In another study by Vasconcelos et al., EC (10 mg/kg) was also shown to benefit colitis induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS) in acute and chronic rat models. In the chronic colitis model, EC treatment was linked to lower macroscopic and microscopic lesion scores and increased glutathione levels, decreased COX-2 expression and increased cell proliferation [117].

Extensive laboratory and epidemiological studies have generally reaffirmed that catechins, depending on their structures and dose, exert preventive effects against chronic inflammatory diseases [118, 119]. However, it should be emphasized that relevant evidence indicated that in the case of application of high concentrations of catechins their pro-inflammatory capacity may exceed the benefitial effect on gut

inflammation. A high dose of EGCG was also demonstrated to act as a prooxidant, instead of antioxidant, by generating ROS, causing even damage to cellular DNA in various kinds of cells [119, 120]. The growing body of evidence indicates that dietary catechins and their condensed sources may play a beneficial role in IBD; however, further clinical and epidemiological trials are greatly needed.

4 Summary

Epidemiological, cell cultures, animal, and human studies support positive effects of flavonoids on health. Over the past decades, flavonoids have attracted interest due to their presumed roles in various beneficial activities, such as antihypertensive, antibacterial, antiinflammatory, antioxidative actions. They were also shown to be protective agents against atherosclerosis. These compounds have also strong chemopreventive potential including anti-inflammatory, antimutagenic, and anticarcinogenic activities. Moreover, increasing numbers of studies have demonstrated that flavonoids may protect against cancer of varios locations, including the stomach, colon, breast, lung, and liver. Furthermore, their consumption may improve postprandial hyperglycemia, protect LDL particles against oxidative modification, reduce neuroinflammation, and improve lipid profile in hyperlipidemic patients. However, there is a great need for better understanding of the underlying molecular mechanisms.

Research challenges and future studies required in flavonoid research include trials to understand the bidirectional relation between flavonoid metabolism and the microbiome. Findings on potential associations between dietary flavonoids intake and chronic diseases risk should ultimately be integrated with studies using appropriate methods to evaluate exposure (i.e., markers of consumption, metabolism, excretion, bioavailability) to overcome the limitations of observational studies. Moreover, detailed research on dietary intakes would provide better insights into the role of specific classes of flavonoids and would show which of them are the most important in disease prevention. It would also indicate the most appropriate level of their consumption. Finally, future reaserch should include prospective clinical trials that would consider evaluation of the extent of absorption and metabolism to establish dose-response relations.

At the present state of knowledge, it should be recommended to consume abundant dietary sources of flavonoids, such as fruits and vegetables, legumes, nuts, spices, and herbs every day.

References

1. Middleton E Jr (1998) Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol 439:175–182
2. Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Scientific World Journal 2013:162750
3. Kozłowska A, Szostak-Węgierek D (2014) Flavonoids-food sources and health benefits. Rocz Panstw Zakl Hig 65:79–85

4. Pollastri S, Tattini M (2011) Flavonols: old compounds for old roles. Ann Bot 108:1225–1233
5. Csepregi K, Neugart S, Schreiner M, Hideg E (2016) Comparative evaluation of total antioxidant capacities of plant polyphenols. Molecules 21:28
6. Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J Nutr Sci 5:e47. https://doi.org/10.1017/jns.2016.41
7. Bhagwat S, Haytowits DB, Holden JM (2013) USDA Database for the flavonoid content of selected foods. Release 3.1. Nutrient Data Laboratory, Beltsville Human Nutrition Research Center Agricultural Research Service U.S. Department of Agriculture, 1–155
8. Zakaryan H, Arabyan E, Oo A, Zandi K (2017) Flavonoids: promising natural compounds against viral infections. Arch Virol 162:2539–2551. https://doi.org/10.1007/s00705-017-3417-y
9. Bhaswant M, Fanning K, Netzel M, Mathai ML, Panchal SK, Brown L (2015) Cyanidin 3-glucoside improves diet-induced metabolic syndrome in rats. Pharmacol Res 102:208–217
10. Kim K, Vance TM, Chun OK (2016) Estimated intake and major food sources of flavonoids among US adults: changes between 1999–2002 and 2007–2010 in NHANES. Eur J Nutr 55:833–843
11. Witkowska AM, Zujko ME, Waskiewicz A, Terlikowska KM, Piotrowski W (2015) Comparison of various databases for estimation of dietary polyphenol intake in the population of Polish adults. Forum Nutr 7:9299–9308
12. Kozłowska A, Przekop D, Szostak-Węgierek D (2015) Flavonoids intake among Polish and Spanish students. Rocz Panstw Zakl Hig 66(4):319–325
13. Wang Y, Qian J, Cao J, Wang D, Liu C, Yang R, Li X, Sun C (2017) Antioxidant capacity, anticancer ability and flavonoids composition of 35 citrus (Citrus reticulata Blanco) varieties. Molecules 22:–114. https://doi.org/10.3390/molecules22071114
14. Wen L, Zhao Y, Jiang Y, Yu L, Zeng X, Yang J, Tian M, Liu H, Yang B (2017) Identification of a flavonoid C-glycoside as potent antioxidant. Free Radic Biol Med 110:92–101
15. Wen L, Jiang Y, Yang J, Zhao Y, Tian M, Yang B (2017) Structure, bioactivity, and synthesis of methylated flavonoids. Ann N Y Acad Sci 1398:120–129
16. Marunaka Y (2017) Actions of quercetin, a flavonoid, on ion transporters: its physiological roles. Ann N Y Acad Sci 1398:142–151
17. Jurikova T, Mlcek J, Skrovankova S, Sumczynski D, Sochor J, Hlavacova I, Snopek L, Orsavova J (2017) Fruits of Black Chokeberry Aronia melanocarpa in the prevention of chronic diseases. Molecules 22. https://doi.org/10.3390/molecules22060944
18. Majewska-Wierzbicka M, Czeczot H (2012) Flavonoids in the prevention and treatment of cardiovascular diseases. Pol Merkur Lekarski 32:50–54
19. Faggio C, Sureda A, Morabito S, Sanches-Silva A, Mocan A, Nabavi SF, Nabavi SM (2017) Flavonoids and platelet aggregation: a brief review. Eur J Pharmacol 807:91–101
20. Lin SH, Huang KJ, Weng CF, Shiuan D (2015) Exploration of natural product ingredients as inhibitors of human HMG-CoA reductase through structure-based virtual screening. Drug Des Devel Ther 9:3313–3324
21. Li D, Zhang Y, Liu Y, Sun R, Xia M (2015) Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients. J Nutr 145:742–748
22. Zhu Y, Huang X, Zhang Y, Wang Y, Liu Y, Sun R, Xia M (2014) Anthocyanin supplementation improves HDL-associated paraoxonase 1 activity and enhances cholesterol efflux capacity in subjects with hypercholesterolemia. J Clin Endocrinol Metab 99:561–569
23. Kianbakht S, Abasi B, Hashem Dabaghian F (2014) Improved lipid profile in hyperlipidemic patients taking Vaccinium arctostaphylos fruit hydroalcoholic extract: a randomized double-blind placebo-controlled clinical trial. Phytother Res 28:432–436
24. Cassidy A, Bertoia M, Chiuve S, Flint A, Forman J, Rimm EB (2016) Habitual intake of anthocyanins and flavanones and risk of cardiovascular disease in men. Am J Clin Nutr 104:587–594
25. Cassidy A, Mukamal KJ, Liu L, Franz M, Eliassen AH, Rimm EB (2013) High anthocyanin intake is associated with a reduced risk of myocardial infarction in young and middle-aged women. Circulation 127: 188–196

26. McCullough ML, Peterson JJ, Patel R, Jacques PF, Shah R, Dwyer JT (2012) Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. Am J Clin Nutr 95:454–464
27. Goetz ME, Judd SE, Safford MM, Hartman TJ, McClellan WM, Vaccarino V (2016) Dietary flavonoid intake and incident coronary heart disease: the REasons for Geographic and Racial Differences in Stroke (REGARDS) study. Am J Clin Nutr 104:1236–1244
28. Jacques PF, Cassidy A, Rogers G, Peterson JJ, Dwyer JT (2015) Dietary flavonoid intakes and CVD incidence in the Framingham Offspring Cohort. Br J Nutr 114:1496–1503
29. Matsuyama T, Tanaka Y, Kamimaki I, Nagao T, Tokimitsu I (2008) Catechin safely improved higher levels of fatness, blood pressure, and cholesterol in children. Obesity (Silver Spring) 16:1338–1348
30. Li B, Yang M, Liu JW, Yin GT (2016) Protective mechanism of quercetin on acute myocardial infarction in rats. Genet Mol Res 15:15017117. https://doi.org/10.4238/gmr.15017117
31. Brull V, Burak C, Stoffel-Wagner B, Wolffram S, Nickenig G, Muller C, Langguth P, Alteheld B, Fimmers R, Naaf S, Zimmermann BF, Stehle P, Egert S (2015) Effects of a quercetin-rich onion skin extract on 24 h ambulatory blood pressure and endothelial function in overweight-to-obese patients with (pre-)hypertension: a randomised double-blinded placebo-controlled cross-over trial. Br J Nutr 114:1263–1277
32. Larson AJ, Symons JD, Jalili T (2012) Therapeutic potential of quercetin to decrease blood pressure: review of efficacy and mechanisms. Adv Nutr 3:39–46
33. Czank C, Cassidy A, Zhang Q, Morrison DJ, Preston T, Kroon PA, Botting NP, Kay CD (2013) Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a (13)C-tracer study. Am J Clin Nutr 97:995–1003
34. Cassidy A (2017) Berry anthocyanin intake and cardiovascular health. Mol Asp Med. https://doi.org/10.1016/j.mam.2017.05.002
35. Cassidy A, Minihane AM (2017) The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. Am J Clin Nutr 105:10–22
36. Babu PVA, Liu D, Gilbert ER (2013) Recent advances in understanding the anti-diabetic actions of dietary flavonoids. J Nutr Biochem 24:1777–1789
37. Liu Y-J, Zhan J, Liu X-L, Wang Y, Ji J, He Q-Q (2014) Dietary flavonoids intake and risk of type 2 diabetes: a meta-analysis of prospective cohort studies. Clin Nutr 33:59–63
38. Tresserra-Rimbau A, Guasch-Ferre M, Salas-Salvado J, Toledo E, Corella D, Castaner O, Guo X, Gomez-Gracia E, Lapetra J, Aros F, Fiol M, Ros E, Serra-Majem L, Pinto X, Fito M, Babio N, Martinez-Gonzalez MA, Sorli JV, Lopez-Sabater MC, Estruch R, Lamuela-Raventos RM (2016) Intake of total polyphenols and some classes of polyphenols is inversely associated with diabetes in elderly people at high cardiovascular disease risk. J Nutr 146:767. https://doi.org/10.3945/jn.115.223610
39. Liu Y, Li J, Wang T, Wang Y, Zhao L, Fang Y (2017) The effect of genistein on glucose control and insulin sensitivity in postmenopausal women: a meta-analysis. Maturitas 97:44–52
40. de Koning Gans JM, Uiterwaal CS, van der Schouw YT, Boer JM, Grobbee DE, Verschuren WM, Beulens JW (2010) Tea and coffee consumption and cardiovascular morbidity and mortality. Arterioscler Thromb Vasc Biol 30:1665–1671
41. Wallace TC, Slavin M, Frankenfeld CL (2016) Systematic review of anthocyanins and markers of cardiovascular disease. Forum Nutr 8. https://doi.org/10.3390/nu8010032
42. JS O, Kim H, Vijayakumar A, Kwon O, Choi YJ, Huh KB, Chang N (2016) Association between dietary flavanones intake and lipid profiles according to the presence of metabolic syndrome in Korean women with type 2 diabetes mellitus. Nutr Res Pract 10:67–73
43. Schloesser A, Esatbeyoglu T, Schultheiss G, Vollert H, Luersen K, Fischer A, Rimbach G (2017) Antidiabetic properties of an apple/kale extract in vitro, in situ, and in mice fed a Western-type diet. J Med Food 20:846–854. https://doi.org/10.1089/jmf.2017.0019
44. Assini JM, Mulvihill EE, Burke AC, Sutherland BG, Telford DE, Chhoker SS, Sawyez CG, Drangova M, Adams AC, Kharitonenkov A, Pin CL, Huff MW (2015) Naringenin prevents

obesity, hepatic steatosis, and glucose intolerance in male mice independent of fibroblast growth factor 21. Endocrinology 156:2087–2102
45. Priscilla DH, Roy D, Suresh A, Kumar V, Thirumurugan K (2014) Naringenin inhibits α-glucosidase activity: a promising strategy for the regulation of postprandial hyperglycemia in high fat diet fed streptozotocin induced diabetic rats. Chem Biol Interact 210:77–85
46. Jennings A, Welch AA, Spector T, Macgregor A, Cassidy A (2014) Intakes of anthocyanins and flavones are associated with biomarkers of insulin resistance and inflammation in women. J Nutr 144:202–208
47. Chahar MK, Sharma N, Dobhal MP, Joshi YC (2011) Flavonoids: a versatile source of anticancer drugs. Pharmacogn Rev 5:1–12
48. Liao W, Chen L, Ma X, Jiao R, Li X, Wang Y (2016) Protective effects of kaempferol against reactive oxygen species-induced hemolysis and its antiproliferative activity on human cancer cells. Eur J Med Chem 114:24–32
49. LY T, Bai HH, Cai JY, Deng SP (2016) The mechanism of kaempferol induced apoptosis and inhibited proliferation in human cervical cancer SiHa cell: from macro to nano. Scanning 38:644–653. https://doi.org/10.1002/sca.21312
50. Chen HJ, Lin CM, Lee CY, Shih NC, Peng SF, Tsuzuki M, Amagaya S, Huang WW, Yang JS (2013) Kaempferol suppresses cell metastasis via inhibition of the ERK-p38-JNK and AP-1 signaling pathways in U-2 OS human osteosarcoma cells. Oncol Rep 30:925–932
51. Kim SH, Hwang KA, Choi KC (2016) Treatment with kaempferol suppresses breast cancer cell growth caused by estrogen and triclosan in cellular and xenograft breast cancer models. J Nutr Biochem 28:70–82
52. Qin Y, Cui W, Yang X, Tong B (2016) Kaempferol inhibits the growth and metastasis of cholangiocarcinoma in vitro and in vivo. Acta Biochim Biophys Sin Shanghai 48:238–245
53. Dang Q, Song W, Xu D, Ma Y, Li F, Zeng J, Zhu G, Wang X, Chang LS, He D, Li L (2015) Kaempferol suppresses bladder cancer tumor growth by inhibiting cell proliferation and inducing apoptosis. Mol Carcinog 54:831–840
54. Li C, Zhao Y, Yang D, Yu Y, Guo H, Zhao Z, Zhang B, Yin X (2015) Inhibitory effects of kaempferol on the invasion of human breast carcinoma cells by downregulating the expression and activity of matrix metalloproteinase-9. Biochem Cell Biol 93:16–27
55. Katiyar SK (2016) Emerging phytochemicals for the prevention and treatment of head and neck cancer. Molecules 21. https://doi.org/10.3390/molecules21121610
56. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61:69–90
57. Xie Q, Chen ML, Qin Y, Zhang QY, HX X, Zhou Y, Mi MT, Zhu JD (2013) Isoflavone consumption and risk of breast cancer: a dose-response meta-analysis of observational studies. Asia Pac J Clin Nutr 22:118–127
58. Wada K, Nakamura K, Tamai Y, Tsuji M, Kawachi T, Hori A, Takeyama N, Tanabashi S, Matsushita S, Tokimitsu N, Nagata C (2013) Soy isoflavone intake and breast cancer risk in Japan: from the Takayama study. Int J Cancer 133:952–960
59. Hua X, Yu L, You R, Yang Y, Liao J, Chen D, Yu L (2016) Association among dietary flavonoids, flavonoid subclasses and ovarian cancer risk: a meta-analysis. PLoS One 11:e0151134. https://doi.org/10.1371/journal.pone.0151134
60. Woo HD, Kim J (2013) Dietary flavonoid intake and smoking-related cancer risk: a meta-analysis. PLoS One 8:e75604. https://doi.org/10.1371/journal.pone.0075604
61. Guo Y, Zhi F, Chen P, Zhao K, Xiang H, Mao Q, Wang X, Zhang X (2017) Green tea and the risk of prostate cancer: a systematic review and meta-analysis. Medicine (Baltimore) 96:e6426. https://doi.org/10.1097/md.0000000000006426
62. Grosso G, Godos J, Lamuela-Raventos R, Ray S, Micek A, Pajak A, Sciacca S, D'Orazio N, Del Rio D, Galvano F (2017) A comprehensive meta-analysis on dietary flavonoid and lignan intake and cancer risk: level of evidence and limitations. Mol Nutr Food Res 61. https://doi.org/10.1002/mnfr.201600930

63. Amawi H, Ashby CR Jr, Tiwari AK (2017) Cancer chemoprevention through dietary flavonoids: what's limiting? Chin J Cancer 36:50. https://doi.org/10.1186/s40880-017-0217-4
64. Xin LT, Liu L, Shao CL, Yu RL, Chen FL, Yue SJ, Wang M, Guo ZL, Fan YC, Guan HS, Wang CY (2017) Discovery of DNA topoisomerase I inhibitors with low-cytotoxicity based on virtual screening from natural products. Mar Drugs 15. https://doi.org/10.3390/md15070217
65. Jablonska-Trypuc A, Matejczyk M, Rosochacki S (2016) Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. J Enzyme Inhib Med Chem 1:7
66. George VC, Rupasinghe HPV (2017) Apple flavonoids suppress carcinogen-induced DNA damage in normal human bronchial epithelial cells. Oxidative Med Cell Longev 2017:1767198. https://doi.org/10.1155/2017/1767198
67. Russo M, Russo GL, Daglia M, Kasi PD, Ravi S, Nabavi SF, Nabavi SM (2016) Understanding genistein in cancer: the "good" and the "bad" effects: a review. Food Chem 196:589–600
68. Jiang Y, Gong P, Madak-Erdogan Z, Martin T, Jeyakumar M, Carlson K, Khan I, Smillie TJ, Chittiboyina AG, Rotte SC, Helferich WG, Katzenellenbogen JA, Katzenellenbogen BS (2013) Mechanisms enforcing the estrogen receptor beta selectivity of botanical estrogens. FASEB J 27:4406–4418
69. Seo HS, Choi HS, Choi HS, Choi YK, Um JY, Choi I, Shin YC, Ko SG (2011) Phytoestrogens induce apoptosis via extrinsic pathway, inhibiting nuclear factor-kappaB signaling in HER2-overexpressing breast cancer cells. Anticancer Res 31:3301–3313
70. Prietsch RF, Monte LG, da Silva FA, Beira FT, Del Pino FA, Campos VF, Collares T, Pinto LS, Spanevello RM, Gamaro GD, Braganhol E (2014) Genistein induces apoptosis and autophagy in human breast MCF-7 cells by modulating the expression of proapoptotic factors and oxidative stress enzymes. Mol Cell Biochem 390:235–242
71. Shike M, Doane AS, Russo L, Cabal R, Reis-Filho JS, Gerald W, Cody H, Khanin R, Bromberg J, Norton L (2014) The effects of soy supplementation on gene expression in breast cancer: a randomized placebo-controlled study. J Natl Cancer Inst. 106. https://doi.org/10.1093/jnci/dju189
72. Bircsak KM, Aleksunes LM (2015) Interaction of isoflavones with the BCRP/ABCG2 drug transporter. Curr Drug Metab 16:124–140
73. Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Barberger-Gateau P (2007) Flavonoid intake and cognitive decline over a 10-year period. Am J Epidemiol 165:1364–1371
74. Calsolaro V, Edison P (2016) Neuroinflammation in Alzheimer's disease: current evidence and future directions. Alzheimers Dement 12:719–732
75. Tramutola A, Lanzillotta C, Perluigi M, Butterfield DA (2016) Oxidative stress, protein modification and Alzheimer disease. Brain Res Bull 133:88–96. https://doi.org/10.1016/j.brainresbull.2016.06.005
76. Magalingam KB, Radhakrishnan AK, Haleagrahara N (2015) Protective mechanisms of flavonoids in Parkinson's disease. Oxidative Med Cell Longev 2015:314560
77. Vauzour D, Vafeiadou K, Rodriguez-Mateos A, Rendeiro C, Spencer JP (2008) The neuroprotective potential of flavonoids: a multiplicity of effects. Genes Nutr 3:115–126
78. Solanki I, Parihar P, Mansuri ML, Parihar MS (2015) Flavonoid-based therapies in the early management of neurodegenerative diseases. Adv Nutr 6:64–72
79. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 1:a006189. https://doi.org/10.1101/cshperspect.a006189
80. Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H (2008) Flavonols and flavones as BACE-1 inhibitors: structure-activity relationship in cell-free, cell-based and in silico studies reveal novel pharmacophore features. Biochim Biophys Acta 1780:819–825
81. Dragicevic N, Smith A, Lin X, Yuan F, Copes N, Delic V, Tan J, Cao C, Shytle RD, Bradshaw PC (2011) Green tea epigallocatechin-3-gallate (EGCG) and other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction. J Alzheimers Dis 26:507–521

82. Fernando W, Somaratne G, Goozee KG, Williams S, Singh H, Martins RN (2017) Diabetes and Alzheimer's disease: can tea phytochemicals play a role in prevention? J Alzheimers Dis 59:481–501. https://doi.org/10.3233/jad-161200
83. Xicota L, Rodriguez-Morato J, Dierssen M, de la Torre R (2017) Potential role of (−)-Epigallocatechin-3-Gallate (EGCG) in the secondary prevention of Alzheimer disease. Curr Drug Targets 18:174–195
84. Chesser AS, Ganeshan V, Yang J, Johnson GV (2016) Epigallocatechin-3-gallate enhances clearance of phosphorylated tau in primary neurons. Nutr Neurosci 19:21–31
85. Magalingam KB, Radhakrishnan A, Haleagrahara N (2013) Rutin, a bioflavonoid antioxidant protects rat pheochromocytoma (PC-12) cells against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity. Int J Mol Med 32:235–240
86. Magalingam KB, Radhakrishnan A, Haleagrahara N (2014) Protective effects of flavonol isoquercitrin, against 6-hydroxy dopamine (6-OHDA)-induced toxicity in PC12 cells. BMC Res Notes 7:49
87. Datla KP, Christidou M, Widmer WW, Rooprai HK, Dexter DT (2001) Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a rat model of Parkinson's disease. Neuroreport 12:3871–3875
88. Gao X, Cassidy A, Schwarzschild MA, Rimm EB, Ascherio A (2012) Habitual intake of dietary flavonoids and risk of Parkinson disease. Neurology 78:1138–1145
89. Socci V, Tempesta D, Desideri G, De Gennaro L, Ferrara M (2017) Enhancing human cognition with cocoa flavonoids. Front Nutr 4:19
90. Mastroiacovo D, Kwik-Uribe C, Grassi D, Necozione S, Raffaele A, Pistacchio L, Righetti R, Bocale R, Lechiara MC, Marini C, Ferri C, Desideri G (2015) Cocoa flavanol consumption improves cognitive function, blood pressure control, and metabolic profile in elderly subjects: the Cocoa, Cognition, and Aging (CoCoA) Study-a randomized controlled trial. Am J Clin Nutr 101:538–548
91. Samieri C, Sun Q, Townsend MK, Rimm EB, Grodstein F (2014) Dietary flavonoid intake at midlife and healthy aging in women. Am J Clin Nutr 100:1489–1497
92. Eleazu C, Obianuju N, Eleazu K, Kalu W (2017) The role of dietary polyphenols in the management of erectile dysfunction-mechanisms of action. Biomed Pharmacother 88:644–652
93. Pavan V, Mucignat-Caretta C, Redaelli M, Ribaudo G, Zagotto G (2015) The old made new: natural compounds against erectile dysfunction. Arch Pharm (Weinheim) 348:607–614
94. Oboh G, Ademiluyi AO, Ademosun AO, Olasehinde TA, Oyeleye SI, Boligon AA, Athayde ML (2015) Phenolic extract from Moringa oleifera leaves inhibits key enzymes linked to erectile dysfunction and oxidative stress in rats' penile tissues. Biochem Res Int 2015:175950
95. Cassidy A, Franz M, Rimm EB (2016) Dietary flavonoid intake and incidence of erectile dysfunction. Am J Clin Nutr 103:534–541
96. Patil KK, Gacche RN (2017) Inhibition of glycation and aldose reductase activity using dietary flavonoids: a lens organ culture studies. Int J Biol Macromol 98:730–738
97. Patel DK, Prasad SK, Kumar R, Hemalatha S (2011) Cataract: a major secondary complication of diabetes, its epidemiology and an overview on major medicinal plants screened for anticataract activity. Asian Pac J Trop Dis 1:323–329
98. Bhatnagar A, Srivastava SK (1992) Aldose reductase: congenial and injurious profiles of an enigmatic enzyme. Biochem Med Metab Biol 48:91–121
99. Patil KK, Meshram RJ, Dhole NA, Gacche RN (2016) Role of dietary flavonoids in amelioration of sugar induced cataractogenesis. Arch Biochem Biophys 593:1–11
100. Mok JW, Chang DJ, Joo CK (2014) Antiapoptotic effects of anthocyanin from the seed coat of black soybean against oxidative damage of human lens epithelial cell induced by H2O2. Curr Eye Res 39:1090–1098
101. Chantrill BH, Coulthard CE, Dickinson L, Inkley GW, Mrris W, Pyle AH (1952) The action of plant extracts on a bacteriophage of pseudomonas pyocyanea and on influenza a virus. Microbiology 6:74–84

102. Li B, Guo QL, Tian Y, Liu SJ, Wang Q, Chen L, Dong JX (2016) New anti-HBV C-boivinopyranosyl flavones from Alternanthera philoxeroides. Molecules 21. https://doi.org/10.3390/molecules21030336
103. He W, Li LX, Liao QJ, Liu CL, Chen XL (2011) Epigallocatechin gallate inhibits HBV DNA synthesis in a viral replication – inducible cell line. World J Gastroenterol 17:1507–1514
104. Zhong L, Hu J, Shu W, Gao B, Xiong S (2015) Epigallocatechin-3-gallate opposes HBV-induced incomplete autophagy by enhancing lysosomal acidification, which is unfavorable for HBV replication. Cell Death Dis 6:e1770. https://doi.org/10.1038/cddis.2015.136
105. Behbahani M, Sayedipour S, Pourazar A, Shanehsazzadeh M (2014) In vitro anti-HIV-1 activities of kaempferol and kaempferol-7-O-glucoside isolated from Securigera securidaca. Res Pharm Sci 9:463–469
106. Liang G, Li N, Ma L, Qian Z, Sun Y, Shi L, Zhao L (2016) Effect of quercetin on the transport of ritonavir to the central nervous system in vitro and in vivo. Acta Pharma 66:97–107
107. Cantatore A, Randall SD, Traum D, Adams SD (2013) Effect of black tea extract on herpes simplex virus-1 infection of cultured cells. BMC Complement Altern Med 13:139
108. de Oliveira A, Adams SD, Lee LH, Murray SR, Hsu SD, Hammond JR, Dickinson D, Chen P, Chu TC (2013) Inhibition of herpes simplex virus type 1 with the modified green tea polyphenol palmitoyl-epigallocatechin gallate. Food Chem Toxicol 52:207–215
109. Liang W, He L, Ning P, Lin J, Li H, Lin Z, Kang K, Zhang Y (2015) (+)-Catechin inhibition of transmissible gastroenteritis coronavirus in swine testicular cells is involved its antioxidation. Res Vet Sci 103:28–33
110. Muller P, Downard KM (2015) Catechin inhibition of influenza neuraminidase and its molecular basis with mass spectrometry. J Pharm Biomed Anal 111:222–230
111. Isaacs CE, Xu W, Merz G, Hillier S, Rohan L, Wen GY (2011) Digallate dimers of (−)-epigallocatechin gallate inactivate herpes simplex virus. Antimicrob Agents Chemother 55:5646–5653
112. Hung PY, Ho BC, Lee SY, Chang SY, Kao CL, Lee SS, Lee CN (2015) Houttuynia cordata targets the beginning stage of herpes simplex virus infection. PLoS One 10:e0115475. https://doi.org/10.1371/journal.pone.0115475
113. Li J, Liu Y, Wang Z, Liu K, Wang Y, Liu J, Ding H, Yuan Z (2011) Subversion of cellular autophagy machinery by hepatitis B virus for viral envelopment. J Virol 85:6319–6333
114. Liu SH, Lu TH, Su CC, Lay IS, Lin HY, Fang KM, Ho TJ, Chen KL, Su YC, Chiang WC, Chen YW (2014) Lotus leaf (Nelumbo nucifera) and its active constituents prevent inflammatory responses in macrophages via JNK/NF-kappaB signaling pathway. Am J Chin Med 42:869–889
115. Fan FY, Sang LX, Jiang M (2017) Catechins and their therapeutic benefits to inflammatory bowel disease. Molecules 22. https://doi.org/10.3390/molecules22030484
116. Bruckner M, Westphal S, Domschke W, Kucharzik T, Lugering A (2012) Green tea polyphenol epigallocatechin-3-gallate shows therapeutic antioxidative effects in a murine model of colitis. J Crohns Colitis 6:226–235
117. Vasconcelos PC, Seito LN, Di Stasi LC, Akiko Hiruma-Lima C, Pellizzon CH (2012) Epicatechin used in the treatment of intestinal inflammatory disease: an analysis by experimental models. Evid Based Complement Alternat Med 2012:508902. https://doi.org/10.1155/2012/508902
118. Chen XQ, Hu T, Han Y, Huang W, Yuan HB, Zhang YT, Du Y, Jiang YW (2016) Preventive effects of catechins on cardiovascular disease. Molecules 21. https://doi.org/10.3390/molecules21121759
119. Chiou YS, Huang Q, Ho CT, Wang YJ, Pan MH (2016) Directly interact with Keap1 and LPS is involved in the anti-inflammatory mechanisms of (−)-epicatechin-3-gallate in LPS-induced macrophages and endotoxemia. Free Radic Biol Med 94:1–16
120. Na HK, Surh YJ (2008) Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. Food Chem Toxicol 46:1271–1278

Bioactive Molecules, Nutraceuticals, and Functional Foods in Indian Vegetarian Diet and During Postpartum Healthcare

4

Jaya Arora and Kishan Gopal Ramawat

Contents

Abstract

Traditionally the majority population of the Indian subcontinent is vegetarian since ancient times due to social and religious guiding principle of the society. Consumption of whole grains of cereals and pulses, fruits, vegetables, and milk products is associated with vegetarian diet. Essentially all meals consist of flatbread, cooked pulses, a vegetable, curd/butter milk, and/or rice. Pulses are consumed in very large quantity, and several salted snacks and sweet preparations are available in the market. Beneficial effects of such products are now being revealed and scientifically validated by modern tools and techniques. In this review, we presented a concise and comprehensive scenario about vegetarian diet in Indian region enforced by caste system associated with religion. This leads to progression of hale and hearty brain and intellectual community of Brahmins consuming only vegetarian diet rich in fruits. Brahmin is a varna (class, caste) in Hinduism specializing as priests, teachers (acharya), and protectors of sacred learning across generations. Marriages within the caste further helped in gradual evolution of Brahmins (a kind of hybridization between superiors). Vegetarian diet is maintained from birth to until death. There is a well-defined and programmed vegetarian herbal diet starting from postpartum care,

J. Arora (✉) · K. G. Ramawat (✉)
Department of Botany, University College of Science, M. L. Sukhadia University, Udaipur, India

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_71

and beneficial effects of such diet are discussed in light of scientific information available. Effect of bioactive molecules present in these foods and safety aspect are also discussed.

Keywords
Vegetarian diet · Postpartum · Functional foods · Bioactive molecules · Caste system · Hindu · Brahmin diet

1 Introduction

Vegetarian diet refers to nonconsumption of meat or animal products including sea animals, fish, and eggs. However, in India milk and milk products are consumed by vegetarians. This tradition of vegetarian diet is part of Hindu philosophy, religion, and society where cruelty to animals is forbidden. Indians in general and majority of Hindus in particular are vegetarian since time immemorial. About 30–40% of the population of the Indian subcontinent is vegetarian, varying between 10% and 62% according to the region [1–4], whereas it is around 10% elsewhere in the world such as Sweden (10%), Austria (9%), Australia (2–11%), and Brazil (7.6%) [4]. Vegetarianism in Western countries is a luxury associated with higher socioeconomic position, greater physical activity, and lower rates of smoking which may result in the observed health benefits [2, 3], whereas in India, it is a tradition- and caste-based lifestyle since centuries. In contrast, vegetarianism is of recent origin in the Western world [5]. A well-planned vegetarian diet has all the essential components required for a healthy living [6].

Traditionally Indian foods can be divided into three categories: cooked vegetables, milk, and fresh fruits. Ayurveda means the science of life. It is an ancient system of healthcare and longevity. Ayurveda takes a holistic view of human beings, their health and illness. It aims at positive health, which has been defined as a well-balanced metabolism coupled with a healthy state of being. Ayurvedic concepts are thus deep rooted in day-to-day Indian culture, and hence food is considered as responsible for physical, mental, and temperamental state of an individual [7, 8].

Several recent studies show that vegetarian diet is associated with protective effect of diet on blood cholesterol [9–11], blood pressure [11–14], fasting blood sugar [15, 16], and cross-sectional evidences suggesting beneficial effects on blood insulin [17, 18], C-reactive protein [17–19], many amino acids [20], and insulin-like growth factors [17, 21]. Consumption of vegetarian or nonvegetarian diet results in different bowel microorganisms [22], which may have beneficial implications for diabetes, cardiovascular disease, and some cancers. Vegetarian diet has also been associated with low rate of mortality, new types of cardiovascular diseases [23–25], and diabetics [15]. Specific works showed reduction in incidence of prostate cancer [26] and colorectal cancers [27, 28], whereas increased incidences of cancer as well as diabetes and cardiovascular diseases were correlated with nonvegetarian diet [29–33]. These studies have shown an advantage for vegetarian diet over

nonvegetarian diet in disease prevention and long-term health. India is a vast country in which food, dialect, and dress change after every 500 km. Shridhar and co-workers [3] found significant cardiovascular health benefits associated with the vegetarian diet in four geographic regions of India. Traditionally food preparations and basic ingredients are more or less same but with different or variable spices and condiments and cooking medium [ghee (cleared butter) or any of the vegetable oils: mustard, ground nut, sesame, or coconut]. Being an old civilization, these cooking practices have been long established about processing of food, its preservation techniques, and their therapeutic effects. This diet has profound effect, as it contains body-healing chemicals, antioxidants, dietary fibers, and probiotics, on the development and social behavior of the population. The food is rich in functional components and provides prophylactic effect to various diseases such as diabetes, cardiovascular diseases related to high cholesterol, and obesity. These effects are further enhanced by processing the food as sprouting, malting, and fermentation [34]. In this chapter, we have reviewed the information about development of socioreligious faith to consume vegetarian diet in majority of the Indian population, and vegetarian diet is blended with herbal medicine during postpartum care for long-term benefit of the mother and the newborn. Available scientific information about these foods is also discussed.

2 Food Habit Influenced by Caste System

Composition of Indian food is influenced by Aryan belief. Aryans were heterogeneous group of people who lived mainly in northern ancient India. They migrated from Indo-Iranian borderlands about 3000–8000 years ago with their cultures and customs [35] and laid down caste system and its governing *Vedas* for society. The four *Vedas*, namely, *Rigveda*, *Samaveda*, *Yajurveda*, and *Atharva veda*, describe the use of different cereal grains and their use in our daily life. Aryans believed that food was not simply meant for body nourishment, but was the basic part of a cosmic moral cycle [36].

The present-day Indian population is descendents of two ancient groups known as "Ancestral North Indians" and "Ancestral South Indians" [37, 38]. The earliest archaeological evidence for agriculture in the northern region is 8000–9000 years old (Mehrgarh in present-day Pakistan) and involved wheat and barley derived from crops originally domesticated in West Asia [39, 40]. In contrast to this, agriculture in southern India is about 4600 years old, mainly cultivating mung bean, horse gram, and local millets (pearl millet, ragi, sorghum) which are not related to west Eurasian agriculture [41]. This is also supported by linguistic correlation of north Indian population speaking Hindi derived from Sanskrit (which is Indo European language), whereas Dravidian languages are not related to any of the Eurasian language [42]. There are 4635 anthropologically well-defined human populations in India; 532 are tribes, including 72 ancestral tribes with the hunter and gatherer lifestyle. Each population differs in terms of language, culture, physical features, and, most importantly, genetic architecture. The total population count of India has reached 1.21 billion [43, 44].

The exact date of origin of caste system is not clear but *Rigveda* contains written description of the system. Caste system was a social arrangement based on occupation. There were four broad categories of castes in a Hindu society, Brahmins, Kshatriya, Vaishya, and Sudra, having a specific job to perform: preaching and teaching, ruler or warrior, agriculture or business, and laborers, respectively. Brahmin is a varna (class, caste) in Hinduism specializing as priests, teachers, and protectors of sacred learning across generations. It is believed that caste system was associated with Indo-Aryan, who established themselves as superior caste and pushed the original Dravidian population to southern part and Sri Lanka. These conclusions are based on mitochondrial DNA and Y chromosome markers [45]. Brahmins and Vaishya were forbidden to eat nonvegetarian food and supposed to eat cereals, fruits, and vegetables by this social system. Now we know that high consumption of fruits and vegetables is associated with reduced risk of stroke and good health. Eating vegetarian diet rich in fruits and vegetable has/had a beneficial effect on brain functioning associated with arranged marriages within the caste resulting in a kind of forced breeding or hybridization between elites. This has a long-term consequential effect on the evolution of Brahmins as preachers and teachers of the society, whereas Kshatriya and Sudra were allowed to eat non-vegetarian diet including spiced food which results in high metabolism and muscle building required for their work [7, 46, 47]. Under this social system, vegetarian food is consumed during all rituals from child birth, in marriages to until death. Hindu rituals are not discussed here.

3 Indian Foods

Since Hippocrates, whose well-known dictum stated "Let food be thy medicine and medicine be thy food," diet has been key for health. It is imperative that food is not medicine for quick change, but long-term preventive measures are important for process of aging and health. Therefore, it is truly said that "you are what you eat" [48]. We have come to know day by day with new reports that bioactive molecules present in food have direct impact on our long-term health besides their nutritional value. It is better to consume what is produced in the region. Long-term consumption of a locally grown food has direct impact on metabolism and well-being of the population.

Whole grains or whole flour is staple food in the Indian subcontinent. Several food plants native to this subcontinent and/or cultivated since ancient times are wheat, rice, barley, cowpea, sugarcane, citrus, mango, onion, eggplant, pigeon pea, *Aegle*, and jujube [41]. There are several preparations of grains (wheat, rice, pulses, pearl millet, maize, pseudocereals), fermented grains/coarse grains (idli, dhokla, dosa, kanji), fruits and green vegetables, and milk products (milk, curd, butter milk, lassi, milk cake, and cottage cheese) [7, 49]. A typical meal consists of flatbread made of whole wheat grain flour, boiled and salted-spiced pulse, a cooked vegetable, and curd or butter milk. Boiled rice may or may not be included. Porridge prepared from a mixture of rice or wheat semolina and pulse is also a common practice. Pulses are consumed in large quantities in daily life as

whole, split, or ground into flour making several types of preparations as boiled and cooked and fried and salted and a variety of desserts. Some representative preparations shown here (Fig. 1) are (A) dal, (B) khaman dhokla, (C) sev, (D) kofta, (E) dahi vada, (F) sambar vada, (I) laddu, and (J) barfi. Besides this, frequent fasting or consumption only of pseudocereals and fruits is common practice in this region. There are convincing evidences that effect of whole extract of medicinal plants is more effective than isolated molecules. Similarly the disease protection offered by all grains in prospective epidemiological studies far exceeds the protection from isolated nutrients and phytochemicals in all grains [50, 51]. Therefore, the concept of functional foods includes foods or food ingredients that exert a beneficial effect on human health and/or reduce the risk of chronic disease beyond basic nutritional functions [52]. Generally, alcohol is not associated with vegetarian diet and forbidden for some caste by social and religious directives.

All grains and millets are the ideal base for a wholesome diet. This is because all variants of grains and millets are low-cost, nutritious, and locally available foodstuff. Moreover, all grains affect glucose and insulin responses, and on the other side, they have slower rate of digestion due to the presence of more dietary fiber, resistant starch, and oligosaccharides in grains [53]. All grains are rich in antioxidants; phenolic compounds; phytate; phytoestrogens such as lignan, plant stanols, and sterols; and vitamins and minerals. Epidemiological studies establish that all grains intake is protective against cancer, cardiovascular diseases, diabetes, and obesity [54]. Asian Indians may be benefitted from ancient whole-grain diet by decreasing the overall amount of dietary carbohydrate and increasing carbohydrate quality in food [55]. Nutrition plays a significant role in the prevention of disease such as diabetes (low glycemic index), cholesterol-lowering effects, ability to decrease the risk of heart diseases, and their protective effects against some cancers. Whole-grain cereals not only contain a large number of nutrients and bioactive compounds, but consumption of pulses provides complementary nutrients and a synergistic health benefit. Epidemiological evidence shows a consistent inverse association between whole-grain intake and the risk of chronic disease [56]. Among these bioactive compounds, γ-aminobutyric acid (GABA), a non-proteinogenic amino acid with several health benefits such as antidiabetic and hypotensive effects (a well-known blood pressure-lowering compound) and depression and anxiety reduction, is of particular interest [57].

India is the largest producer (26%), consumer (nearly one third), and importer of pulses in the world [58]. During 2014–2015, pulses were grown in an area of 25.23 million hectares with a total production of 17.38 million tons and consumption of 22.68 million tons. Pulses have high contents of protein (20–25%), carbohydrates (55–60%), calcium, and iron. Being vegetarian, pulses constitute the major source of protein in Indian diet. Before the Green Revolution of the 1960s in India, traditional meals and recipes were derived from whole-grain carbohydrates and also included amaranth, barley, millet, and other ancient grains that have been grown on the Indian subcontinent for the past few millennia. With more so-called Westernization, food habits are gradually changing particularly in urban populations with increased consumption of refined flour [59].

Fig. 1 Pulses are consumed in large quantities as whole, split, or ground into flour in daily life as boiled and cooked and fried and salted and prepared as various desserts. Some representative preparations are presented here: (**a**) dal, (**b**) khaman dhokla, (**c**) sev, (**d**) kofta, (**e**) dal, (**f**) mixture, (**g**) Dahi Vada, (**h**) Sambar Vada, (**i**) laddu, (**j**) barfi

4 Bioactive Molecules, Nutraceuticals, and Health Benefits

Food provides not only essential nutrients for growth and development but also bioactive molecules for prevention of diseases. Consumption of fruits, vegetables, cereals, and pulses is associated with prevention of several diseases including serious illnesses like cardiovascular disease and cancer. It was inferred that the additive and synergistic effects of phytochemicals present in fruit and vegetables were responsible for their potent antioxidant and anticancer activities and that the benefit of a diet rich in fruit and vegetables is attributed to the complex mixture of phytochemicals present in whole foods. This effect cannot be attained by providing purified bioactive molecules individually [50, 60, 61].

Nutraceutical, a word created by combining "nutrition" and "pharmaceutical," was coined in 1979 by Dr. Stephen L. DeFelice, founder and chairman of the Foundation for Innovation in Medicine, Cranford, New Jersey, USA [62]. It is a food or food product that provides health and medical benefits, including the prevention and treatment of disease. They are the product isolated or purified from foods and generally sold in medicinal forms not usually associated with food and demonstrated to have a physiological benefit or provide protection against chronic disease. Most of the Indian food preparation contains spices and condiments, and these have various bioactive molecules and exert antioxidant effect. Some of the most frequently used are turmeric, asafetida, saffron, cardamom, ginger, clove, fenugreek leaves and seeds, coriander leaves and seeds, mustard leaves and seeds, chillies, nutmeg, mace, cinnamon, nigella seeds, etc. These all are proven effective medicinal herbs and impart beneficial effects on health.

Indian food habits are in practice for at least the last two millennia and mainly comprised of cereals, pulses, fruits, vegetables, and milk [59]. More and more recent researches, particularly epidemiological studies, suggest a beneficial effect of such diet due to increased antioxidant potential of such foods resulting in reduced incidence of cancer, cardiovascular diseases, and stroke. This property is mainly attributed to fruits, and now we recommend eating five fruits a day or one apple a day keeps the doctor away. Slowly and slowly molecular mechanism and mode of action of bioactive molecules present in such foods are being unraveled. Health-promoting effects of isoflavonoids of pulses, antioxidants, and dietary fiber and various carbohydrates and bioactive lipids and consumption of fruits and vegetable are described in detail in this book; hence, details are not presented here.

Cereals (principally corn, wheat, and rice and sorghum and millets to a lesser extent) are staple food in many developing countries of the southern hemisphere with consumption of more than 400 million tons in 2017 [63]. Predominant Indian diet is rich in cereals, pulses, oils, and spices which are source of dietary fiber, vitamin E, carotenoids, and phenolic compounds. This diet exerts strong antioxidant activity (μg α-tocopherol equivalent/100 g fresh weight) and antioxidant activity of

some of the prominent Indian foods such as rice (80–102), whole wheat (220–500), spinach (750–890), amaranth (620–810), coriander leaves (610–750), and fenugreek leaves (520–690) [64]. Daily intake of about 40 g dietary fiber is recommended for an adult which varies in India due to socioeconomic conditions from 60 to 70 g depending upon the type of cereal consumed. Dietary fibers in major cereals and pulses consumed in India are rice (8.3), wheat (17.2), pearl millet (20.5), and mung (15.2), while the soluble dietary fiber content of foods is reported to vary widely: cereals, 8–20%; legumes, 20–50%; vegetables, 25–67%; and fruits, 20–65% [64, 65]. Cereals are not only rich in soluble and non-soluble carbohydrates but also contain vitamins, minerals, phenolics, carotenoids, tocopherols, tocotrienols, anthocyanins, and phytosterols and impart health beneficial effects like immunomodulatory effect; antioxidant activity; antiproliferative and hepatoprotective effects [66, 67]; protection against coronary heart diseases, type 2 diabetes, and cancer; and antiaging, antiallergic, and cholesterol-lowering abilities [68, 69].

Pulses (beans, peas, and lentils) have been consumed for at least 10,000 years and are among the most extensively used foods in the world. Pulses are main source of protein in a vegetarian diet and chickpea is consumed all over the globe. In Indian diet, chickpea (Bengal gram, *Cicer arietinum*) is consumed in large quantities not only in vegetable but in sweet and salted fried preparations, followed by mung bean (green gram, *Vigna radiata*), arhar (pigeon pea, *Cajanus cajan*), urad (black gram, *Vigna mungo*), lentil (*Lens culinaris*), and cowpeas (*Vigna unguiculata*). Pulses have several health-promoting components, including vegetable protein, complex carbohydrate, dietary fibers, vitamins, minerals, oligosaccharides, isoflavones, phospholipids, and antioxidants. Its regular consumption has been associated with protective effects against various diseases. Major bioactive phytoconstituents such as phytic acid, lectins, sterols, saponins, dietary fibers, resistant starch, oligosaccharides, and carotenoids and isoflavones have shown protective effects to various human diseases such as diabetes, obesity, cancer, osteoporosis, and cardiovascular diseases [70–73].

A balance between antioxidants and free radicals is necessary to maintain good physiological functioning of the body, and therefore, great attention has been given to understand the mechanism of free radical action and protective effect of antioxidants present in food. If free radicals overpower the body's ability to control them, a condition known as oxidative stress ensues [74]. Thus free radicals alter the metabolism of lipids, proteins, and DNA leading to deranged physiology or diseased state. Therefore, search for natural and safe antioxidants has been intensified in recent years. A significant role of oxidative stress has been correlated with several diseased conditions like atherosclerosis, arthritis, certain cancers, and process of aging. Oxidative stress is now thought to make a significant contribution to all inflammatory diseases (arthritis, vasculitis, glomerulonephritis, lupus erythematous, adult respiratory disease syndrome), ischemic diseases (heart diseases, stroke, intestinal ischemia), hemochromatosis, acquired immunodeficiency syndrome, emphysema, organ transplantation, gastric ulcers, hypertension and preeclampsia, neurological disorder (Alzheimer's disease, Parkinson's disease, muscular dystrophy), alcoholism, smoking-related diseases, and several others [75]. It is needless to mention that an excess of oxidative stress can result in the

oxidation of lipids and proteins, which always causes changes in their structure and consequently functions.

Fruit consumption is directly related to society's economic status and is variable in different countries. Anticancerous phenolics from *Phyllanthus*, tea polyphenols (epigallocatechin gallate) as antioxidant and anticancer, radioprotective litchi phenolics/flavonoids, hypoglycemic *Syzygium*, quercetin and hydroxyl cinnamates of sweet cherries, xanthones of mangosteen, ellagitannins of pomegranate, ursolic acid of sea buckthorn, muscle relaxative watermelon, anti-cholesterolemic soluble fiber and sterols, cardioprotective saponins, ACE-inhibitory potato hydrolysates, anti-pancreatic cancerous ascorbic acid, and carotenoids including pro-vitamin A are some of the well-known examples [76]. Selected tropical fruits and vegetables and their bioactive molecules and biological effects are presented in Tables 1 and 2. Some of them are grown since millennia, while some are improved recently from their wild relatives. The commonly consumed fruits are mango, banana, papaya, guava, custard apple, pomegranate, apple, sapota, orange, and watermelon. These fruits and vegetables contain diverse bioactive molecules with health benefits ranging from antioxidants, immunomodulators, antidiabetic, and hepatoprotective to anticancer. The other prominent food-based bioactives include tea polyphenols (epigallocatechin gallate) as antioxidant and anticancerous, β-carotene, lycopene and isoflavones for prostate cancer prevention, omega-3 and -6 fatty acids, licorice, quercetin, curcumin as anticancer (antiangiogenesis), curcumin (anti-inflammatory and anticancer).

In an extensive literature survey, Bhattacharya [59] observed that Indian food habit has gradually shifted from large quantities of indigestible fiber carbohydrate, small amounts of digestible carbohydrate, moderate fat, and moderate protein to an increasing intake of high-fiber and refined carbohydrates associated with a decreasing intake of animal proteins. Major shift in intake of high-fiber carbohydrate was observed from1775 to 1947. After 1947, slight change in food habit was observed with increased frequency of small quantities of refined carbohydrates and improvement in protein intake associated with socioeconomic conditions of the country. Therefore, Indian vegetarian food is not a single ingredient but a cocktail of many nutritional materials and herbs to impart nutraceutical effect on human health because of its long-term continuous consumption from childhood.

5 Vegetarian Diet in Postpartum Healthcare

Pregnancy is a unique situation in the life of to-be mother, and diet during this not only affects the health of the mother but also the newborn and has bearing on health throughout life. Similarly, nutrition and functional foods given during the postpartum care have long-term effect on growth, health, and metabolism of the mother and newborn. The postpartum period is commonly defined as beginning 1 h after delivery of the placenta and lasting 6 weeks [122]**.** The postpartum period is a time of increased physical, emotional, and social change for the parents of newborn. In many Southeast Asian cultures including India,

Table 1 Selected subtropical fruits, their bioactive molecules and biological activities

Plant species, name	Part used	Bioactive molecules	Biological activity	References
Aegle marmelos, bael		Coumarins such as marmelosin, umbelliferone, and scopoletin	Antihyperglycemic, chronic diarrhea, dysentery, peptic ulcers, as a laxative	[77, 78]
Artocarpus heterophyllus, jackfruit		Artocarpin, moracin C	Chemoprevention of colorectal cancer, antioxidant activity, anti-inflammatory activity	[79, 80]
Carica papaya, papaya		Papain, carotene	Antioxidant activity	[81, 82]
Citrus reticulata, orange		Ascorbic acid, citric acid, carotene	Antioxidant, antiallergic, improved lipid profile	[83]
Fragaria sp., strawberry		Flavonoids, anthocyanins, hydrolyzable tannins (gallotannins, ellagitannins), phenolic acids (hydroxycinnamic, hydroxybenzoic acids), condensed tannins, vitamin C, folate	Antioxidant, anti-inflammatory, antiatherosclerotic, anticarcinogenic, antidepressant properties	[84, 85]
Malus, apple		Chlorogenic acid, epicatechin, procyanidin B2, phloretin and quercetin, vitamin C, benzoic acids (gallic acid), hydroxycinnamic acids (chlorogenic acid), flavanols (catechin), flavonols (rutin), chalcones (phloridzin), terpenes (ursolic acid)	Antioxidants, antiproliferative, breast cancer treatment	[86, 87]
Mangifera sp., mango		Phenolic compounds, mangiferin, vitamins A and C, β-carotene and	Antioxidant activity, analgesic, antidiabetic, antisclerotic, antimicrobial,	[88–91]

(*continued*)

Table 1 (continued)

Plant species, name	Part used	Bioactive molecules	Biological activity	References
		xanthophylls, cis-9, cis-15-octadecadienoic acid	antiviral; cardio-, hepato-, and neuroprotective; anti-inflammatory; antiallergic; memory improving; radioprotective against X-ray, gamma, and UV radiation	
Musa sp., banana		Flavonoid fraction	Immunomodulatory activity	[92]
Psidium guajava, guava		Flavonoids, carotenoids, polyphenolic compounds, pentacyclic triterpenoids	Antidiarrheal activity, antioxidant activity, antidiabetic, antimicrobial activity	[93]
Phyllanthus emblica Indian gooseberry		Phenols, gallate, mucic acid 1-ethyl 6-methyl ester 2-*O*-gallate	Cardioprotective effect, antioxidant	[94, 95]
Punica granatum, pomegranate		Polyphenols, citric acid, ascorbic acid, ellagitannins, ellagic acid	Antiatherogenic, antioxidant, antihypertensive, anti-inflammatory, cardioprotective, anticancer	[96, 97]
Rubus sp., blackberry, raspberry, dewberry		Phenolics, tannins, stilbenes, flavonoids, anthocyanins; vitamin C, alpha-tocopherol; flavonol glycoside, apigenin, cyanidin-3-glucoside	Antioxidants, caspase-mediated apoptosis in human breast adenocarcinoma cells	[98, 99]
Syzygium cumini, jamun		Polyphenols: resveratrol, anthocyanins, ellagic acid/ ellagitannins	Antidiabetic, antioxidant, antihyperlipidemic, antiulcer, hepatoprotective, antiallergic, antiarthritic, antimicrobial, anti-inflammatory, antifertility, antiplaque,	[100, 101]

(*continued*)

Table 1 (continued)

Plant species, name	Part used	Bioactive molecules	Biological activity	References
			radioprotective, nephroprotective, antidiarrheal activities	
Ziziphus jujuba, jujube		Ascorbic acid; thiamine; riboflavin-bioflavonoids; pectin; mauritine A; amphibine H; jubanine A and B; mucronine D; nummularine B; sativanine E; ziziphines A to Q; betulinic acid; jujubosides A, B, A1, and B1; saponins	Aphrodisiac, hypnotic-sedative, anxiolytic, anticancer (melanoma cells), antifungal, antibacterial, antiulcer, anti-inflammatory, antifertility/ contraception, hypotensive, cardiotonic, antioxidant, immunostimulant, wound healing properties	[102, 103]

postpartum period is considered important from point of view of recovery, by offering a period of confinement ranging from 10 to 45 days. Many activity and dietary restrictions are applied during this period, and the main emphasis is given on proper nutrition.

The following traditions are followed:

1. Covering of head: By north Indian traditions, the mother is supposed to cover the head with scarf to prevent heat loss and catching cold.
2. Daily oil massage is given to the mother and newborn by experienced elderly lady or nurse to impart strength to the body. Sesame oil for the body and coconut oil for the head (or cleared butter for all purposes) are used which are absorbed quickly.
3. Hot/lukewarm water is used for bath.
4. Diet is fortified with cleared butter whether it is bread, mixed porridge, wheat porridge, or vegetables. Too much emphasis is given on consumption of milk, cleared butter, and high-calorie diet (sweet preparations) prepared with herbs.
5. Decoction of cumin, carom, and/or curcuma in milk is given during initial days in the morning.
6. Like headscarf, belly binding with a large cotton cloth is also practiced to facilitate repositioning of the uterus, perfectly position the baby during feeding, and put the stomach muscles back together.

Table 2 Some of the subtropical vegetables, their bioactive molecules and biological activities

Plant species, part used	Principal nutrient source	Bioactive molecules	Biological activity	References
Brassica oleracea, cauliflower, inflorescence		Lectins, essential oils, glucosinolates	Immunomodulatory, hepatoprotective, antioxidant, anticancer effects	[104, 105]
Capsicum annum, chilli, fruit		Capsaicin	Stimulation of gastrointestinal defense and absorption, stimulation of salivary, intestinal, hepatic, and pancreatic secretions	[106]
Chenopodium album, bathua, leaves		Vitamin A, oxalic acid	Anthelmintic, antimicrobial, antioxidant	[107]
Cyamopsis tetragonoloba, cluster bean, immature pods		Flavonoids, isoflavonoids (daidzein, genistein), luteolin, quercetin, kaempferol	Anticancer, antioxidant, antidiabetic, antiulcer, cytoprotective, anticholinergic, hypolipidemic	[108, 109]
Daucus carota, carrot, root		Polyacetylenes, carotenoids (beta-carotene and lutein)	Potential treatment for chronic lymphocytic leukemia	[110, 111]
Lagenaria siceraria, bottle gourd, fruit		Sterols, terpenoids, flavonoids, saponins	Controlling diabetes, hypertension, liver diseases, weight loss, and other associated benefits	[112]
Momordica charantia, bitter gourd, fruits		Cucurbitane-type triterpenoids, triterpene glycosides, phenolic acids, flavonoids, essential oils, saponins, momordicosides Q−U, charantin	Antidiabetic, immunomodulatory effect, antioxidant, anti-inflammatory, anticancer, antibacterial, anti-obesity activity	[113, 114]
Raphanus sativus, radish, root		Raphanusamide, cyanidin, vanillic acid, kaempferol, quercetin	Antiulcer, antimicrobial, antifungal, anti-inflammatory, diuretic, antioxidant, anticarcinogenic,	[115]

(*continued*)

Table 2 (continued)

Plant species, part used	Principal nutrient source	Bioactive molecules	Biological activity	References
			antitumor promotor, and anti-HIV activities	
Solanum lycopersicum, tomato, fruit		Polyphenols, β-carotene, lycopene, vitamin C, quercetin, rutin, hydroxycinnamic acid derivatives	Prevention of cancer and neurodegenerative and cardiovascular diseases, improving immunity	[116, 117]
Solanum melongena, brinjal, fruit		Flavonoids, tropane, glycoalkaloids, arginine, lanosterol, gramisterol, aspartic acid	Analgesic, antipyretic, antioxidant, anti-inflammatory, hypolipidemic, hypotensive, antiplatelet, intraocular pressure reducing	[118]
Spinacia oleracea, spinach, leaves		Ascorbic acid, folate, phylloquinone, alpha-tocopherol, and the carotenoids lutein, violaxanthin, zeaxanthin, and beta-carotene	Protective effect against metabolic syndrome, anti-obesity, anti-inflammatory, anticancer, hypoglycemic, and lipid-lowering effect	[119–121]

In Asia, the dietary choices of women during pregnancy and the postpartum period are principally influenced by traditional healthcare system and cultural beliefs whispered for these periods. It is believed that a "hot-cold balance" should be maintained in order to stay healthy and that this can be achieved by following special prescriptive diets during pregnancy and the initial postpartum period, commonly termed "the confinement period" [123, 124]. Several studies indicate that maternal diet during pregnancy and the postpartum period has not only important effects on the offspring's health but also the diet of a lactating mother which influences the composition of breast milk [125–127].

Vegetarian diet is considered safe for pregnant women and long-term health of the newborn [128]. The major population in the Indian subcontinent is vegetarian, and health of babies is not a problem in general except in cases of malnutrition. Traditionally postpartum care includes hygienic condition with feeding mother with several nutrients and functional foods as enumerated in Table 3. All these herbal plants are used in 50–200 g quality in several preparations for postpartum

Table 3 Bioactive molecules and biological activity of plants used in diet therapy during postpartum healthcare (compiled from [130–134])

Plant species (family), part	Bioactive molecules	Biological activity				
		Total polyphenols (mg GAE/g)	DPPH% inhibition	SOD% inhibition	FRAP (mM Fe^{+2}/g)	Others/remarks
Aegle marmelos (Rutaceae), fruit	*Furocoumarins*, flavonoids, *rutin*, and *marmesin*; a number of essential oils	95.33 ± 2.31	80.25 ± 1.03iu	54.94 ± 0.88	884 ± 5.13	Treatment of chronic diarrhea, dysentery, and peptic ulcers, as a laxative
Anethum graveolens (Apiaceae), fruit	Essential oils, coumarins, flavonoids, phenolic acids, and steroids	4.73 ± 0.23	75.53 ± 2.91	37.25 ± 1.18	62 ± 1.13	Carminative, stomachic, and diuretic
Anogeissus latifolia (Combretaceae), gum	High molecular weight polysaccharic acid (ghattic acid)	300.0 ± 4.55	86.94 ± 2.57	84.95 ± 1.62	975 ± 6.86	Reduction in serum total cholesterol
Areca catechu (Arecaceae), nut	Alkaloids of the pyridine group, ß-sitosterol, leucocyanidins, catechu (tannins, catechin, gallic acid)	393.33 ± 3.77	72.71 ± 0.91	43.23 ± 0.74	1003.33 ± 9.55	Diuretic, digestive, anthelmintic, astringent, and cardiotonic
Asparagus racemosus (Asparagaceae), root tuber	Steroidal saponins, essential oils, asparagine, arginine, tyrosine, flavonoids (kaempferol, quercetin, rutin)	4.13 ± 0.30	26.26 ± 0.71	32.44 ± 0.58	80 ± 1.26	Gastric ulcers and dyspepsia and as a galactogogue
Butea monosperma (Fabaceae), gum	Butrin, isobutrin, butin, palasitrin, and butein	426.66 ± 8.31	87.17 ± 1.39	80.53 ± 1.42	79.0 ± 1.26	Antimicrobial, wound healing, antifungal, antidiarrheal, hypoglycemic, hepatoprotective,

(*continued*)

Table 3 (continued)

Plant species (family), part	Bioactive molecules	Biological activity				
		Total polyphenols (mg GAE/g)	DPPH% inhibition	SOD% inhibition	FRAP (mM Fe^{+2}/g)	Others/remarks
						antioxidant, antihelminthic, anticonvulsive
Cinnamomum zeylanicum (Lauraceae), bark	(E)-Cinnamyl acetate, trans-alpha-bergamotene, and caryophyllene oxide	58.6 ± 0.29	74.31 ± 1.53	79.00 ± 2.53	222 ± 1.98	Benefit in respiratory, digestive, and gynecological ailments
Cocos nucifera (Arecaceae), endosperm	Phenols (catechins, epicatechins, tannins, and flavonoids), triterpenes, steroids	2.46 ± 0.12	3.14 ± 0.37	10.7 ± 0.96	03 ± 0.32	Antihelminthic, anti-inflammatory, antinociceptive, antioxidant, antifungal, antimicrobial, and antitumor activities
Curculigo orchioides (Amaryllidaceae), tuberous roots	Saponins, sapogenins, phenolic glycosides, a triterpene alcohol	11.06 ± 0.92	30.18 ± 0.71	46.32 ± 1.22	90 ± 2.64	Heating, aphrodisiac, alternative, appetizer
Curcuma amada (Zingiberaceae), rhizome	Curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin)	14.93 ± 1.06	84.88 ± 2.04	12.50 ± 0.54	74 ± 1.12	Antibacterial, insecticidal, antifungal, and antioxidant
Curcuma longa (Zingiberaceae), rhizome	Curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin)	16.66 ± 0.42	78.39 ± 1.23	24.05 ± 0.14	72 ± 1.76	Anti-inflammatory, anticancer, antibacterial, antiviral, antioxidant, cardioprotective, hepatoprotective

Desmodium gangeticum (Fabaceae), roots	Pterocarpans (gangetinin, gangetin, desmodin), isoflavones (desmocarpin, genistein)	13.53 ± 0.94	85.62 ± 1.32	25.48 ± 049	208.5 ± 3.56	Febrifuge, digestive, anti-catarrhal, antiemetic, hepatoprotective
Elettaria cardamomum (Zingiberaceae), seeds	1,8-Cineol	3.86 ± 0.12	62.33 ± 1.27	30.18 ± 0.80	02 ± 0.46	Antioxidant activity, anti-inflammatory, antimicrobial potency
Embelia ribes (Myrsinaceae), fruits	Embelin	12.0 ± 1.04	89.99 ± 1.78	23.18 ± 0.46	633 ± 3.60	Anthelmintic, carminative, and stimulant
Gmelina arborea (Lamiaceae), roots	Gmelo furan-a furanosesquiterpenoid, sesquiterpene, gmelinol, ceryl alcohol, hentriacontanol-1, β-sitosterol, *n*-octacosanol	10.26 ± 0.30	89.68 ± 1.53	44.43 ± 1.29	777 ± 2.52	Rejuvenative, aphrodisiac, memory enhancer
Litsea glutinosa (Lauraceae), bark	Steroids and terpenoids, butenolides, litsealactone, tannins, actinodaphnine, boldine, heteropolysaccharide polyuronide	70.0 ± 0.12	79.82 ± 0.84	82.15 ± 1.83	638 ± 7.63	Antioxidant activities, analgesic property, arrest bleeding, aphrodisiac
Mesua ferrea (Clusiaceae), flowers	Mesuol, mammeisin, mesuagin, mammeigin, anthraquinones, xanthones, mesuaferrins	66.66 ± 0.32	87.47 ± 0.87	46.77 ± 1.02	900 ± 3.45	Antioxidant, antineoplastic, antispasmodic, anticonvulsant, hepatoprotective, immunomodulatory

(*continued*)

Table 3 (continued)

Plant species (family), part	Bioactive molecules	Biological activity				
		Total polyphenols (mg GAE/g)	DPPH% inhibition	SOD% inhibition	FRAP (mM Fe^{+2}/g)	Others/remarks
Mucuna pruriens (Fabaceae), seeds	L-Dopa	38.66 ± 1.15	89.85 ± 1.76	17.99 ± 0.42	868 ± 4.04	Male infertility, nervous disorders, and also as an aphrodisiac, antioxidant activity
Myrica esculenta (Moringaceae), bark	Myricanol, aproanthocyanidin, β-sitosterol, taraxerol, myricadiol	45.33 ± 2.04	81.27 ± 3.26	75.39 ± 0.98	800 ± 3.02	Antimicrobial, wound healing, antifungal, hepatoprotective, antioxidant, antihelminthic
Myristica fragrans (Myristicaceae), seeds	Trimyristin, myristic acid, myristicin, safrole, and elimicin	12.0 ± 1.04	89.99 ± 1.78	23.18 ± 0.46	633 ± 3.60	Aromatic, aphrodisiac, diarrhea, anticonvulsant, analgesic, anti-inflammatory, antidiabetic, antifungal antibacterial, antioxidant
Myristica fragrans (Myristicaceae), aril of fruits	Lignin(macelignan), flavonoids, phenolics, tannins, alkaloids, and saponins	40.66 ± 1.15	88.96 ± 1.32	14.2 ± 0.38	588 ± 3.51	Inhibits the enzyme xanthine oxidase
Ocimum basilicum (Lamiaceae), leaves	Terpenoids, alkaloids, flavonoids, tannins, saponin glycosides	9.00 ± 0.72	63.68 ± 1.80	5.04 ± 0.14	08 ± 0.53	Hepatoprotective, antitoxic, immunomodulatory, antihyperglycemic, antifungal, hypolipidemic, anti-inflammatory, antibacterial

Piper cubeba (Piperaceae), fruits	Diarylcyclobutane neolignans, dibenzylbutyrolactone lignan, *O*-ethyl cubebins, monoacetate of dihydrocubebin	15.53 ± 1.85	87.47 ± 0.92	9.72 ± 0.96	485 ± 6.80	Cough, swelling, indigestion, dysmenorrhea, erectile dysfunction
Piper longum (Piperaceae), roots	Alkaloids, tannins, flavonoids	4.13 ± 0.23	45.57 ± 0.12	37.13 ± 0.98	50 ± 0.96	Antioxidant, antibacterial activity
Piper nigrum (Piperaceae), fruits	Piperine	5.13 ± 0.61	57.22 ± 1.05	31.88 ± 0.73	19 ± 0.40	Stimulate digestive enzymes in the pancreas, antioxidant
Quercus infectoria (Fagaceae), galls	Gallic acid, tannic acid	645.33 ± 10.2	90.20 ± 2.4	90.34 ± 0.35	1115 ± 9.29	Astringent, diarrhea, leucorrhea, uterine prolapse, tightening the loose or sagging tissues
Rubia cordifolia (Rubiaceae), roots	Anthraquinone (munjistin, purpurin, and pseudopurpurin), rubiadin	9.60 ± 0.87	71.98 ± 2.21	49.32 ± 0.59	75 ± 1.10	Blood detoxifying, anti-inflammatory, hemostatic, antidysenteric, antipyretic, analgesic, anthelmintic
Solanum surattense (Solanaceae), roots	Alkaloids, sterols, saponins, flavonoids	4.86 ± 0.65	84.41 ± 1.21	24.9 ± 062	520 ± 2.29	Antiasthmatic, hypoglycemic, hepatoprotective antibacterial
Smilax chinensis (Liliaceae), roots	Kaempferol and its glycosides, rutin, engeletin	9.26 ± 0.81	87.36 ± 1.54	55.05 ± 0.83	55 ± 1.62	General health tonic, syphilis, skin diseases and women's health concerns
Solanum indicum (Solanaceae), roots	Flavonoids, steroid, tannin, saponins	14.13 ± 0.80	72.68 ± 1.27	25.11 ± 0.70	733 ± 3.60	Antioxidant, anticancer, anti-inflammatory, diuretic, diaphoretic

(*continued*)

Table 3 (continued)

Plant species (family), part	Bioactive molecules	Biological activity				
		Total polyphenols (mg GAE/g)	DPPH% inhibition	SOD% inhibition	FRAP (mM Fe^{+2}/g)	Others/remarks
						immunomodulatory, neuroprotective effects
Symplocos racemosa (Symplocaceae), bark	Phenolic glycosides (symplocoside), triterpenoids (betulinic acid, acetyloleanolic acid, oleanolic acid) flavonoids (quercetin)	86.0 ± 0.05	75.29 ± 0.10	63.33 ± 1.54	884 ± 4.50	Anticancer, hepatoprotective, antioxidant, antiandrogenic effect, anti-inflammatory, wound healing activity, antidiabetic effects
Syzygium aromaticum (Myrtaceae), flower bud	Clove oil (eugenol, trans-β-caryophyllene)	101.33 ± 0.23	88.13 ± 0.58	55.05 ± 1.05	675.33 ± 5.36	Antibacterial, antifungal, insecticidal, antioxidant, anticarcinogenic activities
Terminalia chebula (Combretaceae), fruits	Tannins (gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulinic acid), chebulinic acid, ellagic acid, anthraquinones	166.0 ± 0.35	78.88 ± 0.85	84.66 ± 2.24	1009 ± 8.98	Antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antimutagenic, antiproliferative, radioprotective, cardioprotective, antiarthritic neuroprotective activities

Trachyspermum ammi (Apiaceae), seeds	Thymol, carvone, limonene, dillapiole	12.46 ± 0.98	88.39 ± 1.01	0.26 ± 0.02	955 ± 7.63	Stimulant, antispasmodic, and carminative
Tribulus terrestris (Zygophyllaceae), fruits	Flavonoids, flavonol glycosides, steroidal saponins, and alkaloids	10.2 ± 0.53	82.64 ± 0.98	1.10 ± 0.06	560 ± 2.80	Diuretic, aphrodisiac, antiurolithic, immunomodulatory, hypolipidemic, hepatoprotective, anti-inflammatory
Vitex negundo (Verbenaceae), seeds	Flavonoids, flavone glycosides, volatile oil, triterpenes, tannins, and lignin	5.46 ± 0.12	53.96 ± 0.96	4.25 ± 0.10	180 ± 1.26	Antinociceptive, anti-inflammatory, antitumor, antioxidant, antimicrobial, antiandrogenic, anti-osteoporotic, hepatoprotective, antihyperglycemic activities
Zingiber officinale (Zingiberaceae), rhizome	Flavonoids, alkaloids, saponins, steroids, terpenoids, tannin	11.4 ± 0.40	87.11 ± 0.61	33.78 ± 0.53	47 ± 0.65	Immunomodulatory, antitumorigenic, antioxidant, anti-inflammatory, antiapoptotic, antihyperglycemic, anti-lipidemic and antiemetic actions

period besides food fortified with fat and sugar to suppress bitter taste of herbs. Ginger is used in separate preparation as considered hot and lodh (*Symplocos racemosa*) in separate preparation being considered as cold. Hot and cold preparations are given separately, hot preferably first. All these preparations are divided into equal proportions to last 40–45 days. Increased milk drinking associated with galactogogue like *Asparagus racemosus* roots facilitates milk production. Bioactive molecules present in these herbal ingredients impart nutrition (high calories) and functional molecules which are helpful in developing immunological, antioxidative, and galactogogue effects. These plants contain a wide variety of free radical-scavenging molecules, such as phenolic compounds (e.g., phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some related endogenous compounds, which have high antioxidant activity [129]. Most of the herbs and foods given during postpartum care in India showed strong antioxidant activity [130]. Other prominent biological activities include stimulant, improves digestion, blood purifier, progesterone production, balance of hormonal cycle, emotional balance, tonic for nerves and stress and wound healing activities. Some of them are well-established medicinal plants used in Ayurveda such as fruits of *Aegle marmelos* and *Terminalia chebula*; rhizome of *Curcuma longa* and *Zingiber officinale*; roots of *Asparagus racemosus*, *Rubia cordifolia*, *Smilax chinensis*, and *Desmodium gangeticum*; and seeds of *Mucuna pruriens* and *Vitex negundo*. Details of their active constituents are given in Table 3. Dried ginger is traditionally used in several food preparations including tea as well as major ingredient in postpartum food preparation. Ginger is known to impart antioxidant, antimicrobial, and anti-inflammatory effects, and modern tools of molecular targets validated these effects [131]. In all 38 plants are described in Table 3. These plants contain very diverse chemicals and their effects as an individual is neither known completely nor is possible to describe in this chapter. It is the combined effect of these herbs that is more important, and we know that such formulation imparts synergistic effect on human health.

Diet therapy includes preparations of medicinal herbs in the form of decoctions, infusions, and cold extracts. The herbs given during this period are traditionally known to strengthen the body and mind and prevent disorders such as postpartum depression (PPD), body aches, insomnia, indigestion, and oxidative stress [135].

Several reports describe the effect of maternal diet particularly rich in high fat on composition of human milk. Bioactive lipids in milk include triacylglycerides, diacylglycerides, saturated and polyunsaturated fatty acids, and phospholipids. Beneficial activities of milk lipids include anticancer, antimicrobial, anti-inflammatory, and immunosuppression properties. Fats are good media for solubility of many organic compounds and facilitate their absorption [136, 137]. Maternal diet has been shown to influence fatty acid composition of breast milk, with changes appearing within 8–10 h after a meal is consumed [138–140]. Fatty acids with a chain length greater than C14:0 originate from the maternal diet or body stores, while fatty acids up to C14:0 originate from de novo synthesis in the breast [138, 141].

The term conjugated linoleic acid (CLA) describes a mixture of positional and geometric isomers of linoleic acid (C18:2n-6) which contain a conjugated double-bond system instead of the more common isolated double bonds. Rumenic or cis-9, trans-11-octadecadienoic acid (cis9,trans11-C18:2) is the most regular CLA isomer and is frequently regarded as the biologically most pertinent one [142]. Humans receive CLA through diet (dairy products and ruminant meat), and it is presumed that the amount of CLA in the milk of breastfeeding women can be augmented by increasing the amount of organic dairy nutrients within their diet [143]. CLA content and both rumenic acid and TVA in human breast milk were increased in the milk of lactating mother consuming diet rich in organic products as compared to control (mothers) eating the same products obtained through conventional methods [144, 145].

Alternative to household preparations, several Ayurvedic formulations are also available for postpartum care such as shatavari kalpa granules (*A. racemosus*) for lactation and dashmool (ten roots, the combination of ten roots is used widely in Ayurveda for many health conditions related to anti-inflammatory, antirheumatic or antiarthritic, analgesic, antispasmodic, adaptogenic, antioxidant, neuroprotective, anti-paralytic, uterine tonic, uterine detoxifier). Out of these ten roots, five (*Aegle marmelos*, *Desmodium gangeticum*, *Gmelina arborea*, *Solanum surrentense*, *Tribulus terrestris*) are used in the household preparations. Besides, there are several herbal preparations that are also available for the newborn mainly for digestive system correction, gas, and flatulence.

6 Conclusions

It is increasingly clear that the regular consumption of traditional food has a long-lasting beneficial effect rather than changed diet regimen. Vegetarian diet has at least no harmful effect. Pulses are traditionally consumed all over India, and now we have new evidence that beneficial bioactive peptides are produced during processing and cooking; hence, the effect is nutraceutical besides nutritional. Similarly, coconut oil is consumed traditionally in almost all food preparations in Kerala state of India, while north Indian consumed sesame or mustard oil. Slight variation in food habits are due to what is produced in that area is preferably consumed in the area, irrespective of the general belief. There is no record of higher disease incidence in Kerala because of the consumption of saturated fat in high amount. Beneficial effects of mustard and sesame oil are being established now, whereas these are used since centuries and soybean or sunflower oil is of recent introduction.

In this review, we have tried to portrait the scenario prevailing in a country like India in relation to vegetarianism and postpartum care influencing the whole life of a newborn. In India, vegetarianism is centuries old and strictly followed by caste system and practiced by religion in well-to-do families. In contrast to this, those who are below poverty line families, they do not follow strict diet rules by caste and religion, and they have their own tribal rules or no rules, hence are not strictly vegetarian. Several herbal

ingredients used during postpartum care need to be evaluated scientifically using modern pharmacological techniques to ascertain their effects.

This will also provide information about necessary inputs required for better health of the mother and child. Recent studies demonstrated health beneficial effects of these centuries-old herbal preparations, and more insight into mode of action and mechanism of action using modern pharmacological tools will shed light on beneficial effects of these herbs and foods.

References

1. Agrawal S, Millett CJ, Dhillon PK et al (2014) Type of vegetarian diet, obesity and diabetes in adult Indian population. Nutr J 13:89
2. Appleby PN, Key TJ (2016) The long-term health of vegetarians and vegans. Proc Nutr Soc 75(3):287–293
3. Shridhar K, Dhillon PK, Bowen L, Kinra S, Bharathi AV et al (2014) The association between a vegetarian diet and cardiovascular disease (CVD) risk factors in India: the Indian Migration Study. PLoS One 9(10):e110586
4. Anonymous (2017) Vegetarianism by country. https://en.wikipedia.org/wiki/Vegetarianism_by_country. Accessed 13 June 2017
5. Fraser GE (2016) The vegetarian advantage: its potential for the health of our planet, our livestock, and our neighbors! Forsch Komplementmed 23:66–68
6. Craig WJ, Mangels AR (2009) Position of the American Dietetic Association: vegetarian diets. J Am Diet Assoc 109:1266–1282
7. Chauhan A, Semwal DK, Mishra SP, Semwal RB (2015) Ayurvedic research and methodology: present status and future strategies. Ayu 36(4):364
8. Ramawat KG, Dass S, Mathur M (2009) The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: Ramawat KG (ed) Herbal drugs: ethnomedicine to modern medicine. Springer, Berlin/Heidelberg
9. West RO, Hayes OB (1968) Diet and serum cholesterol levels: a comparison between vegetarians and non-vegetarians in a Seventh–Day Adventist group. Am J Clin Nutr 21:853–862
10. Appleby PN, Thorogood M, McPherson K, Mann JL (1995) Associations between plasma lipid concentrations and dietary, lifestyle and physical factors in the Oxford Vegetarian Study. J Hum Nutr Diet 8:305–314
11. Fraser G, Katuli S, Anousheh R, Knutsen S, Herring P, Fan J (2014) Vegetarian diets and cardiovascular risk factors in black members of the Adventist Health Study-2. Public Health Nutr 17:1–9
12. Rouse IL, Beilin LJ, Armstrong BK, Vandongen R (1983) Blood pressure lowering effect of a vegetarian diet: controlled trial in normotensive subjects. Lancet 1:5–10
13. Margetts BM, Beilin LJ, Vandongen R, Armstrong BK (1986) Vegetarian diet in mild hypertension: a randomized controlled trial. Br Med J 293:1468–1471
14. Pettersen BJ, Anousheh R, Fan J, Jaceldo-Siegl K, Fraser GE (2012) Vegetarian diets and blood pressure among white subjects: results from the Adventist Health Study – 2 (AHS-2). Public Health Nutr 15:1909–1916
15. Tonstad W, Stewart K, Oda K, Batech M, Herring RP, Fraser GE (2013) Vegetarian diets and incidence of diabetes in Adventist Health Study-2. Nutr Metab Cardiovasc Dis 23:292–299
16. Rosell M, Appleby P, Spencer E, Key T (2006) Weight gain over 5 years in 21,966 meat-eating, fish-eating, vegetarian, and vegan men and women in EPIC-Oxford. Int J Obes (Lond) 30:1389–1396

17. Jaceldo-Siegl K, Fan J, Haddad E, Knutsen S, Bellinger D, Fraser G (2013) Vegetarian dietary patterns associated with biomarkers of cancer risk (Abstract). Am J Epidemiol 177(Suppl 11):S110
18. Kahleova H, Matoulek M, Malinska H et al (2011) Vegetarian diet improves insulin resistance and oxidative stress markers more than conventional diet in subjects with type 2 diabetes. Diabet Med 28:549–559
19. Paalani M, Lee JW, Haddad E, Tonstad S (2011) Determinants of inflammatory markers in a bi-ethnic population. Ethn Dis 21:142–149
20. Schmidt JA, Rinaldi S, Scalbert A et al (2016) Plasma concentrations and intakes of amino acids in male meat eaters, fish-eaters, vegetarians and vegans: a cross-sectional analysis in the EPIC-Oxford cohort. Eur J Clin Nutr 70(3):306–312. https://doi.org/10.1038/ejcn.2015.144
21. Allen NE, Appleby PN, Davey GK, Kaaks R, Rinaldi S, Key TJ (2002) The associations of diet with serum insulin-like growth factor I and its main binding proteins in 292 women meat-eaters, vegetarians, and vegans. Cancer Epidemiol Biomarkers Prev 11:1441–1448
22. Wong JM (2014) Gut microbiota and cardiometabolic outcomes: influence of dietary patterns and their associated components. Am J Clin Nutr 100(Suppl 1):369S–377S
23. Key TJ, Fraser GE, Thorogood M et al (1999) Mortality in vegetarians and nonvegetarians: detailed findings from a collaborative analysis of 5 prospective studies. Am J Clin Nutr 70(Suppl 3):516S–524S
24. Crowe FL, Appleby PN, Travis RC, Key TJ (2013) Risk of hospitalization or death from ischemic heart disease among British vegetarians and nonvegetarians: results from the EPIC-Oxford cohort study. Am J Clin Nutr 97:597–603
25. Fraser GE (2005) A comparison of first event CHD rates in two contrasting California populations. J Nutr Health Aging 9:53–58
26. Tantamango-Bartley Y, Knutsen SF, Knutsen R et al (2016) Are strict vegetarians protected against prostate cancer? Am J Clin Nutr 103:153–160
27. Orlich MJ, Singh PN, Sabaté J et al (2015) Vegetarian dietary patterns and the risk of colorectal cancers. JAMA Intern Med 175:767–776
28. Yokoyama Y, Nishimura K, Barnard ND, Takegami M, Watanabe M, Sekikawa A et al (2014) Vegetarian diets and blood pressure: a metaanalysis. JAMA Intern Med 174:577–587
29. Bernstein AM, Sun Q, Hu FB, Stampfer MJ, Manson JE, Willett WC (2010) Major dietary protein sources and risk of coronary heart disease in women. Circulation 122:876–883
30. Kaluza J, Åkesson A, Wolk A (2015) Long-term processed and unprocessed red meat consumption and risk of heart failure: a prospective cohort study of women. Int J Cardiol 193:42–46
31. Fretts AM, Follis JL, Nettleton JA et al (2015) Consumption of meat is associated with higher fasting glucose and insulin concentrations regardless of glucose and insulin genetic risk scores: a meta-analysis of 50,345 Caucasians. Am J Clin Nutr 102:1266–1278
32. Pan A, Sun Q, Bernstein AM et al (2013) Changes in red meat consumption and subsequent risk of type 2 diabetes mellitus: three cohorts of US men and women. JAMA Intern Med 173:1328–1335
33. Lippi G, Mattiuzzi C, Cervellin G (2016) Meat consumption and cancer risk: a critical review of published metaanalyses. Crit Rev Oncol Hematol 97:1–14
34. Hotz C, Gibson RS (2007) Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. J Nutr 137:1097e100
35. Reich D, Thangaraj K, Patterson N, Price AL, Singh L (2009) Reconstructing Indian population history. Nature 461:489–495
36. Moorjani P, Thangaraj K, Patterson N, Lipson M et al (2013) Genetic evidence for recent population mixture in India. Am J Hum Genet 93:422–438
37. Sarkar P, Dhumal C, Panigrahi SS, Choudhary R (2015) Traditional and Ayurvedic foods of Indian origin. J Ethn Foods 2(3):97–109
38. Achaya KT (1994) Indian food: a historical companion. Oxford University Press, Delhi
39. Renfrew C (1990) Archaeology and language: the puzzle of Indo-European origins. Cambridge University Press, New York
40. Costantini L (1984) The beginning of agriculture in the Kachi Plain: the evidence of Mehrgarh. In: Allchin B (ed) South Asian archaeology 1981. Cambridge University Press, Cambridge

41. Fuller DQ (2011) Finding plant domestication in the Indian subcontinent. Curr Anthropol 52(S4):S347–S362
42. Witzel M (1999) Substrate languages in Old Indo-Aryan (Rigvedic, Middle and Late Vedic). Electron J Vedic Stud 5:1–67
43. Census of India (2001) http://censusindia.gov.in/. Accessed 12 Oct 2017
44. Census of India (2011) http://censusindia.gov.in/. Accessed 12 Oct 2017
45. Tamang R, Thangaraj K (2012) Genomic view on the peopling of India. Investig Genet 3:20
46. Mirmiran P, Noori N, Beheshti M, Azizi ZF (2009) Fruit and vegetable consumption and risk factors for cardiovascular disease. Metabolism 58(4):460–468
47. He FJ, Nowson CA, MacGregor GA (2006) Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. Lancet 367(9507):320–326
48. Brüssow H, Parkinson SJ (2014) You are what you eat. Nat Biotechnol 32:243–245
49. Sen CT (2004) Food culture in India. Greenwood Publishing Group, Santa Barbara
50. Phan MA, Paterson J, Bucknall M, Arcot J (2016) Interactions between phytochemicals from fruits and vegetables: effects on bioactivities and bioavailability. Crit Rev Food Sci Nutr 17:1–20
51. Slavin J (2004) Whole grains and human health. Nutr Res Rev 17(1):99–110
52. Huggett AC, Schliter B (1996) Research needs for establishing the safety of functional foods. Nutr Rev 54:S143–S148
53. Hallifrisch A, Hall J (2000) Textbook of medical physiology. Saunders, Philadelphia
54. Teradal D, Joshi N, Aladakatti RH (2017) Therapeutic evaluation of grain based functional food formulation in a geriatric animal model. J Food Sci Technol 54(9):2789–2796
55. Dixit AA, Azar KMJ, Gardener CD, Palaippan NP (2011) Incorporation of whole grain, ancient grains in to a modern Asian Indian diet to reduce the burden of chronic disease. Nutr Rev 69(8):479–488
56. Rebello CJ, Greenway FL, Finley JW (2014) Whole grains and pulses: a comparison of the nutritional and health benefits. J Agric Food Chem 62(29):7029–7049
57. Nikmaram N, Dar B, Roohinejad S, Koubaa M, Barba FJ, Greiner R, Johnson SK (2017) Recent advances in γ-aminobutyric acid (GABA) properties in pulses: an overview. J Sci Food Agric 97:2681–2689
58. FAO (Food and Agriculture Organization) (2014) Statistics division. Rome. Accessed 25 July 2017
59. Bhattacharya M (2015) A historical exploration of Indian diets and a possible link to insulin resistance syndrome. Appetite 95:421–454
60. Liu RH (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 78(Suppl):517S–520S
61. Liu RH (2013) Health-promoting components of fruits and vegetables in the diet. Adv Nutr 4:384S–392S
62. Kalra EK (2003) Nutraceutical: definition and introduction. AAPS PharmSci 5(3):1–2
63. Food and Agriculture Organization of the United Nations (2017) http://www.fao.org/worldfoodsituation/csdb/en/. Accessed 25 July 2017
64. Rao NBS (2003) Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. Asia Pac J Clin Nutr 12(1):9–22
65. Birch GG, Parker KJ (1982) Dietary fibre. Applied Science Publications, London
66. Arshad MS, Kwon JH, Anjum FM, Sohaib M et al (2017) Wheat antioxidants, their role in bakery industry, and health perspective. In: Wanyera R, Owuoche J (eds) Wheat improvement, management and utilization. InTech, London, UK. https://doi.org/10.5772/67276
67. Nepali S, Ki HH, Lee JH, Lee HY, Kim DK, Lee YM (2017) Wheatgrass-derived polysaccharide has antiinflammatory, anti-oxidative and anti-apoptotic effects on LPS-induced hepatic injury in mice. Phytother Res 31(7):1107–1116
68. Idehen E, Tang Y, Sang S (2017) Bioactive phytochemicals in barley. J Food Drug Anal 25(1):148–161

69. Gangopadhyay N, Hossain MB, Rai DK, Brunton NP (2017) A review of extraction and analysis of bioactives in oat and barley and scope for use of novel food processing technologies. Molecules 20(6):10884–10909
70. Gupta RK, Gupta K, Sharma A, Das M, Ansari IA, Dwivedi PD (2017) Health risks and benefits of chickpea (*Cicer arietinum*) consumption. J Agric Food Chem 65(1):6–22
71. Tang D, Dong Y, Ren H, Li L, He C (2014) A review of phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts (*Vigna radiata*). Chem Cent J 8:4
72. Wahby MM, Mohammed DS, Newairy AA, Abdou HM, Zaky A (2017) Aluminum-induced molecular neurodegeneration: the protective role of genistein and chickpea extract. Food Chem Toxicol 107(Pt A):57–67
73. Boers HM, MacAulay K, Murray P, Dobriyal R, Mela DJ, Spreeuwenberg MA (2017) Efficacy of fibre additions to flatbread flour mixes for reducing post-meal glucose and insulin responses in healthy Indian subjects. Br J Nutr 117(3):386–394
74. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 4(8):118–126
75. Stefanis L, Burke RE, Greene LA (1997) Apoptosis in neurodegenerative disorders. Curr Opin Neurol 10:299–305
76. Shashirekha MN, Mallikarjuna SE, Rajarathnam S (2015) Status of bioactive compounds in foods, with focus on fruits and vegetables. Crit Rev Food Sci Nutr 55(10):1324–1339
77. Ansari P, Afroz N, Jalil S, Azad SB, Mustakim MG, Anwar S, Haque SM, Hossain SM, Tony RR, Hannan JM (2017) Anti-hyperglycemic activity of *Aegle marmelos* (L.) corr. is partly mediated by increased insulin secretion, α-amylase inhibition, and retardation of glucose absorption. J Pediatr Endocrinol Metab 30(1):37–47
78. Shinde PB, Katekhaye SD, Mulik MB, Laddha KS (2014) Rapid simultaneous determination of marmelosin, umbelliferone and scopoletin from *Aegle marmelos* fruit by RP-HPLC. J Food Sci Technol 51(9):2251–2255
79. Sun G, Zheng Z, Lee MH, Xu Y, Kang S et al (2017) Chemoprevention of colorectal cancer by artocarpin, a dietary phytochemical from *Artocarpus heterophyllus*. J Agric Food Chem 65(17):3474–3480
80. Yao X, Wu D, Dong N, Ouyang P, Pu J et al (2016) Moracin C, A phenolic compound isolated from *Artocarpus heterophyllus*, suppresses lipopolysaccharide-activated inflammatory responses in murine raw264.7 macrophages. Int J Mol Sci 17(8):1199
81. Krishna KL, Paridhavi M, Patel JA (2008) Review on nutritional, medicinal and pharmacological properties of Papaya (*Carica papaya* Linn.). Nat Prod Radiance 7(4):364–373
82. Somanah J, Bourdon E, Bahorun T (2017) Extracts of Mauritian *Carica papaya* (var. solo) protects SW872 and HepG2 cells against hydrogen peroxide induced oxidative stress. J Food Sci Technol 54(7):1917–1927
83. Aptekmann NP, Cesar TB (2010) Orange juice improved lipid profile and blood lactate of overweight middle-aged women subjected to aerobic training. Maturitas 67(4):343–347
84. Giampieri F, Forbes-Hernandez TY, Gasparrini M et al (2017) The healthy effects of strawberry bioactive compounds on molecular pathways related to chronic diseases. Ann N Y Acad Sci 1398(1):62–71. https://doi.org/10.1111/nyas.13373
85. Naz S, Farooq U, Khan A et al (2017) Antidepressent effect of two new benzyl derivatives from wild strawberry *Fragaria vesca* var. *nubicola* Lindl. ex Hook.f. Front Pharmacol 8:469
86. González-Aguilar G, Robles-Sánchez RM, Martínez-Téllez MA, Olivas GI, Alvarez-Parrilla E, de la Rosa LA (2008) Bioactive compounds in fruits: health benefits and effect of storage conditions. Stewart Postharvest Rev 3:8
87. Yoon H, Liu RH (2007) Effect of selected phytochemicals and apple extracts on NF-κB activation in human breast cancer MCF-7 cells. J Agric Food Chem 55:3167–3317
88. Khurana RK, Kaur R, Lohan S, Singh KK, Singh B (2016) Mangiferin: a promising anticancer bioactive. Pharm Pat Anal 5(3):169–181

89. López-Cobo A, Verardo V, Diaz-de-Cerio E, Segura-Carretero A, Fernández-Gutiérrez A, Gómez-Caravaca AM (2017) Use of HPLC- and GC-QTOF to determine hydrophilic and lipophilic phenols in mango fruit (*Mangifera indica* L.) and its by-products. Food Res Int 100(Pt 3):423–434
90. Matkowski A, Kuś P, Góralska E, Woźniak D (2013) Mangiferin – a bioactive xanthonoid, not only from mango and not just antioxidant. Mini Rev Med Chem 13(3):439–455
91. Shah KA, Patel MB, Patel RJ, Parmar PK (2010) *Mangifera indica* (Mango). Pharmacogn Rev 4(7):42–48
92. Alese MO, Adewole SO, Akinwunmi KF, Omonisi AE, Alese OO (2017) Aspirin-induced gastric lesions alters EGFR and PECAM-1 immunoreactivity in wistar rats: modulatory action of flavonoid fraction of *Musa paradisiaca*. Maced J Med Sci 5(5):569–577
93. Mittal P, Gupta V, Kaur G, Garg AK, Singh A (2010) Phytochemistry and pharmacological activities of *Psidium guajava*: a review. Int J Pharm Sci Res 1(9):9–19
94. Kumar V, Aneesh KA, Kshemada K et al (2017) Amalaki rasayana, a traditional Indian drug enhances cardiac mitochondrial and contractile functions and improves cardiac function in rats with hypertrophy. Sci Rep 7(1):8588
95. Zhang J, Miao D, Zhu WF et al (2017) Biological activities of phenolics from the fruits of *Phyllanthus emblica* Linn. (Euphorbiaceae). Chem Biodivers. https://doi.org/10.1002/cbdv.201700404
96. Gumienna M, Szwengiel A, Górna B (2016) Bioactive components of pomegranate fruit and their transformation by fermentation processes. Eur Food Res Technol 242:631–640
97. Sahebkar A, Ferri C, Giorgini P, Bo S, Nachtigal P, Grassi D (2016) Effects of pomegranate juice on blood pressure: a systematic review and meta-analysis of randomized controlled trials. Pharmacol Res 115:149–161
98. George BP, Abrahamse H, Hemmaragala NM (2017) Phenolics from *Rubus fairholmianus* induces cytotoxicity and apoptosis in human breast adenocarcinoma cells. Chem Biol Interact S0009-2797(17):30392–30397
99. Figueiras Abdala A, Mendoza N, Valadez Bustos N, Escamilla Silva EM (2017) Antioxidant capacity analysis of blackberry extracts with different phytochemical compositions and optimization of their ultrasound assisted extraction. Plant Foods Hum Nutr. https://doi.org/10.1007/s11130-017-0616-3
100. Shrikanta A, Kumar A, Govindaswamy V (2015) Resveratrol content and antioxidant properties of underutilized fruits. J Food Sci Technol 52(1):383–390
101. Srivastava S, Chandra D (2013) Pharmacological potentials of *Syzygium cumini*: a review. J Sci Food Agric 93(9):2084–2093
102. Tahergorabi Z, Abedini MR, Mitra M, Fard MH, Beydokhti H (2015) "Ziziphus jujuba": a red fruit with promising anticancer activities. Pharmacogn Rev 9(18):99–106
103. Gao QH, Wu CS, Wang M (2013) The jujube (Ziziphus jujuba Mill.) fruit: a review of current knowledge of fruit composition and health benefits. J Agric Food Chem 61(14):3351–3363
104. Duarte CEM, Abranches MV, Silva PF, de Paula SO, Cardoso SA, Oliveira LL (2017) A new TRAF-like protein from *B. oleracea* ssp. botrytis with lectin activity and its effect on macrophages. Int J Biol Macromol 94(Pt A):508–514
105. Morales-López J, Centeno-Álvarez M, Nieto-Camacho A, López MG, Pérez-Hernández E, Pérez-Hernández N, Fernández-Martínez E (2017) Evaluation of antioxidant and hepatoprotective effects of white cabbage essential oil. Pharm Biol 55(1):233–241
106. Maji AK, Banerji P (2016) Phytochemistry and gastrointestinal benefits of the medicinal spice, *Capsicum annuum* L. (Chilli): a review. J Complement Integr Med 13(2):97–122
107. Lone BA, Chishti MZ, Bhat FA, Tak H, Bandh SA, Khan A (2017) Evaluation of anthelmintic antimicrobial and antioxidant activity of *Chenopodium album*. Trop Anim Health Prod. https://doi.org/10.1007/s11250-017-1364-y
108. Morris JB, Wang ML (2017) Functional vegetable guar (*Cyamopsis tetragonoloba* L. Taub.) accessions for improving flavonoid concentrations in immature pods. J Diet Suppl 14(2):146–157

109. Sharma P, Dubey G, Kaushik S (2011) Chemical and medico-biological profile of *Cyamopsis tetragonoloba* (L) Taub: an overview. J Appl Pharma Sci 1:32–37
110. Shakib MC, Gabrial SG, Gabrial GN (2015) Beetroot-carrot juice intake either alone or in combination with antileukemic drug 'chlorambucil' as a potential treatment for chronic lymphocytic leukemia. Maced J Med Sci 3(2):331–336
111. Zaini RG, Brandt K, Clench MR, Le Maitre CL (2012) Effects of bioactive compounds from carrots (*Daucus carota* L.), polyacetylenes, beta-carotene and lutein on human lymphoid leukaemia cells. Anticancer Agents Med Chem 12(6):640–652
112. Prajapati RP, Kalariya M, Parmar SK, Sheth NR (2010) Phytochemical and pharmacological review of Lagenaria sicereria. J Ayurveda Integr Med 1(4):266–272
113. Fachinan R, Fagninou A, Nekoua MP et al (2017) Evidence of immunosuppressive and Th2 immune polarizing effects of antidiabetic *Momordica charantia* fruit juice. Biomed Res Int. https://doi.org/10.1155/2017/9478048
114. Dandawate PR, Subramaniam D, Padhye SB, Anant S (2016) Bitter melon: a panacea for inflammation and cancer. Chin J Nat Med 14(2):81–100
115. Gutierrez RM, Perez RL (2004) Raphanus sativus (Radish): their chemistry and biology. Sci World J 4:811–837
116. Capel C, Yuste-Lisbona FJ, López-Casado G (2017) QTL mapping of fruit mineral contents provides new chances for molecular breeding of tomato nutritional traits. Theor Appl Genet 130(5):903–913
117. Gerszberg A, Hnatuszko-Konka K, Kowalczyk T, Kononowicz AK (2015) Tomato (*Solanum lycopersicum* L.) in the service of biotechnology. Plant Cell Tissue Organ Cult 120(3):881–902
118. Das M, Barua N (2013) Pharmacological activities of *Solanum melongena* linn. (brinjal plant). Int J Green Pharm 7(4):274–277
119. Lester GE, Makus DJ, Hodges DM (2010) Relationship between fresh-packaged spinach leaves exposed to continuous light or dark and bioactive contents: effects of cultivar, leaf size, and storage duration. J Agric Food Chem 58(5):2980–2987
120. Panda V, Mistry K, Sudhamani S, Nandave M, Ojha SK (2017) Amelioration of abnormalities associated with the metabolic syndrome by *Spinacia oleracea* (Spinach) consumption and aerobic exercise in rats. Oxid Med Cell Longev. https://doi.org/10.1155/2017/2359389
121. Roberts JL, Moreau R (2016) Functional properties of spinach (*Spinacia oleracea* L.) phytochemicals and bioactives. Food Funct 7(8):3337–3353
122. Blenning CE, Paladine H (2005) An approach to the postpartum office visit. Am Fam Physician 72:2491–2498
123. Chen LW, Low YL, Fok D et al (2013) Dietary changes during pregnancy and the postpartum period in Singaporean Chinese, Malay and Indian women: the GUSTO birth cohort study. Public Health Nutr 17(9):1930–1938
124. Choudhry UK (1996) Traditional practices of women from India pregnancy childbirth, and newborn care. J Obstet Gynecol Neonatal Nurs 26:533–539
125. Abu-Saad K, Fraser D (2010) Maternal nutrition and birth outcomes. Epidemiol Rev 32:5–25
126. Hayat L, al-Sughayer MA, Afzal M (1999) Fatty acid composition of human milk in Kuwaiti mothers. Comp Biochem Physiol B Biochem Mol Biol 124:261–267
127. Cervera P, Ngo J (2001) Dietary guidelines for the breastfeeding woman. Public Health Nutr 4:1357–1362
128. Piccoli GB, Clari R, Vigotti FN et al (2015) Vegan–vegetarian diets in pregnancy: danger or panacea? A systematic narrative review. BJOG 122:623–633
129. Cai YZ, Sun M, Corke H (2003) Antioxidant activity of betalains from plants of the amaranthaceae. J Agric Food Chem 51:2288–2294
130. Jain N, Goyal S, Ramawat KG (2011) Evaluation of antioxidant properties and total phenolic content of medicinal plants used in diet therapy during postpartum. Int J Pharm Pharm Sci 3(3):248–253
131. Butt MS, Sultan MT (2011) Ginger and its health claims: molecular aspects. Crit Rev Food Sci Nutr 51(5):383–393

132. Ramawat KG, Merillon JM (2013) Handbook of natural products – phytochemistry, botany, metabolism, vol I. Springer, Heidelberg
133. Ramawat KG (2009) Herbal drugs: ethnomedicine to modern medicine. Springer, Heidelberg
134. Khare CP (2007) Indian medicinal plants: an illustrated dictionary. Springer, Berlin/Heidelberg
135. Kuroda S, Watanabe M, Santo T, Shimizuishi Y, Takano T et al (2010) Postpartum increase of serum thioredoxin concentration and the relation to CD8 lymphocytes. Ann Clin Biochem 47:62–66
136. Harzer G, Dieterich I, Haug M (1984) Effects of the diet on the composition of human milk. Ann Nutr Metab 28:231–239
137. Nasser R, Stephen AM, Goh YK, Clandinin MT (2010) The effect of a controlled manipulation of maternal dietary fat intake on medium and long chain fatty acids in human breast milk in Saskatoon, Canada. Int Breastfeed J 5(1):3
138. Innis SM (2007) Human milk: maternal dietary lipids and infant development. Proc Nutr Soc 66:397–404
139. Hachey DL, Thomas MR, Emken EA, Garza C, Brown-Booth L, Adlof RO, Klein PD (1987) Human lactation: maternal transfer to dietary triglycerides labeled with stable isotopes. J Lipid Res 28:1185–1192
140. Emken EA, Adlof RO, Hachey DL, Garza C, Thomas MR, Brown-Booth L (1989) Incorporation of deuterium-labeled fatty acids into human milk, plasma and lipoprotein phospholipids and cholesterol esters. J Lipid Res 30:395–402
141. Thompson BJ, Smith S (1985) Biosynthesis of fatty acids by lactating human breast epithelial cells: an evaluation of the contribution to the overall composition of human milk fat. Pediatr Res 19:139–143
142. Fritsche J, Steinhart H (1998) Analysis, occurrence, and physiological properties of trans fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA): a review. Fett-Lipid 100:190–210
143. Ritzenthaler KL, McGuire MK, Falen R, Shultz TD, Dasgupta N, McGuire MA (2001) Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. J Nutr 131:1548–1554
144. Rist L, Mueller A, Barthel C et al (2007) Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands. Br J Nutr 97(4):735–743
145. Rist L, Zweidler R, von Mandach U (2003) In: Freyer B (ed) Contributions to the 7th research conference on organic agriculture: organic agriculture of the future. University of Natural Resources and Applied Life Sciences, Vienna, pp 237–240

Challenges in Optimal Utilization of Bioactive Molecules Clinically

5

Kotamballi N. Chidambara Murthy, M. Shivapriya, P. Monika, and B. Tejashree

Contents

Abstract

It is an established fact that the plant-based food is known to provide several health benefits beyond basic nutrition to human. However, with increased demand for food, excessive use of pesticides, chemicals, and hormones to increase the yield and quantity has led to serious issues with use of plant-based diet. Additionally, dependency on few major crops, use of convenient, and ready

K. N. Chidambara Murthy (✉)
Central Research Laboratory, Ramaiah Medical College and Hospital, MSRIT Post, Bengaluru, India
e-mail: kncmurthy@gmail.com

M. Shivapriya
College of Horticulture, University of Horticultural Sciences, GKVK, Bengaluru, India
e-mail: priyachidambara@gmail.com

P. Monika · B. Tejashree
Department of Biotechnology, Ramaiah Institute of Technology, MSRIT Post, Bengaluru, India
e-mail: monikamohithe18@gmail.com; tejashree277@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_49

to eat type of food have also lead to excess of energy and high consumption of carbohydrates which resulted to unexpected increase in incidence of obesity, diabetics, and other metabolic syndromes, globally. In view of such issues with plant-based diet, recently a lot of emphasis is laid to the use of health promoting, low calorie and mixed variety of plant-based diet, and to have more benefits. Traditional indigenous and exotic plants are enriched with beneficial nutrients and are sold in today's market as functional food or nutraceuticals. Market value for this class of consumer market is close to 200 billion $ and is expected to grow at a rate of 7.5% compound annual growth rate. However, in this journey from food to nutrition and nutraceuticals, some key components have been neglected, which is critical to get optimum benefits. In this chapter, we would like to discuss some of the key challenges in achieving optimum concept of health promoting molecules from farm to fork. Often, we measure the content of these in plant either after harvesting or during storage and based on which we assume the surety of health benefits. Either due to lack of facility or collaborative efforts, we are not considering critical factors like the right time for harvest of commodity, right storage and processing technique, and right cooking and formulation protocol along with selection of right base and ingredients for food. Some of these aspects are discussed in detail along with problems in achieving the same. Additionally, the chapter also provides necessary information on how to test and ascertain the quality of nutrition and its delivery for optimal benefits.

Keywords
Bioactive molecules · Bioavailability · Bio-efficacy · Food-food interactions · Nutraceuticals

1 Introduction

Bioactive molecules (BAM) in plants: Why and how they exist? Every creature in this universe has been provided with primary means of survival and existence. It is the immune system in human and animals, toxins, or other chemicals in microbes and in plants through secondary metabolites [1]. These secondary metabolites in addition to protecting plants against pest and diseases and abiotic stresses will also serve as metal transporting agents, as agents of symbiosis between microbes and plants, as sexual hormones, and as differentiation effectors. They have a wider variety of diverse chemical structure and biological functions that are species specific. In addition to structural reinforcement of tissue and tree, some of the secondary metabolites have special functions such as chemoattractants for pollinators, indication of ripening, indication of spoilage, etc.

Recent studies have also shown that plants have chemical and physical signal molecules to communicate to each other and sense the environmental alterations [2, 3]. Therefore, it is evident that secondary metabolites act as primary defense system in plants and protect them from both biotic and abiotic stresses. Since secondary metabolites are produced in very low concentration, for commercial application of

potent components, production of secondary metabolites will be induced by means of external factors like stress. Secondary metabolite content in most plants can be enhanced by biotic and abiotic elicitation technique, which is most commonly used in biotechnological route to producing plant metabolites. Some of the examples include production of vincristine and vinblastine by *C. roseus* cell cultures developed from shake flasks to scale up through bioreactor [4]. Other phytochemicals produced using such biotechnological route include medically important molecules like Taxol, berberine, podophyllotoxin, scopolamine, and nutraceuticals like anthocyanin, betacyanin, gingseng, geraniol, etc. [4, 5].

Formation of secondary metabolites: All the secondary metabolites are usually formed from conjugation and other reaction of primary metabolites, specifically amino acids, carbohydrates, and proteins. Among the biochemical pathways, shikimic acid, malonic acid, mevalonic acid, and methylerythritol phosphate (MEP) pathways are responsible of formation of secondary carbon-containing molecules. It is through photosynthesis and carbon source CO_2 compounds and through different pathways that compounds such as phenolics, nitrogen containing compounds, and terpenes are formed. For example, most of the terpenoids are formed from basic unit IPP (isopentenyl diphosphate) and a basic unit C_5H_8, either from mevalonic acid or methylerythritol phosphate (MEP) pathway from sequential combination of three acetyl CoA units to form 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) followed by mevalonic acid and finally isopentenyl diphosphate (IPP). This is formed by dephosphorization in the case of mevalonate pathway. In the case of MEP pathway, it is formed from combination of glyceraldehyde 3-phosphate and pyruvate, through 1-deoxy- D-xylulose 5-phosphate and methylerythritol phosphate (MEP). Similarly, most of the secondary metabolites are formed through established pathways (Table 1). Some of the high-value compounds are produced in plants by induction of stress, alteration in microenvironment, use of elicitors (biotic and abiotic), and genetic manipulation through biotechnological tools. Table 2 shown is the list of secondary metabolites produced through biotechnological routes.

Table 1 Formation of secondary metabolites in plant through different pathways and precursors for the same

Class of secondary metabolite	Initial precursor	Pathway
Purine alkaloids	Histidine and purine	
Imidazole alkaloids	Histidine	
Terpenoids, indole alkaloids, quinoline alkaloids, quinazolidine alkaloids, acridine alkaloids	Shikimate/ Shikimic aid	Shikimate pathway
Flavanoids, stilbenes, coumarins, tannins (condensed), lignans	Cinnamate, chalcone	Phenylpropanoid pathways (PPP)
Polyketides, saturated fatty acids	Acetyl CoA, mevalonate	Mevalonic acid pathways
Vitamin B_1, B_6, isoprenoid	Glucose	Deoxy xylulose pathway

Table 2 Secondary metabolites and bioactive compounds produced through biotechnological route

Name of the metabolite	Route of production	Application	Reference
β-Carboline alkaloids	Suspension culture	Administration of BCA is currently available experimental model to study essential tremor	[6, 7]
Gallotannins	Root culture	Prevention of gushing in beer and carbonated beverages	[8]
Withaferin A	Shoot culture	To study intermediate filament proteins	[9]
Berberine	Root culture	Antibacterial alkaloid	[10]
Vanillin	Genetically modified organism(*Pycnoporus cinnabarinus*)	Flavoring agent	[11]
Catechin	Hairy root culture	Antibacterial agent	[12]
Shikonin from *Lithospermum erythrorhizon*	Stirred tank bioreactor	Inhibit cancer cell glycolysis	[13]
Vinblastine from *Catharanthus roseus*	Air lift reactor	Chemotherapeutic agent	[14]
Capsaicin from *Capsicum*	Immobilized cell reactor + precursor isocapric acid	Digestive stimulant and for treatment of rheumatic disorders	[15]
Diosgenin from *Dioscorea doryophora*	Suspension cell culture	Precursor for medicinal steroids	[16]
Terpenoids	Metabolic engineering of mevalonate pathway	Flavor, fragrances, and cosmetics	[17]
Lycopene	Metabolic engineering of non-mevalonate isopentenyl synthesis pathway	Antioxidant, antitumor agent	[18]

Chemically, three major groups of secondary metabolites are produced in plants, namely, terpenes, phenolics, and N- and S-containing compounds. Terpenes composed of 5-C isopentanoid units are toxins and feeding deterrents to many herbivores. Phenolics synthesized primarily from products of the shikimic acid pathway have several important defensive roles in the plants as well as in humans. Members of the third major group, i.e., N- and S-containing compounds, are synthesized principally from common amino acids, and these have several biological properties in both plant and human [19].

Secondary metabolites role in plant: Unlike primary metabolite's presence, distribution of secondary metabolites within the species and plant part is very specific. They perform several functions in plant which includes protection of

plant against stress, pathogens, and interaction between plants and other organisms [20]. Defense is the primary role of these metabolites in plants, which is executed in multiple ways which includes being toxic to organisms (like pathogenic microbes, insects, and animals), formation of barrier for pathogens, production of enzymes that degrade pathogens, and acquiring immunity after first infection. Antimicrobial compounds which are released whenever there is an interaction from microbes are termed as phytoanticipins; these will be released from constitutively stored precursors during microbe attack [20]. Some of the compounds in this category are saponins, glucosinolates, cyanogenic glucosides, and benzoxazinone glucosides.

2 Alternation in Content of BAM

Bioactive molecules are meant for protection of plant under various stress and diseased conditions. Use of these for human health is one of the ancient systems of medicine which originated in Indian and other South Asian countries centuries back. The same has got great significance in the last few decades, due to established benefit in several diseases including chronic diseases. Although benefits from plant-based formulation is attributed to combined effect of multiple constituents, each of the plant is known to have a specific molecule known to be key ingredient for activity, and often they are termed as marker compound or active principles. As these are naturally produced or accumulated in plants, their concentration and distribution depend on several factors related to both plant and environment. These factors include processing of plant materials as food or formulation along with genetic factors of consumer and other factors including the bioavailability and bio-efficacy. Factors influencing the efficacy of bioactive molecules are summarized in Fig. 1.

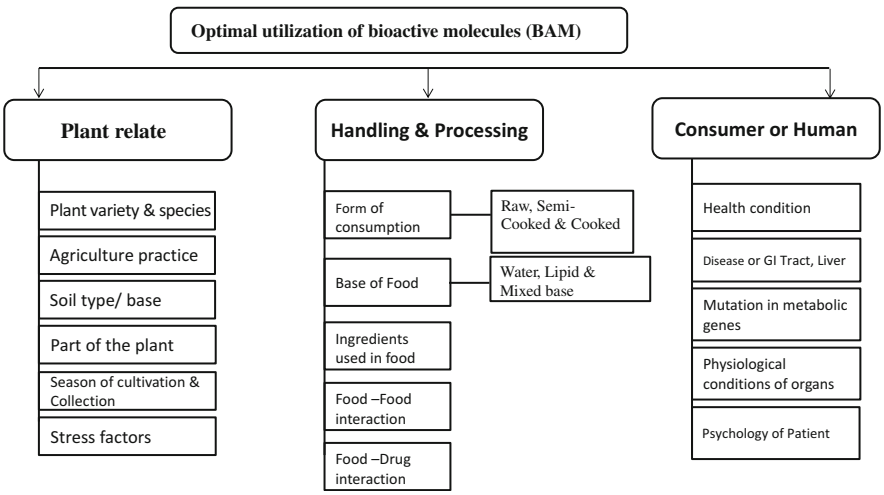


Fig. 1 Various factors affecting optimal utilization of bioactive molecules (BAMs) from food

2.1 Development and Agriculture: Growth of Plant, Season, Postharvest Handling

Accumulation of secondary metabolites at different stages of growth depends on both intrinsic and extrinsic factors. Accumulation of phenolic compounds increases with development, and the highest accumulation is observed during flowering stage. In the case of *Hypericum perforatum* L., increased content of naphthodianthrones at higher temperature and light intensity has been reported [21]. In the case of model plant *Arabidopsis thaliana,* it was observed that both content and composition of glucosinolate changed during different stages of development. Dormant and germinating seeds had the highest concentration (2.5–3.3% by dry weight) of glucosinolates, followed by inflorescences, siliques (fruits), leaves, and roots. Content of aliphatic glucosinolates predominated in most organs; indole glucosinolates make up nearly half of the total composition in roots and late-stage rosette leaves. Seeds possessed much higher concentrations of several types of aliphatic glucosinolates than other organs. Content of methylthioalkyl and hydroxyalkyl glucosinolates and compounds with benzoate esters was high in seed compared to other organs. Based on the age, older leaves had lower glucosinolate concentrations than younger leaves indicating maturity will enhance the secondary metabolites. Content of phytochemicals declined during seed germination and leaf senescence [22]. These results indicate that depending on role of secondary metabolites, accumulation varies with growth and quantity of accumulation will not be same in different parts. Therefore, selection of plant part and stage of development is critical to get optimum content for preparation of herb-based products.

Another important aspect of phytochemical variation is seasonal changes, as mentioned above; accumulation of secondary metabolites is greatly influenced by light, temperature, and length of day and night. These aspects are well established using model plants in laboratory studies; varied content of anthocyanin in grapes in different season and climate has been reported [23]. Accumulation of antimicrobial compounds in bulb and leaf extracts of *Tulbaghia violacea*, *Hypoxis hemerocallidea*, *Drimia robusta*, and *Merwilla plumbea* in different season has been reported by [24]. Results of the study suggest that accumulation of antimicrobial compound was highest in winter and autumn seasons. Total phenolics were found to be highest in spring compared to other seasons. Content of condensed tannin, gallotannin, and flavonoid levels, depending on the sample, was either higher in spring or winter except for *H. hemerocallidea* (corm) which had higher gallotannin levels in autumn. Total saponin content was highest in winter in all the plant studies [24]. Comparison of phytochemicals composition in different parts of commercial broccoli cultivars in spring and fall provided interesting findings in content of glucosinolates, vitamin C, total phenol, and total flavonoid contents and antioxidant activity. The highest total glucosinolate content was found in the florets of plants grown in both seasons. Phenols and flavonoids were highest in leaves, while vitamin C was highest in stems, suggesting that broccoli leaves and stems may be good sources of phytochemicals. All phytochemical contents were generally higher in florets in the spring than in the fall but were higher in leaves and stems during the fall than the spring

[25]. These results indicate that health benefits we get from the plant-based diet have some relation with season. This could be the reason for practice of seasonal plant-based diet in many cultures.

Similar to environment, nutrients supplemented to plants will also have great influence on the accumulation of secondary metabolites. Content of asiaticoside, asiatic acid, and quercetin 3-O-glucuronide was different in three cultivars of *Centella asiatica* grown under different soil nutrition condition. Salt stress is known to increase several secondary metabolites in plants; this includes sorbitol (*Lycopersicon esculentum*), flavonoids (*Hordeum vulgare*), jasmonic acid (*Lycopersicon esculentum*), tropane alkaloids (*Datura innoxia*), anthocyanins (*Grevillea* spec.), trigonelline (*Glycine max*), and glycine betaine (*Triticum aestivum*). Similarly draught stress which sometime occurs naturally is known to increase the accumulation of morphine alkaloids, glycosides, epicatechins, rutine, flavonoids, and anthocyanins, which are well known for the biological activity in human [26]. Under saline stress two cultivars of broccoli, namely, "Naxos" and "Parthenon" have shown accumulation of the highest phenolics [27]. Several plants have demonstrated varied accumulation of secondary metabolites when cultivated under different light and temperature, indicating direct effect from altered photosynthesis and several biosynthetic pathways. Accumulations of lycopene in tomato [28], carotenoids in Brassicaceae [29], and flavonoids and terpenoids in citrus [30] are some of the examples. The same approach is used in the case of plant cell culture to produce high-value secondary metabolites in reactors. These constitute the plant- and environment-associated factors that influence the accumulation of secondary metabolites.

Postharvest-associated factors also play major role in deciding the content of secondary metabolites in food ingredients. This is very important as most of the food ingredients will be stored (transported) under different conditions after harvest before consumed. This includes storage by producers, transportation, storage in market place, and household storage (refrigeration and room temperature). The time between the harvest and consumption of fruits and vegetables will be several weeks and the same is few weeks to months in the case of cereals and pulses. Hence, it is important to evaluate and understand changes in phytochemical content during postharvest handling and storage, which will have great impact on health beneficial activity of ingredients. Modified atmospheric packaging plays an important role in preserving the phytochemical content during postharvest and storage [31]. The level of phytochemicals present in fruit and vegetables may vary within and across cultivars. Available literature correlates the level of phytochemicals with many factors including cultivar type, environmental and agronomic conditions, harvest and food processing operations, and storage factors [32]. Postharvest storage of the vegetables and fruits in cold rooms increases the stress and upregulates the antioxidant pathway and antioxidant production; phytochemical content and also the weight of the vegetable commodities were observed to be significantly different from room temperature-stored vegetables/fruits and cold room-stored vegetables/plants [33]. Total phenolic content gets declined during curing and storage of fruits/vegetables due to anthocyanin degradation; anti-inflammatory factors and

antioxidant activity are reduced during the course of storage [34]. Further, studies also indicated that phytochemicals in fruits such as anthocyanins, phenolics levels changed during ripening and senescence [35]. The optimization of food processing and storage factors is an essential step to reduce the degradation of phytochemicals for potential health benefits.

Processing: Each of the plant food ingredients is subjected to one or the other processing before consumption. This may be cutting or size reduction, germination, drying in the case of those consumed fresh, steaming, boiling, frying, or cooking. These physical processes are known to greatly influence the fate of phytochemicals. One of the studies has shown variation in content of phenolic acids, isoflavones, flavones, and glucosinolates when analysis was done using fresh and fresh-cut samples including tomato, carrot, grape, eggplant, and broccoli. Content of phytochemicals reduced in cut tomato compared to fresh and similar results was observed in the case of eggplant. In other vegetables there was no significant variation between fresh and cut samples. Content of ascorbic acid, carotenoids, anthocyanins, and volatile principles (monoterpenes) decreases when fruit/vegetable is cut in to pieces and stored for a long time before use, irrespective of temperature as these are sensitive to light, temperature, and air [36]. Impact of different processing techniques on biological activity and phytochemicals composition is as shown in Table 3.

Three major medicinal plants, namely, *Ocimum basilicum*, *Senna petersiana*, and *Hypoxis hemerocallidea* have shown higher antimicrobial activity in fresh form compared to stored samples suggesting loss in either content or biological activity [60]. Another study has shown that heating and storage can greatly influence the phenolic content and biological (antioxidant) activity of blue berry extracts [61].

Cooking or formulation: This is another key factor in deciding quality and quantity of phytochemical content in food we finally consume. There are case-specific issues concerned with cooking, which essentially has three major processes, namely, shallow frying, deep frying, semi-boiling and pressure or complete cooking. In all these processes, using temperature-sensitive ingredients like ascorbic acid, some vitamins and enzymes are deactivated. On the other hand, cooking will also help in percolation of active compounds from peelings to edible parts as seen in the case of potato, cashew, and other crops. Content of glucosinolate and sulforaphane is greatly reduced in broccoli florets after cooking, and therefore it is recommended to be consumed raw for optimum health benefits [62]. In addition, cooking will also lead to alteration in chemical nature of phytochemicals like breakdown (pigments), complexation (glucosides), and polymerization (polyphenols). It is also important to consider the biological effects of converted compounds, which may be useful or sometimes harmful (formation of acrylamide is most common example).

3 Hurdles in Release of Bioactive Compounds

In addition to plant-related factors, other factors, which are known to limit or control the bioaccessibility of compounds, are complex nature of plant materials, food matrix, and other factors related to cooking and formulation of food [63]. These

Table 3 Impact of processing on component of food ingredients and biological benefits

Sl. No	Plant source/s	Bioactive molecule/s	Processing method/s used	Impact of processing (+) or (−) on bioactive molecule/s	Reference
1	Oranges and grape fruits	Phenolics	High temperatures or freezing	Affects the content of some polyphenols, including flavonoids, due to the extractability alteration by the rupture of the cell wall	[37]
2	Kale And red cabbage	Phenolics	Peeling, washing, chopping, boiling, frying, and baking (traditional oven or microwave)	Increase in levels of phenolics, as well as antioxidant activity after cooking	[38]
3	White cauliflower	Phenolics	Boiling, steam techniques and stir frying	- boiling results in a decrease of the total phenolic content - steam techniques and stir frying promoted an increase of these compounds	[39]
4	Tomatoes	Phenolics	Boiling, baking, and frying	Significant reduction in total phenolic content	[40]
5	Kale And red cabbage	Phenolics	Steam cooking	Higher contents of bioactive compounds and higher antioxidant activity	[38]
6	Potato "cultivar" (*Solanum tuberosum* L.)	Anthocyanin	Microwave cooking, steamed pressure cooking, boiling, and baking in a hot air oven	Preserves or causes a little increase in the anthocyanin content	[41]
7		Catechin	Heating	Reduction in bioactive compound by epimerization	[42]
8	Blueberry fruits	Anthocyanin	Blanching	Increase in the retention of Anthocyanin levels (23% instead of 12%) and the total anthocyanin content of juice from blanched blueberry is twice the nonblanched one	[43]

(*continued*)

Table 3 (continued)

Sl. No	Plant source/s	Bioactive molecule/s	Processing method/s used	Impact of processing (+) or (−) on bioactive molecule/s	Reference
9	Fruits and vegetables	Catechin, quercetin, and its glycosides	Blanching	Affects color, flavor, texture, and loss of the nutritional and functional quality by promoting the oxidation of phenolic compounds	[44]
10	Blackberry	Phenolics	Pasteurization	Phenolics is lost	[45]
11	Mushroom	Phenolics	Sanitizing using sodium hypochlorite	Disappearance of phenolics and the formation of their oxidation products	[46]
12	Red onion slices	Flavanols and anthocyanins	Sanitizing using sodium hypochlorite	Losses of flavonols (23%) and anthocyanins (13%) due to leaching.	[47]
13	Pineapple, banana, And guava	Phenolic content and flavanoids	Sanitizing using ozone (O_3)	Significant increase in total phenolics Whereas in bananas and pineapples, the flavonoid content increased in response to ozone treatment For guava fruits, the flavonoid content increased and total phenolic decreased inversely	[48]
14	Campbell grapes	Phenolics, flavonoids, and anthocyanin	Microwave heating, ultrasonication and blanching	- microwave heating and ultrasonication increased bioactive contents in non-cold stabilized grape juice than in cold stabilized juices - blanching decreased bioactive compounds both in non-cold stabilized and cold stabilized juice	[49]
15	Pumpkins	Phenols and carotenoids (lutein, a-carotene, and b-carotene)	High pressure processing	No differences in the total bioactive molecule contents	[50]

(*continued*)

Table 3 (continued)

Sl. No	Plant source/s	Bioactive molecule/s	Processing method/s used	Impact of processing (+) or (−) on bioactive molecule/s	Reference
16	Red bell Peppers	Vitamin C	Pulsed electric field treatment	Minimal loss of vitamin C due to improved mass transfer during drying	[51]
17	Beetroot tubers (*B. vulgaris*)	Betalain	Pulsed electric field treatment	90% recovery of metabolite	[52]
18	Oil tocopherol Polyphenol	Rapeseed	Pulsed electric field treatment	Increased content of polyphenols and tocopherols	[53]
19	Carrot	β-Carotene	Boiling	Increase in bioavailability	[54]
20	Kiwifruit puree	Lutein, neo-lutein, β-Carotene, Neoxanthin, Violaxanthin	Pasteurization by Conventional heat	No effect on bioaccessibility	[55]
21	Milk- and soy-based Fruit Beverages	α-Tocopherol, γ-Tocopherol, and δ-Tocopherol	High pressure processing	No effect (α-tocopherol) Decrease (γ- and δ-tocopherol)	[56]
22	Orange juice	Ascorbic acid, phenolics, flavanones, and Carotenoids	Pasteurization	Ascorbic acid, total flavanones, total carotenoids, and provitamin A values of pasteurized orange beverage were lower than those of fermented juice. Total phenolic remained unchanged and was similar to that of original juice	[57]
23	Fruits and vegetables	Anthocyanin	Thermal Processing	Anthocyanin pigments readily degrade during thermal processing which can have a dramatic impact on color quality and may also affect nutritional properties	[58]

(*continued*)

Table 3 (continued)

Sl. No	Plant source/s	Bioactive molecule/s	Processing method/s used	Impact of processing (+) or (−) on bioactive molecule/s	Reference
24	Orange juice	Vitamin C, carotenoids, and flavanones	High pasteurization	Losses in total vitamin C were < 9%, treatments with the higher temperatures tended to show the higher decrease in the content of both forms of vitamin C. Whereas increase in naringenin and hesperetin	[59]

are intermediate to plant- and human-related factors, and major ones include release of bioactive molecules from food matrix, nature of BAM from source, food-food interaction, and food-drug interactions.

Release of bioactive molecules from food matrix: Bioactive molecules are found in plants, fruits, and vegetables and include heterogeneous group of molecules such as polyphenols, carotenoids, tocopherols, phytosterols, and organosulfur compounds [63] with different chemical structures (hydrophilic, hydrophobic). These compounds vary in their distribution in nature (specific to particular vegetable or ubiquitous), concentration in food and human body, site and mode of action, efficiency against oxidative species, and their specificity.

There are many clinical evidences indicating the link between the chronic diseases such as cancer and cardiovascular diseases with diet, especially with plant-based food [64, 65]. Although the composition of the nutrients and other bioactive compounds in food will be mentioned on the labels printed on the package, the bioavailability of the bioactive molecules in human body differs. It is dependent on various factors, which include chemical nature of the nutrients, their release from food matrix, interaction with other food components, presence of suppressors and cofactors, formation of stable components from which nutrients are released, and so on. Recent evidence also suggests that the natural matrix of food and also the microstructure of processed food influence the bioavailability of bioactive molecules in vivo [66].

Food composition database (FCD) provides the information regarding the amount of energy, proteins, fats, vitamins, minerals, and other nutrients present in food. FCD provides the information which is the based on certain chemical assays performed. In the case of complex food, it is based on the nutrient composition of ingredients. FCDs have been methodically compiled over the years in many countries and provide information about the nutrients contained in most consumed foods [67]. FCDs are generally used to assess the nutrient content of diets and to derive nutrition guidelines. They are also often utilized in food policy recommendations and nutrition monitoring.

The FDA has defined bioavailability as the rate and extent to which the active substances or therapeutic moieties contained in a drug are absorbed and become available at the site of action [54]. This definition has to be also applied for the bioactive molecules present in food. Book on Indian nutrition that is known as *Indian Food composition Table* published by the National Institute of Nutrition, Hyderabad, on January 2017 provides extensive details on most of the food ingredients used in different regions of India.

Another term that is commonly used is bioaccessibility, which is defined as the amount of an ingested nutrient that is available for absorption in the gut after digestion [68]. If the amount of recovered nutrient is of relevance, then bioaccessibility term comes into use. However, bioavailability is measured in blood/plasma in vivo, and it will be affected by several factors such as physiological state, dosage, etc.

After consumption, the nutrients that are present in a food or drink will be released, absorbed into the bloodstream, and circulated in the body, and also few of the times, it is transported to their target tissues. Nutrients differ in their bioavailability, which is mainly decided by their solubility and affinity for binding with proteins and biological molecules either in blood or in different organs. Release of the nutrient from the food matrix, effect of digestive enzymes, binding and uptake by the intestinal mucosa, transfer across the gut wall to the blood or lymphatic circulation, systemic distribution and deposition, metabolic and functional use, and excretion can affect nutrient bioavailability. It is mediated by external (characteristics of the food matrix, chemical form of the nutrient, etc.) and consumer internal (gender, age, nutrient status, and life stage) factors. The bioavailability of macronutrients (carbohydrates, proteins, and fats) is usually very high, e.g., more than 90% of the amount ingested will get absorbed and utilized.

Bioaccessibility is the first step to make a nutrient bioavailable, which mainly depends on availability of nutrients for absorption. During this step, the nutrient is released from the food matrix and converted into a chemical form that can bind to and enter the gut cells or pass between them. Chewing, enzymatic digestion of the food in the mouth, mixing with acid and enzymes in the gastric juice, and releasing into the small intestine are the unit operations of the process by which the nutrients are rendered bio-accessible. The small intestine is the major site of nutrient absorption. Enzymes of the pancreatic juice continue breaking down the food matrix. Certain procedures involved in food preparation like cooking, chopping, or pureeing collaborate with mastication and enzymes to the digestibility of food matrices.

The oral bioavailability is limited due to the restricted release of the components from plant matrix, solubility of nutritional molecules in gastrointestinal fluid, the permeability across intestinal epithelial cells, as well as the enzymatic and chemical reactions occurring within the gastrointestinal tract. Following four essential steps are necessary for the effective absorption of bioactive compounds:

1. Release from food matrix
2. Incorporation into bile-salt micelles

3. Absorption by epithelial cells
4. Incorporation into the chylomicrons with secretion into lymphatic system

As mentioned in previous section, processing of food also has an impact on the chemical constituents, physical and sensory properties of the final product. In addition to cooking, industrial food processing technologies may influence the content of bioactive compounds leading to changes in their functional properties (e.g., bioavailability, bioaccessibility, and bioactivity) and potential health benefits. Loss of phytonutrients in foods becomes more significant as food is processed, stored, packaged, and transported. Some of the processes like frying, puffing, semi-cooking, and steaming are known to increase the bioavailability of compounds which are less sensitive to heat. Similarly, processing such as grinding, fermentation, and/or mild heating may improve bioavailability, most likely as a result of disruption of the cell walls of plant tissues, dissociation the nutrient-matrix complexes, or transformation into more active molecular structure. Therefore, attention should be given to the degree of disintegration of the initial tissue structure because of its impact on food quality, functionality, and deterioration. As the demand for functional food increases, intense research efforts for the development of new processing technologies is conducted with the goal of ensuring maximal nutritional and functional properties, as well as the overall quality of a product.

The bioavailability of nutrients and bioactive compounds present in plant products (fruits and vegetables) is presently an extremely important area of food and nutrition research. The objective of these studies is to determine the contribution of actual foods as sources of specific nutrients (e.g., antioxidants) and to establish processing conditions that maximize the health benefits. Although several photochemicals are known to be important in promoting human health, the bioavailability of these bioactive molecules will vary with respect to their microstructure. For example, food microstructure seems to be quite relevant in the bioavailability of several antioxidants. Some of the phytochemicals for which bioavailability has been established and extensively researched includes carotenoids, polyphenols, and folates.

Carotenoids are a family of fat-soluble plant pigments that provide red and orange colors to fruits and vegetables. Their function is to absorb light in photosynthesis, protecting plants against photosensitization. Dietary carotenoids are considered to be beneficial in the prevention of a variety of diseases, including certain cancers and eye disorders. The 5 principal carotenoids found in human plasma as a result of ingestion of plants are α-carotene, β-carotene, cryptoxanthin, lutein, and lycopene, but over 600 carotenoids have been identified to date in plant and marine organisms. Our research has demonstrated both radical scavenging and hepatoprotective activity of carotenoid-rich marine microalgae [69, 70]. Carotenoids present in a wide variety of plants are partially concentrated in chromoplasts or chloroplasts in different ways. The extent of release from the food matrix is highly variable depending on whether carotenoids are non-covalently bound to protein or fiber or not. In general, release of carotenoids from plant foods occurs only when the cells in the food matrix are disrupted, as is usually the case during food preparation,

processing, and/or mastication, but not during digestion, at least in the ileum of humans [71]. One of the studies suggests that size reduction in the case of carrot (pureed fresh carrot) has shown highest release of carotenoids and its isomers compared to raw or boiled carrot [72].

Lycopene, a carotenoid responsible for the distinctive red color of ripe tomatoes, is usually located within cell membranes, and its release is determinant on the bioavailability. Epidemiological studies have suggested that consumption of lycopene may protect against CVD and reduce the risk of several types of cancer, most notably those of the prostate, breast, lung, and digestive tract [73]. Food processing like cooking or heating may improve lycopene bioavailability by breaking down cell walls, which weakens the bonding forces between lycopene and the tissue matrix, thus making it more accessible. One of the studies indicate that bioavailability of lycopene is higher in tomato paste compared to consumption of fresh one [74]. Another clinical study suggests that severely homogenized (blending for 2.5 min using the same blender, followed by processing in a high-pressure homogenizer at 200 bar) tomato had shown the highest amount of lycopene and carotenoid content in serum along with increased antioxidant activity. This was in comparison with treatment of mild homogenization (blending for 2 min) and raw tomatoes to volunteers in food [75]. Clinical study has also shown that lycopene is more bioavailable in its cis form from tangerine compared to all trans form as in the case of tomato juice, which is more of chemical factor [76]. These results indicate that along with physical, chemical factors also plays critical role in deciding bioavailability of lycopene and other phytochemicals.

Xanthophylls are yellow pigments found in plants and are known for various biological effects, which includes lutein, zeaxanthin, capsanthin, canthaxanthin, astaxanthin, echionine, and β-cryptoxanthin. These are oxygenated carotenoids that are synthesized within the plastids. The xanthophylls lutein and zeaxanthin accumulate in the eye lens and macular region of the retina and have been implicated in helping to protect the eye against oxidative damage and cataracts. For optimal absorption of xanthophylls, they must be released from their food matrix and then transferred to lipid micelles in the small intestine. This requires the presence of dietary fat in the small intestine, which stimulates the gallbladder to release bile acids (i.e., emulsifiers). Therefore, fat or fat base is important for all nonpolar phytochemicals including fat-soluble vitamins.

Polyphenols represent a wide variety of compounds belonging to several classes, for example, hydroxybenzoic and hydroxycinnamic acids, anthocyanins, proanthocyanidins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes, and lignans. These are ubiquitously found in all the plants used in diet and nutritional supplements. As mentioned before, phenolic compounds (or polyphenols) are secondary metabolites of the pentosephosphate, shikimate, and phenylpropanoid pathways in plants. Reported bioavailability of polyphenols is highly variable depending on their structure and conjugation. Among polyphenols, phenolic acids account for about one third of the total intake, and flavonoids account for the remaining two thirds. The most abundant flavonoids in the diet are flavanols (catechins plus proanthocyanidins), anthocyanins, and their oxidation products. Chemical structure

is the primary determinant of bioavailability of polyphenols. Studies in human have shown that excretion of polyphenols through urine varies from 0.5 to more than 50% depending the source and structure. Over 27% of caffeic acid was detected in urine after administration of 1000 mg, 24% in the case of hesperidin, 19.8% in the case of genistein from soy milk, and 6–8% of naringin from grapefruit. Most of the polyphenols takes 1–2 h to reach peak plasma concentration, except those absorbed after degradation. Based on the elimination rate and half-life of polyphenols, it is essential for repeated administration or consumption of sources preferably once in 24 h [77].

Folates and folic acid are forms of vitamin B that are necessary for the production and maintenance of new cells, especially important during periods of rapid cell division and growth, such as throughout infancy and pregnancy. Inadequate folate intake has been associated with development of birth defects [78]. Leafy green vegetables such as spinach and turnip greens, dry beans and peas, and some other fruits and vegetables are rich natural sources of folate in nature; folates are covalently bound to macromolecules. Bioavailability of folate from food source mainly depends on factors like nature of food matrix, extent of deconjugation of polyglutamyl folates in the intestine, and stability of certain folates during digestion and in presence of other dietary constituents which may either increase or reduce the stability. Apart from this, in the case of fortified supplements, stability of folate used is also a major concern toward optimum utilization. McNulty and Pentieva from the University of Ulster, UK, have discussed details of these factors along with experimental details in estimating folate bioavailability and bio-efficacy in acute and chronic model [79]. They have concluded that chronic studies on food supplementation can give better understanding on the bioavailability of folate, and this needs to be conducted using robust and highly controlled experiments which can ensure controlled source and delivery of folate equivalent to folic acid. It was also pointed that stability of folate and its analogues in plant sources (green leafy vegetables) after cooking is another major limiting factor.

3.1 Food-Food Interactions

Humans consume verity of food to meet daily needs of energy depending on the ethnicity and region. Additionally different types of foods are consumed on different occasions, which generally consist of several verities made out of multiple ingredients originating from either plant, animal, or microbial source and several chemicals; processed or semi-processed ingredients are also used in the food preparation. As each of the ingredients will have multiple phytochemicals, there will be a good chance of interaction between two or more molecules resulting in useful or deleterious outcome. The classical example of useful interaction is enhancement in iron bioavailability in the presence of ascorbic acid or vitamin C. Conversely, interference of phytic acid in absorption of essential minerals and nutrition is the established example for negative outcome.

Food supplements (e.g., nutrients, vitamins, hormones, amino acids, antioxidants) and functional food (e.g., phytosterols or omega-3 fatty acids enriched

food) occupy a position between food and drugs. Botanical materials represent a large segment of this class of products (e.g., tulsi, *Withania*, soy isoflavones, yam, or hop extracts). In addition to food-food interactions, the interaction between phytochemicals of food and toxic contaminants is another less studied concept. A team of toxicologists made an attempt to study this concept in order to understand the interactions which can lead to additive or subtractive or even synergistic effects, under a project named FOODINTER in Belgium. The communication between scientists and stakeholders (authorities, producers, and consumers) plays an important role in this particular regard. This will help to ensure the safety from food-food interaction and food-contaminants interactions, which accidentally get in to food during storage of food ingredients and processing or preparation of food. Therefore, it is necessary to conduct risk assessment of chemicals, natural compounds, and environmental contaminants present in food supplements which could interact between them or with micro- or macronutrients of normal human diet.

Interaction studies have been performed using existing in vitro models (based on culture of various cell types, prokaryotes and eukaryotes) with mixture of active substances at different concentrations. The project selected Ginkgo biloba, St John's wort, maca, black radish, garlic, and soy isoflavones, which are most commonly used as health supplements to understand the interactions. They studied several key phytochemicals from each of these commodities along with seventeen trace elements As, Ba, Bi, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, Tl, Zn, and Hg, over 23 mycotoxins, more than 15 PHAs, organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and of polybrominated diphenyl ethers (PBDEs), and dioxins were also analyzed in the selected samples. Evaluation parameters like cytotoxicity, inhibition of metabolizing enzymes, and results provided very specific information regarding various contaminants that needs to be considered for limitation in all the products containing selected plants/extracts derived from them. There is a need to create such database on all the major food ingredients used globally. Parameters for analysis and study interactions should be based on the location of cultivation, agriculture and postharvest practices, processing technique used, and method of food preparation [80].

3.2 Food-Drug Interactions

Food-drug interaction is defined as the alteration in the pharmacodynamic, pharmacokinetic properties of the drug due to the interaction with the nutrients present in the food or change in the nutritional composition in of food (in vivo) due to its interaction with drug. Food-drug interactions can result in two main clinical effects: either a decreased bioavailability of a drug, which predisposes to treatment failure, or an increased bioavailability, which increases the risk of adverse events and may even precipitate toxicities [81]. For example, grapefruit juice is a selective intestinal CYP3A4 inhibitor, cytochrome P-450 3A4, in the small intestine, resulting in a significant reduction of drug pre-systemic metabolism [82]. The overall exposure of some drugs can be increased by more than fivefold when consumed with grapefruit

juice and increases the risk of adverse effects. The use of certain drugs may affect GI tract function and may lead to a loss of bodily electrolytes and fluids. People in risk due to the food-drug interactions are usually elderly people, cancer patients, and people with malnutrition, who are more prone for mutation in related to metabolism. However, elderly people are exposed to the highest risk as 30% of the prescribed drugs are consumed by them due to the decrease in physiological function with age [83]. Pharmacokinetic particularities associated with aging are absorption (changes in gastric pH, decreased gastrointestinal blood flow), distribution (decreased lean body mass, water, serum albumin concentration, and binding serum proteins), and elimination (reduced renal function). Therefore, drug bioavailability, volume of distribution, clearance, and half-life of drugs are modified with aging. Water-soluble drugs become more concentrated, and fat-soluble drugs will have longer shelf life due to slow release of the drugs from fat tissue. Food-drug interaction is a serious issue in drugs with very narrow therapeutic window, like chemotherapeutic, immunomodulators, and drugs acting on CNS.

3.3 Types of Drug-Nutrient Interactions

Based on nature and mechanisms, drug-nutrient interactions are classified into four types:

Type I interactions are ex vivo bio-inactivation, which refer to interactions between the drug and the nutritional element or formulation through biochemical or physical reactions, such as hydrolysis, oxidation, neutralization, precipitation, or complexation. They usually occur in the delivery device or outside the body.

Type II interactions affect absorption. They cause either an increase or decrease of the oral bioavailability. The precipitant agents may modify the function of enzymes (type A interactions) or transport mechanisms (type B interactions) that are responsible for biotransformation. Complexation, binding, and/or other deactivating processes occur in the gastrointestinal tract as a result of interaction between drug/food ingredients (type C interaction) and reduce absorption.

Type III interactions affect the systemic or physiologic disposition and occur after the drug or the nutritional element has been absorbed from the gastrointestinal tract and entered the systemic circulation. Changes of the cellular or tissue distribution, systemic transport, or penetration to specific organs or tissues can occur.

Type IV interactions refer to the elimination or clearance of drugs or nutrients, which may involve the antagonism, impairment, or modulation of renal and/or enterohepatic elimination.

Predicting drug interactions during drug development is a challenge for pharmaceutical industries and regulatory agencies. Since the publication of the US Food and Drug Administration's (FDA's) first in vitro and in vivo drug interaction guidance documents in 1997 and 1999, researchers and clinicians have gained a better

understanding of drug interactions [84]. This knowledge has enabled the FDA and the industry to progress and begin to overcome these challenges. The FDA has continued its efforts to evaluate methodologies to study drug interactions and communicate recommendations regarding the conduct of drug interaction studies, particularly for CYP-based and transporter-based drug interactions, to the pharmaceutical industry. A drug interaction website was established to document the FDA's current understanding of drug interactions [http://www.fda.gov/cder/drug/druginteractions/default.htm].

Host factors (includes genetic factors) include genetic and pathological factors that are associated with adverse, toxic effect of selected food ingredients in individuals. Pathological conditions associated with malabsorption mainly associated with mucosal layer. This can be classified as premucosal, which includes impaired digestion and bile acid/enzyme deficiencies; mucosal-associated factors like reduced absorption, bowel resection, and diseases affecting absorption; postmucosal-associated factors including altered nutrient transport and vascular or lymphatic abnormalities. Factors affecting the bioavaibality in human is also depicted in Fig. 2 for better understanding. These conditions can be treated with altering the nutrition in terms of the use of high protein diet, parenteral feeding and designer food with easily digestible and absorbable nutrients [85]. Genetic factors include mutation in metabolization genes such as P450 (CYP2D6), methylene tetrahydrofolate reductase

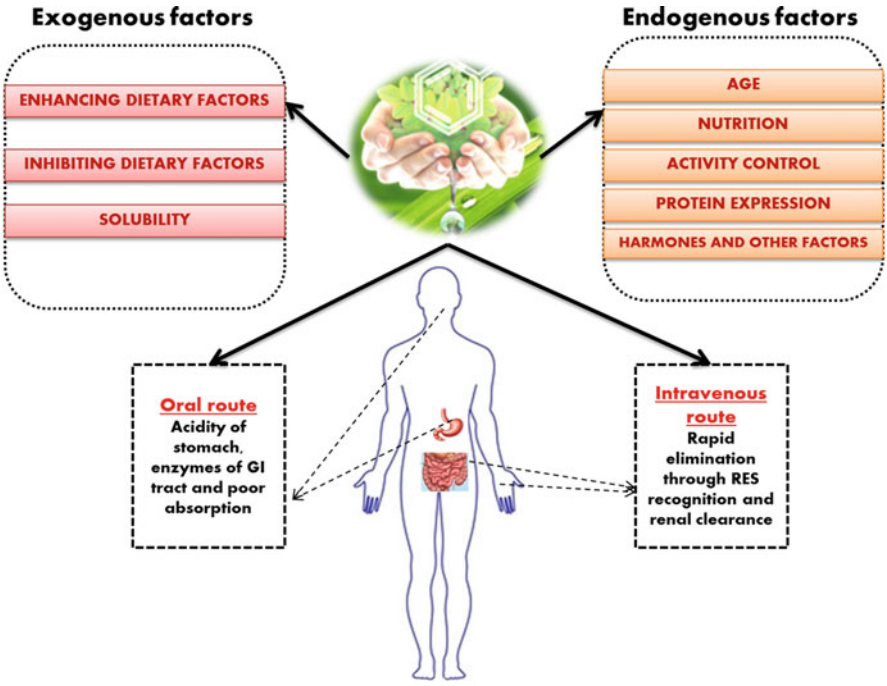


Fig. 2 Factors affecting/influencing bio-availability in human

gene, GST, N-Acetyl transferase, and so on [86]. Impact of metabolizing enzymes on the activity and content of biologically active compounds are shown in Table 4.

4 Models Used to Study Utilization of BAM

There are several in vitro and in vivo models available to understand the utilization of bioactive molecules from food ingredients. Prerequisite for bioavailability study is that we need to identify one or two marker compounds and its metabolites for which there should be very sensitive quantification or analytical method available. Flavonoids, polyphenols, terpenoids, and coumarins are some of the compounds extensively subjected for bioavailability studies in various models.

Among in vitro models, digestion of samples using gastric and intestinal enzymes at both gastric and intestinal pH is most widely used for both food and phytochemicals. It is commonly referred as "in vitro digestion model" [98]. Enzymes of porcine, rabbit, or human origin are used for this study, and some models have shown that the use of minor components such as bile salt, phospholipids, gastric lipase, and emulsifier is also considered for better results [99]. Simulated salivary fluid (SSF, pH 7.0), simulated gastric fluid (SGF, pH 3.0), and simulated intestinal fluid (SIF, pH 7.0) are used in these experiments, which give good indication of release of absorbable form of compound of interest. Use of artificial permeable membrane (also referred as artificial immobilized membrane-AIM), which simulates the permeability of intestinal lining is other model used commonly to estamale bioavailability of natural molecules. This is prepared by covalently binding membrane-forming lipids to an amorphous silica substrate which mimics both hydrophobic and hydrophilic nature of absorbing cells. Another model used is reconstituted or isolated cell membrane, which is more useful to understand specific type of molecular transport across the membrane. Compartment model is a commonly used model, which has two or more compartments connected through semipermeable membrane and movement of molecule from one chamber to another [100].

Common cell-based model used for bioavailability study is CaCo2 cell line, which is a colon adenocarcinoma cell. This cell line is generated to represent mucosal cells, which is primary site of absorption for GI tract. Primarily CaCo2 cells are used to understand the ability of nutrients to cross the intestinal barrier, as well as to study their transport mechanisms. Advantage with this model is it is quick and can provide good idea on permeation and absorption across the intestinal membrane, and it does not require ethical clearance [101]. Other ex vivo models include ligation of the intestine of animals like rat, rabbit, porcine, and pig. In this model, the intestine is ligated on one end, and solution containing food is added inside, and it is immersed in solution mimicking biological fluids [98, 100].

In situ model includes intestinal perfusion in animal, which is mainly done through microsurgery. It this done by cannulation of desired region of the intestine (proximal) after anesthetizing animals and collecting the sample flowing in the intestine at distal end. In this model any region of the small and large intestine can be studied [102]. In vivo animal and human models are the most realistic

Table 4 Major metabolizing enzymes known to interfere with bioactive molecules and their effects (+) or (−) on the bioactive molecules

Sl. No	Metabolizing enzyme/s	Bioactive molecule/s	Effect (+) or (−) on bioactive molecule/s	Reference
1	Transporter enzyme of intestinal or liver cells (e.g., P-glycoproteins	Bioactive molecules	Reduces oral bioavailability	[87]
2	Clearance enzymes	Bioactive molecules	Inhibitory potency against the target	[88]
3	β-Glucuronidase, β-glucosidase, β-galactosidase, mucinase, and nitroreductase	Resveratrol	Significantly reduced activities of these fecal and host colonic mucosal enzymes compared to control animals (21%, 45%, 37%, 41%, and 26%, respectively). The reduced bacterial enzyme activity was associated with a significant reduction in colonic tumor incidence in the resveratrol-fed rats compared to control rats	[89]
4	COX-2, NF-κB, AP-1, TNF-α, IL6, and VEGF (vascular endothelial growth factor)	Resveratrol	Inhibition of enzymes	[90]
5	GSTT2 and COX-2	Quercetin	Modulation of enzymes involved in detoxification and inflammation in LT97 human adenoma cells that could contribute to the chemopreventive potential of polyphenols after degradation in the gut	[91]
6	β -glucuronidase, β -glucosidase, β -galactosidase, mucinase, nitroreductase	Resveratrol	Modulating activity of bacterial enzymes and inhibitory effect on DMH-induced colon carcinogenesis in rats	[92]
7	Cytochromes P450	Flavanoids	Induce the expression of several CYPs and modulate (inhibit or stimulate) their metabolic activity. In addition, some CYPs participate in metabolism of flavonoids. Flavonoids enhance activation of carcinogens and/or influence the metabolism of drugs via induction of specific CYPs	[93]
8	Lipoxygenase, phospholipase, and cyclooxygenase.	Flavanoids	Inhibition of key enzymes involved in the prostaglandin biosynthesis	[94]

(*continued*)

Table 4 (continued)

Sl. No	Metabolizing enzyme/s	Bioactive molecule/s	Effect (+) or (−) on bioactive molecule/s	Reference
9	Kinases	Flavanoids	Inhibit these enzymes by competing with ATP for the binding to the catalytic site. These modes of inhibition provide an explanation for a molecular basis of flavonoid anti-inflammatory effects	[95]
10	Cytochrome P450 monooxygenases	Quercetin	Inhibition of gene expression of CYP1 family enzymes through blocking aryl hydrocarbon receptor which plays an important role in the cancer chemopreventive properties	[96]
11	Cytochrome P450 monooxygenases	Kaempferol	Prevents CYP1A1 gene transcription induced by prototypical aryl hydrocarbon receptor ligand	[97]

models, which involves repeated collection of blood and urine sample at regular intervals to understand the concentration of free-circulating compounds. Based on the loading concentration, both bound and organ absorbed concentrations can be calculated [103].

5 Challenges in Optimizing Mode of Delivery of BAM

As explained in previous sections, phytochemicals or BAM is present in different cellular compartments, which needs to be released from plant cell in to food matrix from where it needs to be released to GI tract for absorption. In this journey, BAM should be stable and withstand the conditions of postharvest, food processing and finally resist gastric pH and microenvironment to successfully be available for absorption. Some of the major challenges in this journey are as follows:

- Good agriculture practices to produce optimum BAM concentration.
- Best postharvest practices to protect or enhance the content of BAM.
- Best food processing methods to retain the quality and quantity of BAM.
- Minimizing the anti-nutritional or inhibitors of BAM activity through physical (soaking, grinding, etc.) or biological (fermentation, germination, etc.) process.
- Avoiding the combination of ingredients or food, those are known to interfere with either absorption or biological activity.
- Use of combination of ingredients or nutrients, which are known to synergize the activity or absorption of each other.

- Finally, to understand the genetic variation (metabolizing, transporting, and cell signaling genes) in individual who have problem in absorption and utilization of specific BAM.

6 Summary

It is a well-established fact that plant-based food can provide more benefits beyond primary nutrition to human. Often we give more importance to primary nutrition and focus on providing food security through agriculture and food industry practices. With increase in incidence of chronic disease, it is essential to provide more quality food which can provide good content of phytochemicals to support key biological functions like enhanced immunity, increase radicals scavenging ability, detoxify xenobiotics, and avoid infections. To achieve this, it needs integral efforts of agriculturist, food processing industry, food scientists, cook, and finally smart consumer. Often, aspects related to safe and efficient delivery of BAM is neglected due to lack of evidences, integration of experts and also complexity of processes involved. Therefore, it is essential to integrate the interdisciplinary expertise to achieve optimum outcome. This can greatly help in achieving good health through nutrition and can greatly help to reduce the burden of chronic diseases globally.

Acknowledgment Authors would like to acknowledge the support of the management of Gokula Education Foundation (medical) for their constant support and encouragement for research and also would like to acknowledge the help of research collaborators Prof. Bhimanagouda S Patil and Dr. G. K. Jayaprakasha, at VFIC, Texas A&M University for their support for research.

References

1. Bennett RN, Wallsgrove RM (1994) Secondary metabolites in plant defence mechanisms. Newphytologist 127:617–633
2. Austin AT, Ballaré CL (2014) Plant interactions with other organisms: molecules, ecology and evolution. Newphytologist 204:257–260
3. Becard G (2017) Plant defense and Allelochemicals in: how plants communicate with their biotic environment, vol 82. Academic, Förlag
4. Alam MM, Naeem M, Khan MMA, Uddin M (2017) Vincristine and vinblastine anticancer Catharanthus alkaloids: pharmacological applications and strategies for yield improvement. In: Catharanthus roseus. Springer, Cham, pp 277–307
5. Ochoa-Villarreal M, Howat S, Hong S, Jang MO, Jin YW, Lee EK, Loake GJ (2016) Plant cell culture strategies for the production of natural products. BMB Rep 49(3):149
6. Sasse F, Heckenberg U, Berlin J (1982) Accumulation of β-Carboline alkaloids and serotonin by cell cultures of Peganum harmala L: I. Correlation between plants and cell cultures and influence of medium constituents. Plant Physiol 69(2):400–404
7. Laviță SI, Aro R, Kiss B, Manto M, Duez P (2016) The role of β-carboline alkaloids in the pathogenesis of essential tremor. Cerebellum 15(3):276–284
8. Taniguchi S, Yazaki K, Yabu-Uchi R, Kawakami KY, Ito H, Hatano T, Yoshida T (2000) Galloylglucoses and riccionidin a in Rhus javanica adventitious root cultures. Phytochemistry 53(3):357–363

9. Ray S, Jha S (2001) A in shoot cultures of Withania somnifera. Planta Med 67(5):432–436
10. Sato F, Yamada Y (1984) High berberine-producing cultures of Coptis japonica cells. Phytochemistry 23(2):281–285
11. Falconnier B, Lapierre C, Lesage-Meessen L, Yonnet G, Brunerie P, Colonna-Ceccaldi B, Corrieu G, Asther M (1994) Vanillin as a product of ferulic acid biotransformation by the white-rot fungus Pycnoporus cinnabarinus. J Biotechnol 37(2):123–132
12. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, Cazin M, Cazin JC, Bailleul F et al (2000) Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J Ethnopharmacol 72:35–42
13. Scragg A (1994) Secondary products from cultured cells and organs: II. Large scale culture. Plant Cell Cult 145:199–216
14. Zhao J, Zhu WH, Hu Q (2000) Enhanced ajmalicine production in Catharanthus roseus cell cultures by combined elicitor treatment: from shake-flask to 20-l airlift bioreactor. Biotechnol Lett 22(6):509–514
15. Johnson TS, Ravishankar G, Venkataraman L (1990) *In vitro* capsaicin production by immobilized cells and placental tissues of Capsicum annuum L. grown in liquid medium. Plant Sci 70(2):223–229
16. Tsukamoto T, Kawasaki T, Yamauchi T (1956) Saponins of Japanese Dioscoreaceae. V. On the Structure of Dioscin. Pharmaceutical bulletin 4(1):35–42
17. Aharoni A, Jongsma MA, Bouwmeester HJ (2005) Volatile science? Metabolic engineering of terpenoids in plants. Trends Plant Sci 10(12):594–602
18. Kim SW, Keasling J (2001) Metabolic engineering of the nonmevalonate isopentenyl diphosphate synthesis pathway in Escherichia coli enhances lycopene production. Biotechnol Bioeng 72(4):408–415
19. Mazid M, Khan T, Mohammad F (2011) Role of secondary metabolites in defense mechanisms of plants. Biology Med 3:232–249
20. Piasecka A, Jedrzejczak-Rey N, Bednarek P (2015) Secondary metabolites in plant innate immunity: conserved function of divergent chemicals. Newphytologist 206:948–964
21. Radušienė J, Karpavičienė B, Stanius Ž (2012) Effect of external and internal factors on secondary metabolites accumulation in St. John's worth . Bot Lith 18(2): 101–108
22. Brown PD, Tokuhisa JG, Reichelt M, Gershenzon J (2003) Variation of glucosinolate accumulation among different organs and developmental stages of Arabidopsis thaliana. Phytochemistry 62(3):471–481
23. Downey MO, Dokoozlian NK, Krstic MP (2006) Cultural practice and environmental impacts on the flavonoid composition of grapes and wine, a review of recent research. Am J Enol Vitic 57(3):257–268
24. Ncube B, Finnie J, Van Staden J (2011) Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. South Afr J Botany 77:387–396
25. Bhandari SR, Kwak JH (2014) Seasonal variation in phytochemicals and antioxidant activities in different tissues of various broccoli cultivars. African J Biotechnol 13:604–615
26. Akula R, Ravishankar GA (2011) Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behav 6:1720–1731
27. Dominguez-Perles R, Martinez-Ballesta MC, Riquelme F, Carvajal M, Garcia-Viguera C, Moreno DA (2011) Novel varieties of broccoli for optimal bioactive components under saline stress. J Sci Food Agricult 9:1638–1647
28. Dumas Y, Dadomo M, Di Lucca G, Grolier P (2003) Effects of environmental factors and agricultural techniques on antioxidantcontent of tomatoes. J Sci Food Agricult 83:369–382
29. Morison J, Lawlor D (1999) Interactions between increasing CO2 concentration and temperature on plant growth. Plant Cell Environ 22:659–682
30. Zandalinas SI, Sales C, Beltrán J, Gómez-Cadenas A, Arbona V (2017) Activation of secondary metabolism in citrus plants is associated to sensitivity to combined drought and high temperatures. Front Plant Sci 7:1–17

31. Diaz-Mula HM et al (2011) Modified atmosphere packaging of yellow and purple plum cultivars. 2. Effect on bioactive compounds and antioxidant activity. Postharvest Biolo Technol 61:110–116
32. Tiwari U, Cummins E (2011) Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations. Food Res Int 50:497–506
33. Javanmardi J, Stushnoff C, Locke E, Vivanco JM (2006) Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chem 83:547–550
34. Grace MH et al (2014) Phytochemical changes in phenolics, anthocyanins, ascorbic acid, and carotenoids associated with sweet potato storage and impacts on bioactive properties. Food Chem 145:717–724
35. Gomez et al (2011) Postharvest studies beyond fresh market eating quality: Phytochemical antioxidant changes in peach and plum fruit during ripening and advanced senescence. Postharvest Biol Technol 60:220–224
36. Tiwari U, Cummins E (2013) Factors influencing levels of phytochemicals in selected fruit and vegetables during pre-and post-harvest food processing operations. Food Res Int 50(2):497–506
37. Peleg H, Naim M, Rouseff RL, Zehavi U (1991) Distribution of bound and free phenolic acids in oranges (Citrus sinensis) and grapefruits (Citrus paradisi). J Sci Food Agric 57:417–426
38. Murador D, Braqa AR, Da Cunha D, de Rosso V (2015) Alterations in phenolic compound levels and antioxidant activity in response to cooking technique effects. Food Chem 196:1101–1107
39. Ahmed FA, Ali RFM (2013) Bioactive compounds and antioxidant activity of fresh and processed white cauliflower. Biomed Res Int 1–9
40. Sahlin E, Savage G, Lister C (2004) Investigation of the antioxidant properties of tomatoes after processing. J Food Compos Anal 17:635–647
41. Lachman J, Hamouz K, Orsák M et al (2012) Impact of selected factors – cultivar, storage, cooking and baking on the content of anthocyanins in coloured-flesh potatoes. Food Chem 133:1107–1116
42. Wang H (2000) Epimerisation of catechins in green tea infusions. Food Chem 70:337–344
43. Rossi M, Giussani E, Morelli R, Lo Scalzo R, Nanic RC, Torreggiani D (2003) Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. Food Res Int 36:999–1005
44. Richard-Forget FC, Gauillard FA (1997) Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. J Agric Food Chem 45:2472–2476
45. Azofeifa G, Quesada S, Pérez AM, Vaillant F, Michel A (2015) Pasteurization of blackberry juice preserves polyphenol-dependent inhibition for lipid peroxidation and intracellular radicals. J Food Compos Anal 42:56–62
46. Choi SW, Sapers GM (1994) Effects of washing on polyphenols and polyphenol oxidase in commercial mushrooms (*Agaricus bisporus*). J Agric Food Chem 42:2286–2290
47. Pérez-Gregorio MR, Regueiro J, González-Barreiro C, Rial-Otero R, Simal-Gándara J (2011) Changes in antioxidant flavonoids during freeze-drying of red onions and subsequent storage. J Food Control 22:1108–1113
48. Alothman M, Kaur B, Fazilah A, Bhat R, Karim AA (2010) Ozone-induced changes of antioxidant capacity of fresh-cut tropical fruits. Innov Food SciEmerg Technol 11:666–671
49. Cabrera SG, Moon K-D (2015) Effects of Processing Treatments on the Bioactive Compounds of Campbell Grape Juice. Asia Pacific J Multidiscipl Res 3:1–4
50. González-Cebrino F, Durán R, Delgado-Adámez J, Contador R, Bernabé RR (2005) Impact of high pressure processing on color, bioactive compounds, polyphenol oxidase activity, and microbiological attributes of pumpkin puree. Food Sci Technol Int 22(3):235–245
51. Ade-Omowaye BIO, Taiwo KA, Eshtiaghi NM, Angersbach A, Knorr D (2003) Comparative evaluation of the effects of pulsed electric field and freezing on cell membrane permeabilisation and mass transfer during dehydration of red bell peppers. Innovat Food Sci Emerg Technol 4:177–188

52. Fincan M, DeVito F, Dejmek P (2004) Pulsed electric field treatment for solid-liquid extraction of red beetroot pigment. J Food Eng 64:381–388
53. Guderjan M, Elez-Martinez P, Knorr D (2007) Application of pulsed electric fields at oil yield and content of functional food ingredients at the production of rapeseed oil. Innovat Food Sci Emerg Technol 8:55–62
54. Hedrén E, Diaz V, Svanberg U (2002) Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. Eur J Clin Nutr 56(5):425–430
55. Benlloch-Tinoco M, Kaulmann A, Corte-Real J, Rodrigo D, Martinez-Navarrete N, Bohn T (2015) Chlorophylls and carotenoids of kiwifruit puree are affected similarly or less by microwave than by conventional heat processing and storage. Food Chemistry 187:254–262
56. Cilla A, Alegria A, De Ancos B, Sanchez-Moreno C, Cano MP, Plaza L, Clemente G, Lagarda MJ, Barbera R (2012) Bioaccessibility of tocopherols, carotenoids, and ascorbic acid from milk- and soy-based fruit beverages: influence of food matrix and processing. J Agric Food Chem 60(29):7282–7290
57. Blanca EL, Isabel C, Ángel GI, DámasoHornero M, Griselda HM, Sonia M, Federico F et al (2016) Effect of thermal processing on the profile of bioactive compounds and antioxidant capacity of fermented orange juice. Int J Food Sci Nut 67:779–788
58. Ankit P, Nigel P, O'Donnellb C, Tiwarib BK (2010) Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends Food Sci Technol 21:3–11
59. Concepcioa N, SaaNchez-Moreno LP, Elez-Martiänez P, De Ancos BA, Martiän-Belloso O, Pilar Cano M (2005) Impact of High Pressure and Pulsed Electric Fields on Bioactive Compounds and Antioxidant Activity of Orange Juice in Comparison with Traditional Thermal Processing. J Agric Food Chem 53:4403–4409
60. Laher F, Aremu AO, Van Staden J, Finnie JF (2013) Evaluating the effect of storage on the biological activity and chemical composition of three south African medicinal plants. South African J Botany 88:414–418
61. Srivastava A, Akoh CC, Yi W, Fischer J, Krewer G (2007) Effect of storage conditions on the biological activity of phenolic compounds of blueberry extract packed in glass bottles. J Agricult Food Chem 55:2705–2713
62. Jones RB, Frisina CL, Winkler S, Imsic M, Tomkins RB (2010) Cooking method significantly effects glucosinolate content and sulforaphane production in broccoli florets. Food Chem 123:237–242
63. Rein MJ, Renouf M, Cruz-Hernandez C, Actis-Goretta L, Thakkar SK, da Silva PM (2013) Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. British J Clin Pharmacol 75:588–602
64. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113:71–88
65. Liu RH (2013) In vitro models of the intestinal barrier. Advances Nutr 4:384S–392S
66. Parada J, Aguilera J (2007) Food microstructure affects the bioavailability of several nutrients. J Food Sci 72:502–509
67. Shi J, Le Maguer M (2000) Lycopene in tomatoes: chemical and physical properties affected by food processing. Crit Rev Biotechnol 20:293–334
68. Van het Hof KH, West CE, Weststrate JA, Hautvast JG (2000) Dietary factors that affect the bioavailability of carotenoids. J Nutr 130:503–506
69. Chidambara Murthy KN, Rajesha J, Vanitha A, Swamy MM, Ravishankar G (2005) Protective effect of *Dunaliella salina* – a marine micro alga, against carbon tetrachloride-induced hepatotoxicity in rats. Hepatol Res 33:313–319
70. Chidambara Murthy KN, Vanitha A, Rajesha J, Swamy MM, Sowmya P, Ravishankar GA (2005) In vivo antioxidant activity of carotenoids from *Dunaliella salina* – a green microalga. Life Sci 76:1381–1390
71. Hedrén E, Mulokozi G, Svanberg U (2002) In vitro accessibility of carotenes from green leafy vegetables cooked with sunflower oil or red palm oil. Int J Food Sci Nutr 53:445–453

72. Aherne SA, Daly T, Jiwan MA, O'Sullivan L, O'Brien NM (2010) Bioavailability of β-carotene isomers from raw and cooked carrots using an in vitro digestion model coupled with a human intestinal Caco-2 cell model. Food Res Int 43:1449–1454
73. Omoni AO, Aluko RE (2005) The anti-carcinogenic and anti-atherogenic effects of lycopene. Trends Food Sci Technol 16(8):344–350
74. Gärtner C, Stahl W, Sies H (1997) Lycopene is more bioavailable from tomato paste than from fresh tomatoes. Am J Clin Nutr 66:116–122
75. Hof KH, de Boe VH, Tijburg LBM, Lucius BRHM, Zijp I, West CE, Hautvast JG, Weststrate JA (2000) Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. J Nutr 130:1189–1196
76. Cooperstone JL, Ralston RA, Riedl KM, Haufe TC, Schweiggert RM, King SA, Timmers CD, Francis DM, Lesinski GB, Clinton SK (2015) Enhanced bioavailability of lycopene when consumed as cis-isomers from tangerine compared to red tomato juice, a randomized, cross-over clinical trial. Molec Nutr Food Res 59:658–669
77. Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130:2073S–2085S
78. Takimoto H, Tamura T (2006) Increasing trend of spina bifida and decreasing birth weight in relation to declining body-mass index of young women in Japan. Med Hypoth 67:1023–1026
79. McNulty H, Pentieva K (2004) Folate bioavailability. Proc Nutr Soc 63:529–536
80. Mormont M, Muller M, Maghuin-Rogister G, Scippo M L, Van Der Heiden E, Ribonner L, Larondelle, Y et al (2009) Food interactions: effects on health, consumer perception and impact on agro-food industries,"FOODINTER", Belgian Science Policy
81. Genser D (2008) Food and drug interaction: consequences for the nutrition/health status. Ann Nutr Metabol 52:29–32
82. Dahan A, Altman H (2004) Food–drug interaction: grapefruit juice augments drug bioavailability – mechanism, extent and relevance. Eur J Clin Nutr 58(1):1–9
83. Chan LN, Shike M, Ross AC, Caballero B, Cousins RJ (2006) Drug-nutrient interactions. In: Modern Nutrition in Health and Disease. Lippincott Williams & Wilkins, Baltimore, pp 1540–1553
84. Huang SM, Strong JM, Zhang L, Reynolds KS, Nallani S, Temple R, Abraham S, Habet SA, Baweja RK, Burckart GJ (2008) New era in drug interaction evaluation: US Food and Drug Administration update on CYP enzymes, transporters, and the guidance process. J Clin Pharmacol 48(6):662–670
85. Blaauw R (2011) Malabsorption: causes, consequences, diagnosis and treatment. South African J Clin Nutr 24(3):125–127
86. Wormhoudt LW, Commandeur JN, Vermeulen NP (1999) Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. Crit Rev Toxicol 29(1):59–124
87. Veber Daniel F, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD (2002) Molecular Properties That Influence the Oral Bioavailability of Drug Candidate. Journal of Medicinal Chemistry 45:2615–2623
88. Gao J, Sudoh M, Aube J, Borchardt RT (2001) Transport characteristics of peptides and peptidomimetics: I. N-methylated peptides as substrates for the oligopeptide transporter and P-glycoprotein in the intestinal mucosa. J Pept Res 57:316–329
89. Tulipani S, Urpi-Sarda M, García-Villalba R, Rabassa M, López-Uriarte P, Bullo M et al (2012) Urolithins are the main urinary microbial-derived phenolic metabolites discriminating a moderate consumption of nuts in free-living subjects with diagnosed metabolic syndrome. J Agric Food Chem 60(36):8930–8940
90. Namasivayam N (2011) Chemoprevention in experimental animals. Ann N Y AcadSci 1215:60–71
91. Miene C, Weise A, Glei M (2011) Impact of polyphenol metabolites produced by colonic microbiota on expression of COX-2 and GSTT2 in human colon cells (LT97). Nutr Cancer 63(4):653–662

92. Sengottuvelan M, Nalini N (2006) Dietary supplementation of resveratrol suppresses colonic tumour incidence in 1, 2-dimethylhydrazine-treated rats by modulating biotransforming enzymes and aberrant crypt foci development. Br J Nutr 96(1):145–153
93. Petr H, Pavel T, Marie S (2001) Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. Chemico-Biological interactions 139:1–21
94. Manthey JA (2000) Biological properties of flavonoids pertaining to inflammation, microcirculation. Postharvest biology and technology 41:151–155
95. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM (2001) Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metabol 86(8):3815–3819
96. Kang ZC, Tsai SJ, Lee H (1999) Quercetin inhibits benzo[a]pyrene-induced DNA adducts in human Hep G2 cells by altering cytochrome P-450 1A1 expression. Nutr Cancer 35 175–179
97. Ciolino HP, Daschner PJ, Yeh GC (1999) Dietary flavonols quercetin and kaempferol are ligands of aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. Biochem J 340:715–722
98. Hur SJ, Lim BO, Decker EA (2011) In vitro human digestion models for food applications. Food Chem 125(1):1–12
99. Minekus M, Alminger M, Alvito P, Balance S, Bohn T, Bourlieu C, Carriere F, Boutrou et al (2014) A standardised static in vitro digestion method suitable for food–an international consensus. Food Function 5(6):1113–1124
100. Ting Y, Zhao Q, Xia C, Huang Q (2015) Using in vitro and in vivo models to evaluate the oral bioavailability of nutraceuticals. J Agric Food Chem 63(5):1332–1338
101. Au AP, Reddy MB (2000) Caco-2 cells can be used to assess human iron bioavailability from a semipurified meal. J Nutr 130(5):1329–1334
102. Le Ferrec E, Chesne C, Artusson P, Brayden D, Fabre DG, Gires P, Guillou F, Rousset M, Rubas W, Scarino ML (2001) *In Vitro* models of the intestinal barrier. Atla 29:649–668
103. Carbonell-Capella JM, Buniowska M, Barba FJ, Esteve MJ, Frígola A (2014) Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables, a review. Comprehensive Rev Food Sci Food Safety 13:155–171

Bioactive Food Components in the Prevention of Cardiovascular Diseases

6

Arti Parihar and Mordhwaj S. Parihar

Contents

Abstract

Cardiovascular diseases (CVDs) remain one of the leading causes of death globally. The risk factors such as lipids, lipoproteins, and inflammation play a critical role in CVD. Lifestyle factors directly influence the risk of CVD. Understanding of the risk factors and the disease-causing mechanisms will lead to novel therapeutic treatments. Emerging data have explored the utility of natural food-based strategies in the management of diseases. Increasing interest has been grown up in recent years on the health-related products of food industry and to understand how foods products can help and maintain the individual cardiovascular health. Plant extracts rich in bioactive components could be used as the

A. Parihar (✉)
Bellingham Technical College, Bellingham, WA, USA
e-mail: asp1959@gmail.com

M. S. Parihar
School of Studies in Zoology and Biotechnology, Vikram University, Ujjain, Madhya Pradesh, India
e-mail: mdsparihar@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_55

functional ingredients for providing many health benefits. The recent advances in health benefits of bioactive components provide novel therapeutic approaches, which have played an important role in the reductions of CVD worldwide. This chapter presents a critical review of the potential benefits of bioactive foods consumed through diet to reduce the incidence of cardiovascular disease.

Keywords
Cardiovascular disease · Coronary heart disease · Atherogenesis · Dyslipidemia · Inflammation · Active ingredients · Bioactive components of foods

1 Introduction

Cardiovascular diseases (CVDs) cover a wide group of disorders that involve the cardiac muscles and the vascular system. CVDs are regarded as the most common human health problem throughout the world. The mortality rates differ considerably from country to country with Japan and other Mediterranean countries having the lowest rates [1]. CVD is the major cause of the death increasing day by day in developed and developing countries [2, 3]. Heart failure is more common in countries with lower socioeconomic status and with those who tend to adopt unhealthier lifestyle, such as smoking and careless dietary habits [4–6]. CVD alone is responsible for taking 17.7 million lives every year, and atherosclerosis has been recognized as the prominent symptomatic anomaly responsible for CVD-related deaths [7]. The most important CVDs includes coronary heart disease, cerebrovascular disease, peripheral arterial disease, heart failure, rheumatic heart disease, congenital heart disease, deep vein thrombosis, and pulmonary embolism. Heart attacks and strokes that usually caused by a blockage of blood flow to the heart or brain are the most important acute manifestations of CVD in human. The causes of this global epidemic have been largely explained to originate from lifestyle factors which directly impact the novel pathways of CVD risk [8]. The increased lipids in the blood and build-up of fatty deposits on the inner walls have been strongly correlated with the increased incidence of CVD. Usually, combinations of risk factors are involved in causing the heart attacks and strokes. The most important factors associated with the incidence of CVD are lack of physical activity, drinking alcohol, use of tobacco, unhealthy diet, obesity, hypertension and hyperlipidemia. Diabetes mellitus is regarded as another epidemiological factor associated with an increasing prevalence of CVD [9, 10].

Atherosclerosis, mainly located in the intima of middle sized and large arteries, is the major cause of myocardial infarction, heart failure, and stroke. Dyslipidemias in vascular endothelium [11] and cholesterol deposition are the major contributors of atherosclerosis. Low-density lipoprotein (LDL) cholesterol when oxidized is pro-inflammatory and immunogenic and acts as an independent risk factor for CVD. The increase in oxidized LDL cholesterol results in endothelial dysfunction and directly influences the development of atherosclerosis. The decline in high-density lipoprotein (HDL) cholesterol increases the susceptibility to atherosclerosis substantially while the increase in the HDL cholesterol may reduce the incidence of coronary

heart disease (CHD) [12] and reduces the risk of CVD [13]. Appropriate levels of HDL cholesterol may also be responsible for clearing oxidized LDL cholesterol from the tissue by obstructing the monocytes attachment to the endothelium layer of blood vessels. Maintaining the HDL cholesterol level may also initiate the nitric oxide release that stops vascular bed atherogenesis [14]. Since the built up of atherosclerosis lesions require a long period of time, therefore, initiation of early lipid management may help prevention of atherosclerotic vascular diseases [15]. To manage the lipid level in patients with dyslipidemia, alternatives therapies have been designed in recent years [16]. Lifestyle modifications have been suggested as the primary prevention strategy in managing cardiovascular risk whereas food supplements were prescribed to patients to reduce the risk factors and symptomatic relief from CVD. The natural foods obtained from medicinal plants have been tried that might confer some benefit on some patient. However, more research is required that may include clinical trials with long follow-up outcomes to conclude their effectiveness against CVD illnesses.

The past few years have witnessed the extensive expansion of research on the association between dietary food and CVD. Certain foods have been recognized showing the effectiveness in reducing the risk of chronic diseases. These specific foods and food components appear as therapeutic strategies in the reduction and prevention of the risk of CVD. In recent years, a considerable importance has been given to functional foods. Apart from their basic nutritional effects, the functional foods exhibit an important role in disease prevention, or slowing the progression of many chronic illnesses. These functional foods work on two basic principles either possess a component with positive health benefits or remove a component with negative effects on the body functions. The relationship between the nutritional value of food components and the prevention of several chronic diseases led to increase in their demand in the market. However, to sustain in the market these foods must be safe, healthy, and delicious. They may be composed of a single compound that physiologically active or may include the addition of other food components to make them functional including omega fatty acids, prebiotics, phytochemicals, and bioactive peptides.

Bioactive food components are physiologically active constituents that are present in minute quantities in plant products and lipid-rich foods [17]. They are being extensively studied to evaluate their beneficial effects on health. These compounds vary widely in chemical structure and function and are grouped accordingly. Scientific evidence indicates that those certain bioactive food components participate in the prevention of CVD [17, 18]. Oral supplements of bioactive food components when taken along with the routine diet may increase the absorption of nutrients that will have clinical benefit in some diseases. Usually, bioactive components of foods are taken in addition to a healthy diet but they do not serve as the substitution to conventional food [19]. Their consumption as part of basic nutrition exerts useful physiologic effects in reducing the risk of diseases [20]. They may act at the different metabolic pathways that control various metabolism including lipid disorders in humans. By virtue of their targeted actions on various metabolic pathways, they are believed to have the therapeutic advantages for reducing the risk of CVD by combating the inflammation and dyslipidemias [21]. They have well-illustrative

beneficial biological effects such as antilipidemic, antihypertensive, antiglycemic, antithrombotic, and antiatherogenic. With the perceptible health benefits of bioactive components against various diseases, a wide array of the metabolomics and physiological relevance of these compounds were established [22]. A recent study in the United States, adults showed the associations of suboptimal intakes of dietary factors with mortality due to CVDs [4]. These functional foods, apart from providing basic nutritions, provide therapeutic benefits in the management of chronic diseases [23]. In this chapter, we thoroughly examined the beneficial effects of bioactive food components in reducing the risk factors of CVD including hypertension, dyslipidemias, oxidative stress, and inflammation.

2 Risk Factors of Cardiovascular Disease

A number of risk factors such as changes in lifestyle, age, physical inactivity, blood pressure, smoking, alcohol, obesity, hyperlipidemia, and diabetes have been listed for the pathogenesis of CVD [24–26]. Inflammation has been regarded as the principal molecular mechanisms responsible for atherogenesis [27–29]. During inflammation nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a family of transcription factors that regulate varied processes in immune cells, initiates encoding of a number of genes responsible for cytokines and chemokines production [30]. The NF-κB signaling and cytokine secretion are found increased in atherosclerosis [31]. The IkappaB kinase/NF-kappaB (IKK/NF-κB) signaling pathway plays an important role in inflammation. In addition, this signaling pathway also regulates many other biological functions, including growth and survival of cells. These authors further elaborated that the activation of IKK/NF-κB pathway, which is an important regulator of inflammation, in cardiovascular tissues produce an excessive inflammatory response that causes cardiomyopathy leading to heart failure. Oxidative stress is another risk factor affecting the cardiovascular tissue in many ways. Oxidative stress can influence the functioning of endothelial layer of blood vessels, thereby leading to cardiovascular disease [32]. Reactive oxygen species (ROS) can have a direct effect on cardiac cells by oxidizing cellular constituents and disrupting the functions of proteins and enzymes [33]. The increase in ROS during the myocardial ischemia, hypoxia, and reoxygenation is the principal causes of reperfusion injury in cardiac tissues. During reoxygenation, increase in ROS cause direct oxidative damage to cellular components [34]. The obesity has been extensively correlated with the incidence of CVD, particularly among women [35]. Studies have shown an important connection between the obesity and dyslipidemia and the metabolic syndrome [36]. Dyslipidemia is mainly characterized by elevated levels of LDL-cholesterol and decline in HDL-cholesterol [37]. Hypercholesterolemia with total cholesterol level above 190 mg/L is the major form of dislipidemia and considered as one of the major risk factors for the CVD.

Hypertension is one of the major risk factors for cardiovascular-related illness. It is believed that hypertension is the single greatest contributor to cardiovascular disease [38]. With aging population throughout the world, the prevalence of

hypertension has a steep increase [39]. In addition to its detrimental effects on cardiovascular tissues, the hypertension is also associated with other cardiovascular risk factors, such as metabolic syndrome [40] and renal disease [41]. Changes in lifestyle, lowering sodium intake, reducing alcohol consumption, and weight reduction or physical exercise may lower blood pressure and thus reduce CVD [42]. Cigarette smoking is another important risk factor for CVD. An estimated 34.7% of all deaths resulting from cigarette smoking is related to CVD. Tobacco use cause impairment in endothelium-dependent vasodilation in the coronary microcirculation. The vasodilation response which was partly initiated by the release of nitric oxide (NO) was reduced in individuals who smoked [43]. Sera from smokers contain a reduced expression of endothelial nitric oxide synthase (eNOS) [44], whereas a brief exposure to tobacco smoke has caused the production of peroxynitrite ($ONOO^-$) [45]. Consumption of tobacco has been linked with the increase in the oxidation of LDL cholesterol, platelet aggregation, and impairment of endothelial layer [46]. Smoking for long-term increases the prevalence of hypertension, stroke, and atherosclerosis. Alcohol consumption is also associated with CVD. Although moderate consumption of alcohol cause lower risk of coronary heart disease, excess consumption of alcohol is detrimental to cardiovascular tissue [47]. These studies suggest that lifestyle changes such as physical inactivity, tobacco smoking, and heavy alcohol consumption are the risk factors contribute to CVD. The oxidizing chemicals, nicotine, carbon monooxide, and other particulate matters are believed to be accountable for cardiovascular disease.

3 Diet and Cardiovascular Disease

The life style and dietary patterns are often directly related with the development of hypertension, diabetes, and CVD. The nutritional diets may have a direct impact on the functioning of circulatory system physiology, thus affecting the occurrences of these diseases [48]. The metabolic abnormalities and the risk factors for CVD including dyslipidemia, central obesity, and hypertension are mostly depend upon the intake of an excess of total energy such as consuming high calorie or fatty meal [49, 50]. Individuals with dyslipidemia and high cholesterol levels were advised to take a diet rich in bioactive substances, fiber, and antioxidants and minimize the consumption of saturated and *trans* fatty acids. The dietary interventions that interfere with the reduction of plasma cholesterol and triglyceride levels may be effective in the prevention and management of CVD [51]. The diet consisting of nutritionally poor foods with high calorie and highly processed with deficiency of functional foods has been found to enhance systemic inflammation [52]. Food rich in fruits and vegetables along with moderate amounts poultry, fish, and meat has been associated with the reduction of inflammation thus prevent the occurrence of CVD. The direct correlation between serum total cholesterol and the heart attack and stroke was known since long time. Similarly, the importance of the Mediterranean diet in reducing the risk of coronary heart disease (CHD) [53–58] was also known since many years ago. Several studies were conducted describing the lifestyle and diets of

a population in the Mediterranean region and their relation to the rates of heart diseases [59, 60]. The traditional Mediterranean diet consisting of cereals, olive oil, fish, legumes, fruits, vegetables, dairy and meat products and moderate quantity of wine is quite beneficial for the heart. Consuming such diet provides a low risk of CHD and prevents the heart disease. Studies in individuals who opted for Mediterranean food [61] have shown a reduction in the incidence of CHD. Similarly, the choice of the Mediterranean diet with extra-virgin olive oil or nuts among individuals who are at high cardiovascular risk showed a reduction in CVD [62]. The studies performed on the composition of diet that should be useful for the prevention of CVD is limited. Thus, there is an urgent need to undertake such meticulously designed studies on the dietary food components that may be useful in reducing the lifestyle generated cardiovascular risk.

4 Types of Food Components Used for Prevention of Cardiovascular Disease

A critical role of lifestyle and diet in the prevention and treatment of CVD has become widely accepted. Besides classical food components, i.e., carbohydrates, proteins, fats, vitamins, and minerals, the diet prescribed to promote health and prevent diseases also includes the foods derived from medicinal plants known by various terms such as medicinal foods, bioactive foods, functional foods, or therapeutic foods [63], the consumption of which is believed to reduce the risk of many diseases including CVD [64]. The functional food refers to products with certain health benefit and reduced the risk of diseases. Foods such as fruits vegetables, cereals, fish, and red wine can be considered as functional foods that can prevent or cure several diseases. Medicinal food refers food product that can be considered to have therapeutic value in treating or preventing certain diseases and plays beneficial effects on physiological functions of a specific tissue. Medicinal foods are considered important in reducing the risk of certain diseases. Bioactive food components refer to constituents in foods supply with the basic human nutritional needs and taken with diet or as supplements. These food components exhibit the power to regulate one or more processes of metabolism that exerts the health benefits in reducing the risk of chronic human diseases [65]. These food components taken in appropriate quantities must be a part of the standard diet and consumed on a regular basis in order to beneficially affecting at various metabolic targets.

5 Bioactive Food Components in the Prevention of Cardiovascular Disease

One of the most favorite topics in the modern world is how to keep healthy and reduce the diseases caused by aging or changes in lifestyle. The most efficient ways to decrease the risk of common diseases, including CVD and cancer, are by limiting intake of carbohydrate/fat enriched food, limiting consumption of alcohol, limiting

salt intake, and adding the plant-derived food items, including vegetables, fruits, whole grains, legumes, nuts, and oils in the routine diet. Generally, bioactive foods are referred to those compounds that have potent antioxidative, anti-inflammatory, antithrombotic and immunomodulatory properties. These compounds possess the property to protect the body against the inflammation, cholesterol accumulation in the cardiovascular tissues, and protect against the oxidative stress, thus prevent the development of cardiovascular diseases and cancer as well as other pathological conditions such as neurodegenerative diseases [18]. Some of the most important bioactive components found in fruits and vegetables that possess the potential for promoting a healthy metabolism and in the prevention of diseases are flavonoids, carotenoids, organosulfur compounds, phytoestrogens, tocopherols, and L-ascorbic acid (Table 1). Figure 1 shows the schematic presentation of source and effects of some of the important bioactive compounds. The detailed account of occurrences and biological effects of these bioactive compounds and their role in the prevention of CVD are described individually in the following sections.

5.1 Carotenoids (Lycopene)

Carotenoids belong to a class of natural fat-soluble pigments found mainly in plants. The dietary carotenoids including lycopene, beta-carotene, lutein, beta-cryptoxanthin, zeaxanthin, and astaxanthin provide health benefits in decreasing the risk of many chronic diseases. Lycopene, the major carotenoid obtained from plants such as tomatoes, watermelon, and pink or red grapefruit, is very important in attenuating the risk of CVD. Lycopene extracted from plant sources predominantly exists in an all-*trans* isomer; however, *cis*-lycopene is the more bioavailable form [66]. The isomerization of *trans-cis* isoforms occurs with exposure to light of heat or in the gastric cavity under acid conditions [67]. Heat generated during cooking and processing converts some of the *trans*-lycopene to *cis*-lycopene increasing its bioavailability in the tissue [68, 69]. Thus the bioavailability of lycopene is greater in tomato paste and tomato puree than from fresh tomatoes [70, 71]. Thus the bioavailability of dietary lycopene is greater after cooking or when consumed with oil and other dietary fats [72, 73]. In addition, other nutrition present in tomatoes such as carotenes and lutein would also be absorbed by cells. Thus consuming whole processed tomato products will have more advantages than consuming lycopene alone. Although some studies concluded that consumption of a carotenoid-rich diet have no positive effect on plasma antioxidant status or markers of oxidative stress [74], but the majority of epidemiological and clinical studies have shown beneficial effects of lycopene or tomato-based food preparation in cardiovascular health. Lycopene supplementation has been shown to improve biomarkers associated with CVD. Studies involving a large group of people reported the association of higher intake of tomato with a reduced risk of CVD and myocardial infarction [75]. Lycopene level in the blood serum also improved the common carotid artery intima-media thickness which is an indicator of early atherosclerosis [76]. The intake of lycopene caused the reduction in cholesterol synthesis and enhanced the

Table 1 List of important bioactive food components that may influence cardiovascular diseases

Category	Bioactive components	Source	Cardiovascular effects	References
Carotenoids	Lycopene (carotenoid)	Tomatoes, watermelon, and pink or red grapefruit	Hypolipemic, inhibitor of proinflammatory and prothrombotic factors	[77–80]
Flavonoids	Genistein (Isoflavone)	Soybean, soy products	Antiatherosclerotic by inhibiting the expression of ICAM-1 and VCAM-1 and NFk-B on human endothelial cells	[105]
	Sulforaphane (isothiocyanate)	Cruciferae Family	Anti-inflammatory and antioxidant by stimulating Nrf2	[119]
	Apigenin (flavone)	Celery, parsley, and chamomile	Myocardial ischemia/ reperfusion injury reduction, inhibition of lymphocyte proliferation, and proinflammatory cytokine expression	[110, 112, 113]
	Quercetin (Flavanols)	Onions and shallots	Antiplatelet aggregation activity, reduction of mycocardial infarction	[121, 122]
	Resveratrol (polyphenolic compound)	Grapes, red wine	Reduction in LDL oxidation, vasorelaxation, reduction of platelet aggregation, antiatherosclerotic,	106–109
	Anthocyanins (glycosides of anthocyanidins)	Fruits and vegetables	Antiatherosclerotic, reduction in CVD mortality, protect DNA damage	[131–141]
	Hesperetin (flavanone)	Citrus plants	Block oxidized LDL-induced endothelial apoptosis	[94]
	Catechins (Flavanol)	Green tea	Reduction in blood cholesterol; antihypertensive; triglyceride, total cholesterol and low-density lipoprotein cholesterol	[91, 93]
Vitamins	Ascorbic acid	Fruits and vegetables	Prevent HDL from lipid oxidation	[144]
	Alpha-tocopherol	Oils, nuts, and leafy green vegetables	Prevent HDL from lipid oxidation	[147–150]

Abbreviations: *ICAM-1* Intercellular adhesion molecules-1, *VCAM-1* Vascular cell adhesion molecule-1, *Nrf2* nuclear factor-erythroid 2, *LDL* Low-density lipoprotein, *HDL* High-density lipoprotein, *CVD* Cardiovascular disease, *DNA* deoxyribonucleic acid

Fig. 1 Schematic diagram illustrating the role of bioactive components of food in the prevention of cardiovascular diseases (CVDs). As shown, bioactive components act on cells or tissue by targeting many physiological and metabolic processes. Together regulating multiple processes, these components of food prevent or cure cardiovascular diseases

degradation of LDL [77]. Oxidative damage related parameter such as lipid peroxidation and LDL oxidation were significantly declined in the subjects who were prescribed lycopene supplementation [78]. Similarly, individuals who were treated with lycopene (40 mg/day) for 6 weeks showed decline in triglyceride levels and LDL cholesterol levels whereas the HDL cholesterol was significantly increased [79]. The increase in HDL cholesterol and decline in total cholesterol/HDL cholesterol ratio was also reported in a study comprised of healthy men and women consuming the soy-tomato beverage that contain 21 mg lycopene/day on an average daily [80]. These studies suggest that lycopene or tomato-based products with a significant amount of lycopene improves the cardiovascular health by reducing the triglycerides, scavenging LDL, and maintaining HDL level thus reducing the risk of CVD.

5.2 Phenolic Acids and Flavonoids

Flavonoids are a group of polyphenolic compounds found in significant amount in many fruits, vegetables, grains, and beverages. They are classified as flavonols, flavones, flavanones, flavan-3-ols, anthocyanidins, and isoflavones. The flavonols

include compounds namely isorhamnetin, kaempferol, myricetin, and quercetin. Apigenin and luteolin compounds are grouped under flavones. Flavanones include compounds eriocitrin, hesperetin, and naringenin whereas flavan-3-ols include compounds such as catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, and gallocatechin. Anthocyanidins include compounds such as cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin, whereas compounds daidzein and genistein are grouped under isoflavones. These compounds have varied chemical structure and biological functions and are grouped accordingly. The isoflavones genistein, daidzein, and glycitein occur predominantly in legumes. Genistein and daidzein found in soy, and resveratrol occurs in grape skin and red wine, are nonsteroidal polyphenolic compounds and considered as a phytoestrogen. The flavonoids contain sulfur are grouped under organosulfur compounds, prominent among are sulforaphane found in crucifer plants and γ-glutamyl-S-allyl-L-cysteines and S-allyl-L-cysteine sulfoxides found in garlic (Allium sativum L., family Liliaceae). Anthocyanins are a group of most studied flavonoid occurs in a wide variety of plant kingdom. Anthocyanins are providing the bright red-orange to blue-violet colors in many fruits and vegetables. Their consumption has been estimated to be up to ninefold higher than that of other dietary flavonoids [81].

Phenolic acid and flavonoids have three most important health benefit effects on the prevention of cardiovascular tissue. The most important effect is attributed to their antioxidant activity [82]. Secondly they have prominent effects on the prevention of atherosclerosis [83], and lastly, they have a significant effect on the platelet aggregation [84]. In vitro studies indicate that flavonoids inhibit the oxidation of LDL and decrease the thrombotic tendencies [85]. They delay the development of atherosclerotic plaque and prevent the development of atherosclerosis by reducing the endothelial dysfunction. Another mechanism by which flavonoids help prevent CVD is through their effect on platelet aggregation. Flavonoids interact with membrane lipids and modifying the membrane fluidity [86], which is partly responsible for the antiaggregatory and disaggregatory effects on human platelets.

Catechins are polyphenolic compounds found in food and in all kinds of tea with cardioprotective properties [87]. Catechins have an important role in the prevention of cardiovascular disease [88, 89]. Catechins have been shown to reduce the accumulation of cholesterol and its oxidation products in the walls of arteries, thus help in smooth blood circulation [90]. Supplementation of catechins may reduce the serum triglyceride, total cholesterol, and low-density lipoprotein cholesterol, thus preventing atherosclerosis [91]. Catechins may improve the vascular endothelial environment by eliminating ROS [92]. In addition, catechins exhibit very strong antihypertensive activity [93]. Both (−)epigallocatechin gallate and hesperetin flavonoids block oxidized LDL-induced endothelial apoptosis thus may function as antiatherogenic agents [94]. However, there is no clear evidence to suggest a beneficial effect of tea catechins on the prevention of CVD.

Phytoestrogens are polyphenolic compounds occur in many legumes, beans, nuts, soybeans, seeds of sesame and so on, and mimic the properties of estrogens. These compounds include certain isoflavonoids, flavonoids, stilbenes, and lignans [95]. The best-studied dietary phytoestrogens are the soy isoflavones and the flaxseed

lignans. Isoflavones, such as genistein and daidzein, occur in soybeans, legumes, lentils, and chickpeas. Lignans are the most abundant nonflavonoids occur in most cereals, linseed, fruits, and vegetables. The phytoestrogens are the most intensively studied bioactive components of food with regard to their health benefits. Isoflavone content of foods such as soybeans is sold as nutritional supplements. Good scientific evidence supports the observation that phytoestrogens may play a beneficial role in reducing the risk of cardiovascular disease [18]. Diet is the chief source of phytoestrogens in human. The bioavailability of phytoestrogens in body organs may be dependent on the metabolism of intestinal bacteria. After ingestion in their *beta*-glycosidic forms, most of these phytoestrogens are hydrolyzed in the intestine to their aglycones. Then in the intestinal wall and liver aglycones are absorbed and glucuronidated [96, 97].

Isoflavones have beneficial biological effects in the cardiovascular system. They exert antiestrogenic effects by competitive inhibition at the estrogen receptor and help maintain cellular proliferation, vascular reactivity, lipid profiles, and thrombosis [98]. Epidemiologic studies reported that consumption of dietary isoflavones from soy products protects women not only from cardiovascular disease but also from breast and uterine cancer [99–102]. The lipid-lowering functions of isoflavones will have profound effects on cardiovascular protection. Although very little evidence presented to show the protective roles of phytoestrogens for cardiovascular disease, clinical studies involving Japanese women concluded that intake of high isoflavone was correlated with the reduced risk of cerebral and myocardial infarctions [103]. Phytoestrogens intake could delay the progression of atherosclerosis in vascular tissue by their pathophysiologic effects on lipid profile, reactive oxygen species production, inflammation, and tissue damage [104]. Atherosclerosis is initiated when monocytes bind to the endothelium layer of blood vessel, migrate into the tunica intima, and later develop into the foam cells. Lipid-induced and oxidant-sensitive transcription of adhesion molecules and chemokines promote the monocyte binding to endothelium. Genistein has been reported to be capable of inhibiting the expression of intercellular adhesion molecules-1 and vascular cell adhesion molecule-1 (ICAM-1 and VCAM-1) on human endothelial cells cocultured with monocytes [105], thus protecting against atherosclerosis. Resveratrol, a phytoestrogen found in high concentration in red wine, has been proposed to be the agent responsible for cardiovascular protection [106]. Its protective role in the cardiovascular system occurs by mechanisms of stimulation of reduction of low-density lipoprotein oxidation, vasorelaxation, suppression of platelet aggregation, anti-atherosclerotic properties, and also providing defense against ischemic-reperfusion injury [107]. This compound has the ability to stimulate Ca^{++}-activated K^{+} channels so as to increase endothelium nitric oxide signaling, thus exerting vasorelaxant activity [108, 109].

Apigenin, a flavonoid found in many vegetables such as celery (Apium graveolens), parsley and chamomile have demonstrated to possess the cardio-protective effects. Apigenin ameliorates myocardial ischemia/reperfusion injury via the inactivation of p38 mitogen-activated protein kinase [110]. In a cardiac hypertrophy model, the supplementation of apigenin reduces hypertension,

cardiomyocyte cross-sectional area, and serum angiotensin II [111]. Similarly, in an autoimmune myocarditis mice model, dietary apigenin cause inhibition of lymphocyte proliferation thus mediating the protection of cardiac tissue [112]. Apigenin caused reduction of LPS-induced mortality in mice by inhibiting proinflammatory cytokine expression [113] and decrease heart injury by suppressing sphingosine kinase 1/sphingosine 1-phosphate signaling pathway [114]. In a recent study, Li et al. [115] demonstrated that apigenin protects cardiac tissue damage, cardiac injury, cardiomyocyte cell death, and cardiac dysfunction against LPS- induced toxicity by its anti-inflammatory and antioxidant effect.

Organosulfur compounds are chiefly found in cruciferous vegetables, as well as in garlic and onions. Vegetables belong to Cruciferae family such as cabbage, broccoli, and kale contain rich amount of sulfur-containing compounds known as glucosinolates. Isothiocyanates are biologically active breakdown products of glucosinolates. Different types of glucosinolates are found in different cruciferous vegetables, each of which upon hydrolysis forms a different isothiocyanate [116]. For example, glucosinolate glucoraphanin, most abundantly present in 3-day-old broccoli sprouts, is converted to the isothiocyanate sulforaphane by the endogenous enzyme, myrosinase. Sulforaphane protects the chronic diseases by upregulating the "phase 2 response" known as Kelch-like ECH-associated protein 1- nuclear factor erythroid 2 p45-related factor 2-antioxidant responsive element (Keap1-Nrf2-ARE) pathway. Sulforaphane has been regarded as one of the most potent known naturally occurring inducers of cytoprotective phase 2 enzymes [117]. Glucosinolates are rapidly hydrolyzed by myrosinase, generating metabolites when raw cruciferous vegetables are cut while processing the food. While cooked vegetables inactivate myrosinase, so as to prevent the glucosinolates breakdown. Light steam cooking for about 5 min will preserve some of the myrosinase and thus allow the conversion of isothiocyanate. Most of the absorption of glucosinolates occurs in the small intestine, however, a large proportion of it reaches the colon [118] where it generates a broad range of metabolites depending on the pH and the presence of cofactors. The sulforaphane has been shown to target pro-inflammatory pathways by stimulating Nrf2 induced antioxidant enzymes [119] and downregulating the expression of NF-κB target genes which code for proinflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1β), and IL-6 [119].

Allium vegetables are recognized to be a good source of organosulfur compounds [120] and its supplementation in human exhibits antiplatelet aggregation activity. Similarly, the quercetin, the main polyphenol found in onions and shallots, also inhibited platelet aggregation in vivo [121] and in vitro [122]. Higher intake of cruciferous and allium vegetables (per serving 75 g/d) were associated with lower risk of atherosclerotic vascular disease mortality [120]. This study was conducted in older Australian women with 15 years of follow-up and concluded that higher cruciferous and allium vegetables intakes render lower risk of atherosclerotic vascular disease (ASVD) mortality in old individuals and protect vascular health. Resveratrol has recently become a well-known potent antioxidant and is found in red grapes, blueberries, cranberries, and other types of Vaccinium berries [123].

It is also known for its antithrombotic, anti-inflammatory, anticarcinogenic, and lifespan elongation effects, as well as its positive role in protection against insulin resistance [18, 124–126].

Anthocyanins are the polyphenolic compounds, possessing a characteristic C3–C6–C3 carbon structure and present in fruits, vegetables, berries, and red wine [127]. Anthocyanins are readily absorbed and metabolized in the tissue. These phenolic compounds found in the circulation and urine as intact, methylated, glucuronide derivatives, and/or sulfoconjugated forms [128–130]. Their anti-inflammatory and antioxidative properties show beneficial effects in the cardiovascular system. Compounds containing anthocyanin have been shown to reduce atherosclerosis in rodents [131]. Epidemiological studies have examined the relationship between total anthocyanin intake and risk of developing CVD. In a 16-year follow-up study period of consuming strawberry daily (intake of 0.2 mg/d of anthocyanins), the postmenopausal women participating in the Iowa Women's Health Study showed a significant reduction in CVD mortality [132]. Intake of blueberries also showed a significant decrease in coronary heart disease mortality. Moderate consumption of red wine will have a preventive effect in CVD-related mortality [133, 134]. The beneficial effects of anthocyanins in the prevention of CVD are strongly linked to the protection against reactive oxygen species-induced oxidative stress. Other mechanisms of anthocyanin beneficial role on cardiovascular tissues may be via providing protection from DNA damage, inhibiting enzyme, regulating immune responses through increased cytokine production, and exhibiting anti-inflammatory activity [135–139]. There is a significant reduction in ischemia in patients with vascular diseases who were consuming anthocyanin [140]. Inhibition of platelet activity and experimental coronary thrombosis in vivo was significantly achieved by commercial grape juice (10 mL/kg) [141]. These studies suggest that anthocyanins have a wide range of protective effects against CVD. However, epidemiological studies are insufficient to provide comprehensive information about their usefulness in CVD.

5.3 Vitamins

A substantial body of evidence has presented describing the possible role of several vitamins in the reduction of CVD risk. L-ascorbic acid or vitamin C found in a wide variety of fruits and vegetables has received a considerable attention in the past two decades as a powerful dietary antioxidant with a possible role in heart health. It is well known that HDL participates in the removal of cholesterol from sites of atherosclerotic lesion. In addition, the HDL also performs other functions that potentially have cardioprotective properties. Some of the beneficial functions of HDL include the inhibition of platelet activation, anticoagulant and profibrinolytic activities, preservation of vascular endothelial function, and protection of LDL from oxidation [142]. However, HDL is also vulnerable to lipid oxidation with ensuing loss of some cardioprotective properties [143]. Vitamin C has been shown to prevent the lipid oxidation in HDL and thus conserving the cardioprotective properties

of this lipoprotein [144]. Vitamin C also has been found to inhibit the oxidation of LDL-protein, thereby contributing to reduce atherosclerosis [145]. Although the cardiovascular other functions of vitamin C in cardiovascular diseases are not fully understood, it has been linked to improve the lipid profiles, protect arteries from arterial stiffness, and improve the endothelial function [145]. Vitamin E that includes tocopherols and tocotrienols was found in many types of oils, nuts, and leafy green vegetables and exhibit cardiovascular protective property [146]. Several studies have demonstrated the protective role of vitamin E in preventing HDL from lipid oxidation with subsequent cardioprotective benefits [147–150]. Initial studies found no correlation between serum or plasma vitamin E concentrations and death from cardiovascular diseases [151, 152]. Epidemiological studies in Finnish men found no association between serum vitamin E level and a coronary end point [153]. An extensive study on vitamin E supplementation was carried out in the USA involving 87,425 female nurses [154]. Their findings concluded that the benefit function of vitamin E supplementation was only apparent with the continued consumption for greater than 2 years. The benefit of vitamin E intake appeared to occur with both supplemental and dietary vitamin E. Overall studies suggest that there exists a relationship between consumption of vitamin E and the incidence of CVD [146].

6 Conclusion

Changes in lifestyle, dietary habits, and environmental and physiological factors directly influence the risk factors of CVD. The health benefits of fruits and vegetables are known since ancient times. However, investigations on their bioactive components and their physiological and metabolic targets attracted the significance of these compounds in preventing or treating certain chronic human diseases. Studies have shown that fruitful changes in lifestyle and regular consumption of recommended bioactive food components will help prevent chronic cardiovascular-related illness. Bioactive components of food are a challenging area in terms of its ability to regulate the metabolic process and control chronic diseases. Exact mechanism of their effects and appropriate doses on various signaling pathways needs to work out. The appropriate human dose and the risk of side effects of most of the bioactive food components are not known. Awareness among people and confidence among physicians on dietary recommendations to patients to prevent or to treat the disease are warranted. The key is to encourage people to make habit of consuming bioactive food components as part of their daily diet so as to prevent or eliminate lifestyle or age-related chronic diseases.

References

1. Ferrieres J (2004) The French paradox: lessons for other countries. Heart 90(1):107–111
2. Martínez-Augustin O, Aguilera CM, Gil-Campos M et al (2012) Bioactive anti-obesity food components. Int J Vitam Nutr Res 82:148–156

3. World Health Organization: Obesity and Overweight (2017). http://www.who.int/mediacentre/factsheets/fs311/en/
4. Micha R, Peñalvo JL, Cudhea F et al (2017) Association between dietary factors and mortality from heart disease, stroke, and type 2 diabetes in the United States. JAMA 317(9):912–924
5. Fiscella K, Tancredi D (2008) Socioeconomic status and coronary heart disease risk prediction. JAMA 300(22):2666–2668
6. Clark AM, DesMeules M, Luo W et al (2009) Socioeconomic status and cardiovascular disease: risks and implications for care. Nat Rev Cardiol 6:712–722
7. World Health Organization: Cardiovascular disease (2017). http://www.who.int/mediacentre/factsheets/fs317/en/
8. Mozaffarian D, Furberg CD, Psaty BM et al (2008) Physical activity and incidence of atrial fibrillation in older adults: the cardiovascular health study. Circulation 118(8):800–807
9. Gersh BJ, Sliwa K, Mayosi BM et al (2010) Novel therapeutic concepts: the epidemic of cardiovascular disease in the developing world: global implications. Eur Heart J 31(6):642–648
10. Yang W, Lu J, Weng J et al (2010) Prevalence of diabetes among men and women in China. N Engl J Med 362(12):1090–1101
11. Mallika V, Goswami B, Rajappa M (2007) Atherosclerosis pathophysiology and the role of novel risk factors: a clinicobiochemical perspective. Angiology 58:513–512
12. Gordon DJ, Probstfield JL, Garrison RJ et al (1989) High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 79:8–15
13. Xavier HT, Izar MC, Faria NJR et al (2013) V Brazilian guideline on dyslipidemia and prevention of atherosclerosis Brazilian. Arq Bras Cardiol 101:1–20
14. Beisiegel U (1998) Lipoprotein metabolism. Eur Heart J 19(Suppl A):A20–A23
15. Rosin S, Ojansivu I, Kopu A, Keto-Tokoi M, Gylling H (2015) Optimal use of plant Stanol Ester in the Management of Hypercholesterolemia. Cholesterol 2015:706970
16. Hunter PM, Hegele RA (2017) Functional foods and dietary supplements for the management of dyslipidaemia. Nat Rev Endocrinol 13(5):278–288
17. Kitts DD (1994) Bioactive substances in food: identification and potential uses. Can J Physiol Pharmacol 72:423–434
18. Kris-Etherton PM, Hecker KD, Bonanome A et al (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113(suppl 9B):71S–88S
19. Weaver CM (2014) Bioactive foods and ingredients for health. Adv Nutr 5:306S–311S
20. Langella C, Naviglio D, Marino M et al (2015) Study of the effects of a diet supplemented with active components on lipid and glycaemic profiles. Nutrition 31:180–186
21. Scicchitanoa P, Camelib M, Maielloc M et al (2014) Nutraceuticals and dyslipidaemia: beyond the common therapeutics. J Funct Foods 2014:11–32
22. Badimon L, Chagas P, Chiva-Blanch G (2017) Diet and cardiovascular disease: effects of foods and nutrients in classical and emerging cardiovascular risk factors. Curr Med Chem 24:1. [Epub ahead of print]
23. USDA (2010) Dietary guidelines for Americans. Available from: http://www.health.gov/dietaryguidelines/2010.asp
24. Massaro M, Scoditti E, Carluccio MA et al (2010) Nutraceuticals and prevention of atherosclerosis: focus on omega-3 polyunsaturated fatty acids and Mediterranean diet polyphenols. Cardiovasc Ther 28:e13–e19
25. Chomistek AK, Manson JE, Stefanick ML et al (2013) Relationship of sedentary behavior and physical activity to incident cardiovascular disease: results from the Women's health initiative. J Am Coll Cardiol 61:2346–2354
26. Do KA, Gree A, Guthrie JR et al (2000) Longitudinal study of risk factors for coronary heart disease across the menopausal transition. Am J Epidemiol 151:584–593
27. Libby P (2002) Inflammation in atherosclerosis. Nature 420:868–874
28. Plutzky J (2001) Inflammatory pathways in atherosclerosis and acute coronary syndromes. Am J Cardiol 88:10K–15K
29. Ross R (1999) Atherosclerosis – an inflammatory disease. N Engl J Med 340:115–126
30. Pahl HL (1999) Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18:6853–6866

31. Cuaz-Perolin C, Billiet L, Bauge E et al (2008) Antiinflammatory and antiatherogenic effects of the NF-kappaB inhibitor acetyl-11-keto-beta-boswellic acid in LPS-challenged ApoE−/− mice. Arterioscler Thromb Vasc Biol 28(2):272–277
32. Elahi M, Matata B (2005) Blood-dependent redox activity during extracorporeal circulation in health and disease. Cardiology 1:156–157
33. Wilson SH, Best PJ, Edwards WD et al (2002) Nuclear factor-kappa B immunoreactivity is present in human coronary plaque and enhanced in patients with unstable angina pectoris. Atherosclerosis 160(1):147–153
34. Granger DN, Kvietys PR (2015) Reperfusion injury and reactive oxygen species: the evolution of a concept. Redox Biol 6:524–551
35. Hubert HB, Feinleib M, McNamara PM et al (1983) Obesity as an independent risk factor for cardiovascular disease: a 26 year follow-up of participants in the Framingham heart study. Circulation 67(5):968–977
36. Klop B, Elte JWF, Cabezas MC (2013) Dyslipidemia in obesity: mechanisms and potential targets. Forum Nutr 5(4):1218–1240
37. Esmaillzadeh A, Azadbakht L (2008) Food intake patterns may explain the high prevalence of cardiovascular risk factors among Iranian women. J Nutr 138(8):1469–1475
38. Lopez AD, Mathers CD, Ezzati M et al (2006) Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 367:1747–1757
39. Kjeldsen SE (2017) Hypertension and cardiovascular risk: general aspects. Pharmacol Res. pii: S1043-6618 (17)31118-0. [Epub ahead of print]
40. Malik S, Wong ND, Franklin SS et al (2004) Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease and all causes in United States adults. Circulation 110:1245–1250
41. Tasic I, Lovic D (2018) Hypertension and cardiometabolic disease. Front Biosci (Schol Ed) 10:166–174
42. Watkins PJ (2003) ABC of diabetes: cardiovascular disease, hypertension and lipids. Br Med J 326:874–876
43. Kugiyama K, Yasue H, Ohgushi M et al (1996) Deficiency in nitric oxide bioactivity in epicardial coronary arteries of cigarette smokers. J Am Coll Cardiol 28(5):1161–1167
44. Barua RS, Ambrose JA, Srivastava S et al (2003) Reactive oxygen species are involved in smoking-induced dysfunction of nitric oxide biosynthesis and upregulation of endothelial nitric oxide synthase: an in vitro demonstration in human coronary artery endothelial cells. Circulation 107(18):2342–2347
45. Deliconstantinos G, Villiotou V, Stavrides JC (1994) Scavenging effects of hemoglobin and related heme containing compounds on nitric oxide, reactive oxidants and carcinogenic volatile nitrosocompounds of cigarette smoke: a new method for protection against the dangerous cigarette constituents. Anticancer Res 14(6B):2717–2726
46. Bloomer RJ (2007) Decreased blood antioxidant capacity and increased lipid peroxidation in young cigarette smokers compared to nonsmokers: impact of dietary intake. Nutr J 6:39–43
47. Wakabayashi I (2009) Impact of body weight on the relationship between alcohol intake and blood pressure. Alcohol Alcohol 44(2):204–210
48. Ravera A, Carubelli V, Sciatti E et al (2016) Nutrition and cardiovascular disease: finding the perfect recipe for cardiovascular health. Forum Nutr 8(6):363–390
49. Saura-Calixto F, Goni I (2009) Definition of the Mediterranean diet based on bioactive compounds. Crit Rev Food Sci Nutr 49(2):145–152
50. Moller DE, Kaufman KD (2005) Metabolic syndrome: a clinical and molecular perspective. Annu Rev Med 56:45–62
51. Vincent-Baudry S, Defoort C, Gerber M et al (2005) The Medi-RIVAGE study: reduction of cardiovascular disease risk factors after a 3-mo intervention with a Mediterranean-type diet or a low-fat diet. Am J Clin Nutr 82:964–971
52. Alissa EM, Ferns GA (2012) Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. J Nutr Metab 2012:1–16

53. Keys A, Menotti A, Karvonen MJ et al (1986) The diet and 15-year death rate in the seven countries study. Am J Epidemiol 124:903–915
54. Knoops KT, de Groot LC, Kromhout D et al (2004) Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. JAMA 292: 1433–1439
55. Fidanza F (1991) The Mediterranean Italian diet: keys to contemporary thinking. Proc Nutr Soc 50:519–526
56. Kromhout D, Keys A, Aravanis C et al (1989) Food consumption patterns in the 1960s in seven countries. Am J Clin Nutr 49:889–894
57. Panagiotakos DB, Pitsavos C, Polychronopoulos E et al (2004) Can a Mediterranean diet moderate the development and clinical progression of coronary heart disease? A systematic review. Med Sci Monit 10(8):RA193–RA198
58. Martinez-Gonzalez MA, Bes-Rastrollo M, Serra-Majem L et al (2009) Mediterranean food pattern and the primary prevention of chronic disease: recent developments. Nutr Rev 67(suppl 1):S111–S116
59. Kafatos A, Diacatou A, Voukiklaris G et al (1997) Heart disease risk-factor status and dietary changes in the Cretan population over the past 30 y: the seven countries study. Am J Clin Nutr 65:1882–1886
60. Menotti A, Keys A, Kromhout D et al (1993) Inter-cohort differences in coronary heart disease mortality in the 25-year follow-up of the seven countries study. Eur J Epidemiol 9:527–536
61. Martinez-Gonzalez MA, Garcia-Lopez M, Bes-Rastrollo M et al (2011) Mediterranean diet and the incidence of cardiovascular disease: a Spanish cohort. Nutr Metab Cardiovasc Dis 21:237–244
62. Estruch R, Ros E, Martinez-Gonzalez MA (2013) Mediterranean diet for primary prevention of cardiovascular disease. N Engl J Med 369:676–677
63. Nagai T, Inoue R (2004) Preparation and functional properties of water extract and alkaline extract of royal jelly. Food Chem 84:181–186
64. Mozaffarian D (2016) Dietary and policy priorities for cardiovascular disease, diabetes, and obesity – a comprehensive review. Circulation 133(2):187–225
65. Parihar P, Parihar MS (2017) Metabolic enzymes deregulation in heart failure: the prospective therapy. Heart Fail Rev 22(1):109–121
66. Boileau AC, Merchen NR, Wasson K et al (1999) Cis-lycopene is more bioavailable than trans-lycopene in vitro and in vivo in lymph-cannulated ferrets. J Nutr 129:1176–1181
67. Re R, Fraser PD, Long M, Bramley PM et al (2001) Isomerization of lycopene in the gastric milieu. Biochem Biophys Res Commun 281:576–581
68. Nguyen ML, Schwartz SJ (1998) Lycopene stability during food processing. Proc Soc Exp Biol Med 218:101–105
69. Gupta R, Kopec RE, Schwartz SJ et al (2011) Combined pressure-temperature effects on carotenoid retention and bioaccessibility in tomato juice. J Agric Food Chem 59:7808–7817
70. Porrini M, Riso P, Testolin G (1998) Absorption of lycopene from single or daily portions of raw and processed tomato. Br J Nutr 80:353–361
71. van het Hof KH, de Boer BC, Tijburg LB et al (2000) Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. J Nutr 130:1189–1196
72. Gartner C, Stahl W, Sies H (1997) Lycopene is more bioavailable from tomato paste than from fresh tomatoes. Am J Clin Nutr 66:116–122
73. Brown MJ, Ferruzzi MG, Nguyen ML et al (2004) Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. Am J Clin Nutr 80:396–403
74. Paterson E, Gordon MH, Niwat C et al (2006) Supplementation with fruit and vegetable soups and beverages increases plasma carotenoid concentrations but does not alter markers of oxidative stress or cardiovascular risk factors. J Nutr 136(11):2849–2855

75. Sesso HD, Liu S, Gaziano JM et al (2003) Dietary lycopene, tomato-based food products and cardiovascular disease in women. J Nutr 133(7):2336–2341
76. Rissanen TH, Voutilainen S, Nyyssönen K et al (2003) Serum lycopene concentrations and carotid atherosclerosis: the Kuopio Ischaemic Heart Disease Risk Factor Study. Am J Clin Nutr 77(1):133–138
77. Arab L, Steck S (2000) Lycopene and cardiovascular disease. Am J Clin Nutr 71(6 Suppl): 1691S–1695S
78. Agarwal S, Rao AV (1998) Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. Lipids 33(10):981–984
79. Shen YC, Chen SL, Wang CK (2007) Contribution of tomato phenolics to antioxidation and down-regulation of blood lipids. J Agric Food Chem 55(16):6475–6481
80. Bohn T, Blackwood M, Francis D et al (2013) Bioavailability of phytochemical constituents from a novel soy fortified lycopene rich tomato juice developed for targeted cancer prevention trials. Nutr Cancer 65(6):919–929
81. Wallace TC (2011) Anthocyanins in cardiovascular disease. Adv Nutr 2:1–7
82. Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. J Nutr Biochem 13:572–584
83. Tripoli E, La Guardia M, Giammanco S et al (2007) Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review. Food Chem 104:466–479
84. Lamuela-Raventos RM, Romero-Perez AI, Andres-Lacueva C et al (2005) Review: health effects of cocoa flavonoids. Food Sci Technol Int 11(3):159–176
85. Benavente-Garcia O, Castillo J, Marin FR et al (1997) Uses and properties of citrus flavonoids. J Agric Food Chem 45(12):6505–6515
86. Furusawa M, Tsuchiya H, Nagayama M (2003) Anti-platelet and membrane-rigidifying flavonoids in brownish scale of onion. J Health Sci 49(6):475–480
87. Chen XQ, Hu T, Han Y et al (2016) Preventive effects of Catechins on cardiovascular disease. Molecules 21(12):1759–1765
88. Legeay S, Rodier M, Fillon L et al (2015) Epigallocatechin gallate: a review of its beneficial properties to prevent metabolic syndrome. Nutrients 7(7):5443–68
89. Vita JA (2005) Polyphenols and cardiovascular disease: effects on endothelial and platelet function. Am J Clin Nutr 81:292S–297S
90. Koo SI, Green NS (2007) Tea as inhibitor of the intestinal absorption of lipids: potential mechanism for its lipid-lowering effect. J Nutr Biochem 18(3):179–183
91. Ahmad RS, Butt MS, Sultan MT et al (2015) Preventive role of green tea catechins from obesity and related disorders especially hypercholesterolemia and hyperglycemia. J Transl Med 13:79–85
92. Kipshidze N, Dangas G, Tsapenko M et al (2004) Role of the endothelium in modulating neointimal formation-Vasculoprotective approaches to attenuate restenosis after percutaneous coronary interventions. J Am Coll Cardiol 2004(44):733–739
93. Bogdanski P, Suliburska J, Szulinska M et al (2012) Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves, parameters associated with insulin resistance in obese, hypertensive patients. Nutr Res 32:421–427
94. Choi JS, Choi YJ, Shin SY et al (2008) Dietary flavonoids differentially reduce oxidized LDL-induced apoptosis in human endothelial cells: role of MAPK- and JAK/STAT-signaling. J Nutr 138(6):983–990
95. Dixon RA (2004) Phytoestrogens. Annu Rev Plant Biol 55:225–261
96. Doerge DR, Chang HC, Churchwell MI (2000) Analysis of soy isoflavone conjugation in vitro and in human blood using liquid chromatography-mass spectrometry. Drug Metab Dispos 283:298–307
97. Rowland I, Faughnan M, Hoey L (2003) Bioavailability of phyto-estrogens. Br J Nutr 89(Suppl 1):S45–S58
98. Lissin LW, Cooke JP (2000) Phytoestrogens and cardiovascular health. J Am Coll Cardiol 35:1403–1410

99. Colditz GA, Willett WC, Stampfer MJ et al (1987) Menopause and the risk of coronary heart disease in women. N Engl J Med 316:1105–1110
100. Parker WH, Broder MS, Chang E et al (2009) Ovarian conservation at the time of hysterectomy and long-term health outcomes in the nurses' health study. Obstet Gynecol 113:1027–1037
101. Shimazu T, Inoue M, Sasazuki S et al (2011) Plasma isoflavones and the risk of lung cancer in women: a nested case–control study in Japan. Cancer Epidemiol Biomarkers Prev 20:419–427
102. Kurahashi N, Iwasaki M, Sasazuki S et al (2007) Soy product and isoflavone consumption in relation to prostate cancer in Japanese men. Cancer Epidemiol Biomarkers Prev 16:538–545
103. Kokubo Y, Iso H, Ishihara J, Okada K et al (2007) Association of dietary intake of soy, beans, and isoflavones with risk of cerebral and myocardial infarctions in Japanese populations: the Japan public health center based (JPHC) study cohort I. Circulation 116:2553–2562
104. Gencel VB, Benjamin MM, Bahou SN et al (2012) Vascular effects of phytoestrogens and alternative menopausal hormone therapy in cardiovascular disease. Mini Rev Med Chem 12(2):149–174
105. Takahashi M, Ikeda U, Masuyama JI et al (1996) Monocyte-endothelial cell interaction induces expression of adhesion molecules on human umbilical cord endothelial cells. Cardiovasc Res 32:422–429
106. Huang FC, Kuo HC, Huang YH et al (2017) Anti-inflammatory effect of resveratrol in human coronary arterial endothelial cells via induction of autophagy: implication for the treatment of Kawasaki disease. BMC Pharmacol Toxicol 18(1):3–11
107. Hao HD, He LR (2004) Mechanisms of cardiovascular protection by resveratrol. J Med Food 7(3):290–298
108. Pirola L, Frojdo S (2008) Resveratrol: one molecule, many targets. IUBMB Life 60(5):323–332
109. Harikumar KB, Aggarwal BB (2008) Resveratrol: a multitargeted agent for age-associated chronic diseases. Cell Cycle 7(8):1020–1035
110. Yang X, Yang J, Hu J et al (2015) Apigenin attenuates myocardial ischemia/reperfusion injury via the inactivation of p38 mitogen activated protein kinase. Mol Med Rep 12(5):6873–6878
111. Zhu ZY, Gao T, Huang J et al (2016) Apigenin ameliorates hypertension-induced cardiac hypertrophy and down-regulates cardiac hypoxia inducible factor-1 alpha in rats. Food Funct 7(4):1992–1998
112. Zhang S, Liu X, Sun C et al (2016) Apigenin attenuates experimental autoimmune myocarditis by modulating Th1/Th2 cytokine balance in mice. Inflammation 39(2):678–686
113. Nicholas C, Batra S, Vargo MA et al (2007) Apigenin blocks lipopolysaccharide-induced lethality in vivo and proinflammatory cytokines expression by inactivating NF-kappaB through the suppression of p65 phosphorylation. J Immunol 179(10):7121–7127
114. Zhang T, Yan T, Du J et al (2015) Apigenin attenuates heart injury in lipopolysaccharide-induced endotoxemic model by suppressing sphingosine kinase 1/sphingosine 1-phosphate signaling pathway. Chem Biol Interact 233:46–55
115. Li F, Lang F, Zhang H et al (2017) Apigenin alleviates endotoxin-induced myocardial toxicity by modulating inflammation, oxidative stress, and autophagy. Oxidative Med Cell Longev 2017:1–10
116. Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56(1):5–51
117. Dinkova-Kostova AT (2010) The effectiveness of the isothiocyanate sulforaphane in chemoprotection. Acta Hortic 867:27–36
118. Barba FJ, Nikmaram N, Roohinejad S et al (2016) Glucosinolates and their breakdown products: impact of processing. Front Nutr 3:24
119. Dong Z, Shang H, Chen YQ et al (2016) Sulforaphane protects pancreatic acinar cell injury by modulating Nrf2-mediated oxidative stress and NLRP3 inflammatory pathway. Oxidative Med Cell Longev 2016:1–12
120. Blekkenhorst LC, Bondonno CP, Lewis JR et al (2017) Cruciferous and allium vegetable intakes are inversely associated with 15 year atherosclerotic vascular disease deaths in older adult women. J Am Heart Assoc 6:1–22

121. Briggs WH, Xiao H, Parkin KL et al (2000) Differential inhibition of human platelet aggregation by selected allium thiosulfinates. J Agric Food Chem 48(11):5731–5735
122. Hubbard GP, Stevens JM, Cicmil M et al (2003) Quercetin inhibits collagen-stimulated platelet activation through inhibition of multiple components of the glycoprotein VI signaling pathway. J Thromb Haemost 1(5):1079–1088
123. Rimando AM, Kalt W, Magee JB et al (2004) Resveratrol, Pterostilbene, and Piceatannol in Vaccinium Berries. J Agric Food Chem 52(15):4713–4719
124. Baur JA, Pearson KJ, Price NL et al (2006) Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444(7117):337–342
125. Huang JP, Huang SS, Deng JY et al (2010) Insulin and resveratrol act synergistically, preventing cardiac dysfunction in diabetes, but the advantage of resveratrol in diabetics with acute heart attack is antagonized by insulin. Free Radic Biol Med 49(11):1710–1721
126. Lagouge M, Argmann C, Gerhart-Hines Z et al (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127(6):1109–1122
127. Zafra-Stone S, Yasmin T, Bagchi M et al (2007) Berry anthocyanins as novel antioxidants in human health and disease prevention. Mol Nutr Food Res 51(6):675–683
128. Mazza G, Kay CD, Cottrell T et al (2002) Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. J Agric Food Chem 50(26):7731–7737
129. Felgines C, Talavera S, Gonthier MP et al (2003) Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates in humans. J Nutr 133(5):1296–1301
130. Kay CD, Mazza GJ, Holub BJ (2005) Anthocyanins exist in the circulation primarily as metabolites in adult men. J Nutr 135(11):2582–2588
131. Jiang Y, Dai M, Nie WJ et al (2017) Effects of the ethanol extract of black mulberry (Morus nigra L.) fruit on experimental atherosclerosis in rats. J Ethnopharmacol 200:228–235
132. Mink PJ, Scrafford CG, Barraj LM et al (2007) Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr 85(3):895–909
133. Rimm EB, Giovannucci EL, Willett WC et al (1991) Prospective study of alcohol consumption and risk of coronary disease in man. Lancet 338(8765):464–468
134. Klatsky AL (2001) Could abstinence from alcohol be hazardous to your health? Int J Epidemiol 30:739–742
135. Acquaviva R, Russo A, Galvano F et al (2003) Cyanidin and cyanidin 3-O-beta-D-glucoside as DNA cleavage protectors and antioxidants. Cell Biol Toxicol 19:243–252
136. Lazze MC, Pizzala R, Savio M et al (2003) Anthocyanins protect against DNA damage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells. Mutat Res 535:103–115
137. Lefevre M, Wiles JE, Zhang X et al (2008) Gene expression microarray analysis of the effects of grape anthocyanins in mice: a test of a hypothesis-generating paradigm. Metabolism 57(7 Suppl 1):S52–57
138. Ramirez-Tortosa C, Andersen ØM, Gardner PT et al (2001) Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. Free Radic Biol Med 31:1033–1037
139. Rossi A, Serraino I, Dugo P et al (2003) Protective effects of anthocyanins from blackberry in a rat model of acute lung inflammation. Free Radic Res 37:891–900
140. Sumner MD, Elliott-Eller M, Weidner G et al (2005) Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease. Am J Cardiol 96:810–814
141. Demrow HS, Sllane PR, Folts JD (1995) Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries. Circulation 91:1182–1188
142. Nofer JR, Kehrel B, Fobker M et al (2002) HDL and arteriosclerosis: beyond reverse cholesterol transport. Atherosclerosis 161:1–16
143. Francis GA (2000) High density lipoprotein oxidation: in vitro susceptibility and potential in vivo consequences. Biochim Biophys Acta 1483:217–235

144. Hillstrom RJ, Yacapin-Ammons AK et al (2003) Vitamin C inhibits lipid oxidation in human HDL. J Nutr 133(10):3047–3051
145. Moser MA, Chun OK (2016) Vitamin C and heart health: a review based on findings from epidemiologic studies. Int J Mol Sci 17(8):1328–1336
146. Spencer AP, Carson DS, Crouch MA (1999) Vitamin E and coronary artery disease. Arch Intern Med 159(12):1313–1320
147. Laureaux C, Therond P, Bonnefont-Rousselot D et al (1997) α-tocopherol enrichment of high-density lipoproteins: stabilization of hydroperoxides produced during copper oxidation. Free Radic Biol Med 22:185–194
148. Arrol S, Mackness MI, Durrington PN (2000) Vitamin E supplementation increases the resistance of both LDL and HDL to oxidation and increases cholesteryl ester transfer activity. Atherosclerosis 150:129–134
149. Schnel JW, Anderson RA, Stegner JE et al (2001) Effects of a high polyunsaturated fat diet and vitamin E supplementation on high-density lipoprotein oxidation in humans. Atherosclerosis 159:459–466
150. Jaouad L, Milochevitch C, Khalil A (2003) PON1 paraoxonase activity is reduced during HDL oxidation and is an indicator of HDL antioxidant capacity. Free Radic Res 37:77–83
151. Salonen JT, Salonen R, Penttila I et al (1985) Serum fatty acids, apolipoproteins, selenium and vitamin antioxidants and the risk of death from coronary artery disease. Am J Cardiol 56:226–231
152. Kok FJ, deBruijn AM, Vermeeren R et al (1987) Serum selenium, vitamin antioxidants, and cardiovascular mortality: a 9-year follow-up study in the Netherlands. Am J Clin Nutr 45:462–468
153. Salonen JT, Salonen R, Seppänen K et al (1988) Relationship of serum selenium and antioxidants to plasma lipoproteins, platelet aggregability and prevalent ischemic heart disease in eastern Finnish men. Atherosclerosis 70:155–160
154. Stampfer MJ, Hennekens CH, Manson JE et al (1993) Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 328(20):1444–1449

Capsicum annuum Bioactive Compounds: Health Promotion Perspectives

7

Muhammad Imran, Masood Sadiq Butt, and Hafiz Ansar Rasul Suleria

Contents

M. Imran
National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

Department of Diet and Nutritional Sciences, Faculty of Health and Allied Sciences, Imperial College of Business Studies, Lahore, Pakistan
e-mail: mic_1661@yahoo.com

M. S. Butt
National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad, Pakistan
e-mail: drmsbutt@yahoo.com

H. A. R. Suleria (✉)
UQ School of Medicine, Translational Research Institute, The University of Queensland, Brisbane, QLD, Australia

Department of Food, Nutrition, Dietetics and Health, Kansas State University, Manhattan, KS, USA
e-mail: hafiz.suleria@uqconnect.edu.au

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_47

Abstract

Capsicum annum L. commonly known as bell pepper exhibits proven health as well as medicinal significance. It can be consumed either in fresh or processed form and is rich source of vitamin C, provitamin A, and calcium. Array of bioactive compounds especially antioxidants in its phytochemical profile make it an ideal choice for preventing cell damage, cancer insurgence, diabetes prevalence, cardiovascular disorders, cataracts, Alzheimer's, and Parkinson's disease. Major antioxidant compounds in capsicum are carotenoids, tocopherols, and capsaicinoids (capsacicin). Their anticancer role is attributed to their ability to act as scavengers of singlet molecular oxygen, reactive oxygen species (ROS), peroxyl radicals, and reactive nitrogen species (RNS). Capsaicinoids intake effectively reduced the triacyclglycerols, plasma total cholesterol (PTC), and non-high-density lipoprotein cholesterol, and thereby helps in the prevention of cardiovascular ailments. It also exhibit effective and proactive contribution against age-related ailments. Capsaicin exposure expressively repressed the initial adipogenic differentiation, maturation, and lipogenesis of adipocytes. Capsaicin also has ability to target the TRPV1 receptors in the C-fibers lead to their stimulation followed by desensitization that helps to improve the neurogenic bladder. So, it may serve as a potential emerging treatment for patients who are nonrespondent to conventional therapy especially those with neurogenic bladder.

Keywords

Bell pepper · Capsaicinoids · Antioxidant · Neurogenic bladder

List of Abbreviations

18α-GA	18 alpha-glycyrrhetinic acid
ABCA1	ATP-binding cassette transporter
ABCG1	ATP-binding cassette transporter-G1
ABCG5	ATP-binding cassette transporter-G-5
AdipoR2	Adiponectin gene/protein and its receptor
ADP	Adenosine diphosphate
ALCAM	Activated leukocyte cell adhesion molecule
AMPK	Activation of activated protein kinase
Apo-A1	Apolipoprotein-A1
apoM	Apolipoprotein M
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BCC	Basal carcinoma cells
BUN	Blood urea nitrogen
C/EBPα	C-enhancer-binding proteins
Ca^{2+}	Calcium
CaMK-II	Calmodulin-dependent protein kinase II
Capz	Capsazepine
CCMSs	Capsaicin-chitosan microspheres

CD36	Cluster of differentiation-36
COX-2	Cyclooxygenase-2
CRP	C-reactive protein levels
CRT	Calreticulin
Cx43	Connexin 43
DCs	Dendritic cells
DHC	Dihydrocapsaicin
DNA	Deoxyribonucleic acid
EC-LPS	Lipopolysaccharide from *Escherichia coli*
EMT	Epithelial mesenchymal transition
eNOS	Endothelial nitric oxide synthase
ERK	Extracellular signal-regulated kinases
FABP4	Fatty acid binding protein-4
FADD	Fas-associated protein with death domain
FAK	Focal adhesion kinase
GC	Gastric cancer
GDM	Gestational diabetes mellitus
Glu	Glutamate
GSH	Glutathione
GSSG	Oxidized glutathione
HDL-C	High density lipoprotein
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase
HO-1	Heme oxygenase-1
HSL	Hormone sensitive lipase
HUVECs	Human umbilical vein endothelial cells
ICD	Immunogenic cell death
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
KA	Kainic acid
Klf2	Kruppel-like factor 2
LDL-C	Low-density lipoprotein-cholesterol
LDL-R	Low-density lipoprotein receptor
LPS	Lipopolysaccharide
MDA	Malondialdehyde
MS–MS	Mass spectrometry
NDO	Eurogenic detrusor overactive
NET	Neuroendocrine tumor cells
NF-κB	Nuclear factor-kappa B
NOD/SCID	Nonobese diabetic/severe combined immunodeficiency
NPC1	Niemann-Pick C1 protein
OH	Hydroxyl
PC-3	Pancreatic cancer
p-CaM	Adhesion molecule
PCR	Polymerase chain reaction
PPARdelta	Peroxisome proliferator-activated receptor delta

PPARgamma	Peroxisome proliferator-activated receptor gamma
PPARα	Peroxisome proliferator-activated receptor-alpha
PPARγ	Peroxisome proliferator-activated receptor gamma
PPARγ	Peroxisome proliferator-activated receptor-gamma
PTPϵ	Protein-tyrosine phosphatase ϵ
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SRA-1	Steroid receptor RNA activator 1
SRB1	Scavenger receptor class B member 1
TG	Triglycerides
TIMP-1	Tissue inhibitors of metalloproteinases-1
TNF-α	Tumor necrosis factor-alpha
TRP	Transient receptor potential
TRPV1	Transient receptor potential vanilloid subtype 1
UCP2	Uncoupling protein 2
UV	Ultraviolet
VEGFA	Vascular endothelial growth factor-A
VLDL-C	Very low-density lipoprotein- cholesterol
WT	Wild-type

1 Introduction

The bell pepper (*Capsicum annum* L.), a member of *Solanaceae* family, is most commonly consumed fruity vegetable and well known for its antioxidant significance [1]. *Solanaceae* family include over 90 genera and 2000 species, among them *Capsicum annum*, *Capsicum chinense*, *Capsicum baccatum*, *Capsicum pubescens*, and *Capsicum frutescens* are most common. Bell pepper exhibits medicinal as well as food value in all over the world, and both warm and dry climate are considered suitable for its cultivation [2]. About 30 species are included in the genus *Capsicum*, out of which only five are domesticated while rest of these are considered as wild. Major cultivars include *C. baccatum*, *C. chinense*, *C. frutescens*, *C. annuum*, and *C. pubescens. Capsicum chinense* is known as pungent fruit among the domesticated species [3]. These are also a rich source of neutral phenolic compounds, especially luteolin, quercetin, and capsaicinoids [4]. The utilization of bioactive compounds plays key health-promoting functions such as provide protection against oxidative damage to cells, cancer insurgence, diabetes prevalence, cardiovascular disorders, cataracts, Alzheimer's, and Parkinson's disease [1]. These chemical compounds also prevent oxidation of structural lipids of brain cells and are vital for their proper functioning [4].

Different consumable forms of pepper are fresh, processed (sauces), or preserves (dehydrated powder). Its industrial utilization is mainly due to its unique composition and aromatic properties. *Capsicum annuum* has worldwide existence [5] and has different types of peppers such as sweet bell and hot peppers, cherry, serrano, and

cayenne [6, 7]. These serve as a domineering nutrient source in human diet [8, 9]. It has a significant source of ascorbic acid (vitamin C) and carotene (provitamin A). As per estimates, about 60% and 100% recommended daily amounts (RDA) for vitamin A and C can be achieved by the intake of 50–100 g fresh pepper. Ripened pepper fruit contain phytochemicals with antioxidant and anticancer functions [10]. Among the different antioxidants found in pepper, chlorophyll, carotenoids, tocopherols, and capsaicinoids are most important constituents [8].

The pungent values of chili fruit are due to the presence of alkaloids compound, i.e., capsaicinoids, which is in capsicum placenta during its early maturity and can be used in the human diet as food, medicine, and pharmaceutics. Capsaicin along with dihydrocapsaicin collectively constitute about 90% of the capsicum capsaicinoids, while the rest include nordihydrocapsaicin, homodihydrocapsaicin, homocapsaicin, norcapsaicin, and nornorcapsaicin [11].

Carotenoids a colored pigment found in peppers impart yellow to red color. It also exhibits antioxidant properties and prevents tissues damage by acting as singlet molecular oxygen, reactive oxygen species (ROS), peroxyl radicals, and reactive nitrogen species (RNS) scavenger [12] (Table 1).

2 Chemistry of Capsicum

Flavonoids along with some other polyphenols are pervasive phytochemicals and considered as essential functional ingredients found in different types of peppers (green, sweet, and hot). So far various attempts have been made to detect, quantify, and characterize different types of flavonoid in peppers [14, 15]. They are also rich in glycosides as well as aglycones of quercetin, myricetin, luteolin, kaempferol, and apigenin. Structural studies of flavonoids showed that position and numbers of hydroxyl groups along with a double bond at the C2-C3 position are mainly responsible for their strong antioxidant and anticancer activities. Role of structure in determining antioxidant potential confirms the myricetin as one of the most potent flavonoids found in pepper [16, 17]. Mass spectrometry (MS–MS) fragmentation and ultraviolet (UV) spectra-based structural study was carried out to identify C- and O-glycosides in peppers. Results showed that the pericarp of pepper fruit contains four quercetin (3-*O*-rhamnoside, 3-*O*-rhamnoside-7-*O*-glucoside, 3-*O*-glucoside-7-*O*-rhamnoside, and quercetin glycosylated), two luteolin *O*-glycosides (apiosyl-acetyl-glucoside and 7-*O*-2-apiosyl-glucoside), five luteolin C-glycosides (6-C-hexoside, 8-C-hexoside, 6-C-pentoside-8-C-hexoside, 6-C-hexoside-8-C-pentoside, and 6,8-di-C-hexoside) along with two apigenin C-glycosides (6-C-pentoside-8-C-hexoside and 6, 8-di-C-hexoside) [18].

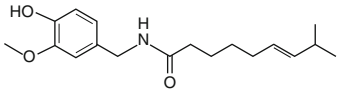
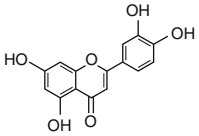
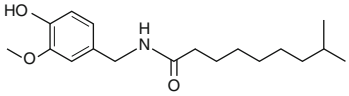
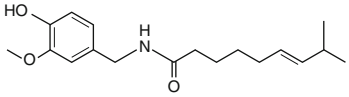
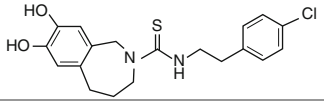
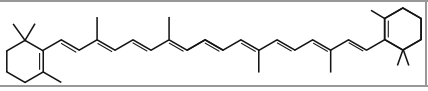
Flavonoid levels vary and mainly depend on genetics, developmental stage, and ecological situations. Such as red fruits of most cultivars have shown highest quantities of flavonoids. On the other hand, green fruits were found to be rich in quercetin 3-*O*-R-L-rhamnopyranoside whose level decreased during ripening [19]. Bioactive compounds include both essential and nonessential substances of natural occurrence in food and are well known for their impact human health.

Table 1 Nutritional composition of *Capsicum annum* [13]

Nutrient	1.80 g of capsicum
Water	0.145 g.
Energy	5.724 kcal
Carbohydrate	1.019 g
Fiber, total dietary	0.490 g
Protein	0.216 g
Fat (Total lipid)	0.311 g
Ash	0.109 g
Calcium	2.664 mg
Iron (Fe)	0.140 mg
Sodium (Na)	0.540 mg
Copper (Cu)	0.007 mg
Phosphorus (P)	5.274 mg
Selenium (Se)	0.158 mcg
Manganese (Mn)	0.036 mg
Magnesium (Mg)	2.736 mg
Zinc (Zn)	0.045 mg
Potassium (K)	36.252 mg
Saturated fatty acids (SFA)	0.059 g
Monounsaturated fatty acids (MUFA)	0.050 g
Thiamin	0.006 mg
Vitamin B6	0.037 mg
Niacin	0.157 mg
Riboflavin	0.017 mg

These are usually present in insignificant amounts in various foods and influence many cellular and physiological functions [20]. The bell pepper exhibits excellent antioxidant activity due to the presence of phytochemicals, such as flavonoids, capsaicinoids, phenolic compounds, carotenes, and ascorbic acid [21, 22]. Presence of these valuable micronutrients in bell pepper increases its significance against emerging diseases, especially dementia, diarrhea, and dysentery, along with many other ailments [23, 24]. However, quantities of these bioactive components in bell pepper depends on their farming conditions, state of maturity at harvesting, and varietal purity, and postharvest management [21, 24]. In red pepper, capsanthin, cryptocapsin, and capsorubin represent oxygenated carotenoids and exhibit strong antioxidant activity [25]. These phytochemicals pose generous ability to counteract free radicals involved in lipids oxidation, proteins, and cell deoxyribonucleic acid (DNA) damage and chronic human ailments [26]. In addition to capsaicinoids and carotenoids, flavonoids also exhibit antioxidants, anti-inflammatory, antiallergic, and antibacterial significance [14, 27, 28] (Table 2).

Table 2 Bioactive compounds and their potential metabolic roles

Name	Chemical structure	Metabolic role	References
Capsaicin		Anticancer	[29, 30]
Luteolin		Antidiabetic	[4]
Dihydrocapsaicin		Cardiopreventive	[31]
Capsaicin		Antirheumatoid and antiosteoarthritis	[32]
Capsazepine		Anticancer	[33]
β-Carotene		Antitumorigenesis	[34]

3 Health Perspectives

3.1 Anticancer and Antimalignant Activities

Meticulous in vitro and in vivo probing in mice demonstrated that capsaicin suppresses basal carcinoma cells (BCC) (5637 and T24) and nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mouse by inhibiting their proliferation, persuading cell cycle detention at G0/G1 phase, and diminishing ROS production. It also enhanced the Forkhead box O3 also known as FOXO3a and enhanced the catalase and superoxide dismutase concentration [29, 35]. Capsaicin stimulates autophagic process in BC cells by altering redox homeostasis, changing adenosine diphosphate/adenosine diphosphate (ADP/ATP) ratio, and inducing mitochondrial depolarization along with activation of activated protein kinase (AMPK) pathway. This enhanced capsaicin-prompted cell decrease in BC cells, proving that capsaicin promoted autophagy serves as a prosubsistence progression. Furthermore, BC cells showed distinctive mesenchymal properties of the epithelial-mesenchymal transition (EMT), such as overextended appearance and enhanced expression of the integrin-like kinase, anti-apoptotic Bcl-2 proteins, vimentin, and α5 and β1 integrin subunits

on capsaicin treatment. It rises CD24 and vascular endothelial growth factor-A (VEGFA), promotes the expression of Dhh/Ptch2/Zeb2 members of the Hedgehog signaling pathway and tissue inhibitors of metalloproteinases-1 (TIMP-1), and decreases CD44 and activated leukocyte cell adhesion molecule (ALCAM) mRNA expression levels. Amantini et al. [36] reported the involvement of the Hedgehog signaling pathway in the CPS-influenced autophagy and EMT phenotype. While increased resistance to commonly used BC curing drugs such as mitomycin C, gemcitabine, and doxorubicine was observed in capsaicin-resilient EMT-positive BC cells.

Capsaicin administration at the rate of 200 μM significantly inhibited the colon cancer cells proliferation; repressed TNF-α, IL-1β, IFN-γ, IL-10, and IL-1ra synthesis and exudation in a dose-dependent manner; and stimulated the IL-6 in human peripheral blood mononuclear cells (PBMCs) and HT-29 or RKO cells [37]. In addition its intake effectively reduce the proliferation of renal carcinoma cells, attenuation of transient receptor potential vanilloid type 1 (TRPV1) representative opponent capsazepine (CPZ), induction of apoptosis, upregulation of proapoptotic genes (Fas-associated protein with death domain (FADD), c-myc, Bax and cleaved-caspase-3, -8, and -9), and downregulation of antiapoptotic gene Bcl2. It also stimulated the p-38 and the Jun N-terminal kinase (JNK)-MAPK pathways [38]. Capsaicin in gastric cancer (GC) cell lines exerts its effect via significantly suppressing cell progression, but in GC cell lines, it causes change in histone acetylation. Subsequent investigations confirmed the vital role of hMOF, i.e., a major histone acetyltransferase for H4K16 in capsaicin-tempted epigenetic vicissitudes. Reduction in hMOF activity was noticed in GC tissues, which could be reinstated by capsaicin both in vivo and in vitro [39].

Immunogenic cell death (ICD) is due to early calreticulin (CRT) surface exposure. Cisplantin and capsaicin can persuade the cell apoptosis in human osteosarcoma cells (MG-63). Capsaicin treatment causes CRT translocation from the intracellular space to the cell surface which increased the MG-63 cells phagocytosis and IFN-γ secretion stimulation [40], induces apoptosis, lowers the bladder cancer lines growth, and downregulates the tNOX expression. Moreover, capsaicin effectively decreases the expressions of various protein involved in cell cycle progression, thereby enhanced the phenomenon of cell cycle arrest, repressed the extracellular signal-regulated kinases (ERK) activation, reduced paxillin and focal adhesion kinase (FAK) phosphorylation, and cell migration [41].

Capsazepine (Capz) is a synthetic analog of capsaicin having anticancer effects. The mode of action of the capsaicin against prostate cancer cells involves; inhibition of signal transducer and activator of transcription-3 (both constitutive and induced) activation, suppression of the upstream janus kinases (JAK1/2) and c-Src activation and low concentration level of oncogenes protein products, which in turn induce apoptosis is, minimize proliferation and invasion. The phosphatase inhibitor pervanadate overturned the Capz-induced STAT3 inhibition, representing that the consequence of Capz based on a protein tyrosine phosphatase. Moreover, siRNA-mediated knockdown of protein-tyrosine phosphatase ϵ (PTPϵ) reversed the Capz-induced initiation of PTPε and reticence of STAT3 activation, indicating that PTPε is

crucial for Capz-dependent STAT3 dephosphorylation. Finally, intraperitoneal Capz administration decreased tumor growth in a xenograft mouse prostate cancer model and reduced p-STAT3 and Ki-67 expression [42].

Capsaicin treatment of U251 cells decreased cell viability and altered the punctate patterns of LC3. In U251 human glioma cells, dihydrocapsaicin (DHC) exerts its effect by inducing apoptosis in a dose- and time-dependent manner and through decreased cell viability. The apoptosis effect of capsaicin is mainly attributed to mitochondrial depolarization, caspase-9 and -3, and enhanced ROS synthesis along with increase in calcium (Ca^{2+}) level [42].

3.2 Cardiovascular Role

Different body organs such as brain, gut, bladder, dorsal root ganglia, and sensory nerves contain transient receptor potential vanilloid subtype 1 (TRPV1), which serves as a receptor for capsaicin. TRPV1 activation results in increased intracellular calcium signaling which in turn induce various physiological effects. It is also involved in swelling, oxidation trauma, and associated discomfort [38, 43].

Capsaicinoids intake significantly reduced the plasma total cholesterol, non-high-density lipoprotein cholesterol, and triacyclglycerols but not cause any change in high-density lipoprotein cholesterol level. Fecal excretion of total acidic sterols is increased by dietary capsaicinoids possibly by increasing cholesterol 7-alpha-hydroxylase and decreasing liver X receptor α. Capsaicinoids reduced the cholesterol absorption by decreasing plasma campesterol/cholesterol ratio as confirmed by plasma sterol analysis. Capsaicinoids impede cyclooxygenase-2 (COX-2) expression, thereby positively influence the endothelium-dependent relaxations but negatively affect the endothelium-dependent contractions.

In atherosclerotic plaque mice, dihydrocapsaicin (DHC) treatment expressively reduced the cellular cholesterol content but increased the apoA1-mediated cholesterol efflux. Results also showed decreased in plasma triglycerides (TG), low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C), interleukin-beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor (TNF-α), and C-reactive protein (CRP) levels while apoA1 and high-density lipoprotein (HDL-C) levels in plasma were significantly increased. Results of another study reported a significant reduction in atherosclerotic lesion in $apoE^{-/-}$ mice when treated with DHC. Moreover, combined treatment with liver X receptor alpha (LXR-alpha) siRNA and peroxisome proliferator-activated receptor gamma (PPARγ) siRNA induces the upregulation of DHC on ATP-binding cassette transporter (ABCA1), ATP-binding cassette transporter-G1, ATP-binding cassette transporter-G-5 (ABCG5), scavenger receptor class B member 1 (SRB1), Niemann-Pick C1 protein (NPC1), cluster of differentiation-36 (CD36), low-density lipoprotein receptor (LDL-R), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), apolipoprotein-A1 (Apo-A1), and apo-E expression markedly stopped but decreased the DHC regulation on steroid receptor RNA activator 1 (SRA-1) expression distinctly compensated. LXRα siRNA treatment

demonstrated nonsignificantly affect the DHC-induced PPARγ expression but with significantly decreased DHC-based LXRα expression [31].

3.3 Antidiabetic Role

Peripheral neuropathy is a chronic diabetes complication mellitus and is characterized by severe pain. Antidepressants and antiepileptic administration serves as effective drug treatment options. Nociceptive sensory neurons, as well as other tissues containing transient receptor potential vanilloid 1 (TRPV1), are triggered via capsaicin-regulated modulation. TRPV1 activation induces calcium influx and promotes Kruppel-like factor 2 (Klf2), uncoupling protein 2 (UCP2), LXRα, endothelial nitric oxide synthase (eNOS), peroxisome proliferator-activated receptor delta (PPAR delta), and gamma (PPAR gamma) expression. In addition, TRPV1 activation provokes Ca influx which minimizes shear stress and stimulates eNOS, COX-2, thrombomodulin, and NRF2-responsive antioxidant enzymes expression along with decreased expression of proinflammatory proteins. LXRα activation via TRPV1-mediation validates effective cholesterol transfer thereby prevent plaque buildup. In gastrointestinal (GI) tract, capsaicin-based stimulation of TRPV1 increases metabolic rate via compassionately promoted activation of brown fat. TRPV1 activation demonstrated defensive antioxidant property through the improved expression of uncoupling protein-2 (UCP2) in the liver and vascular endothelium during nonalcoholic fatty liver disease and hyperglycemia perspective, respectively [44, 45]. Capsaicin supplementation effectively decreased the fasting lipid metabolic syndromes and postprandial hyperglycemia as well as hyperinsulinemia in women with gestational diabetes mellitus (GDM) and also reduced the prevalence of large-for-gestational-age newborns [46].

Capsaicin reduces viability and propagation, and induces apoptotic death in pancreatic neuroendocrine tumor (NET) cells. In addition, it also causes loss of mitochondrial membrane potential; minimizes ATP, reactive oxygen species (ROS), and mitochondrial Bcl-2 protein synthesis; and increases cytochrome c levels [47]. In male KKAy mice, 0.015% capsaicin supplementation significantly reduced the plasma and/or liver fasting glucose/insulin and triglyceride concentrations, along with decreased macrophage intrusion and inflammatory adipocytokine genes expression (e.g., monocyte chemoattractant protein-1 and interleukin-6). In addition, it also causes increase in adiponectin gene/protein and its receptor (AdipoR2) expression as well as activation of hepatic AMP-motivated protein kinase, i.e., a marker of fatty acid oxidation. It decreases metabolic dysregulation in diabetic and/or obese KKAy mice through improved adiponectin and its receptor expression [48].

Apolipoprotein M (apoM), mainly found in kidney and liver tissues, is linked with increased prevalence and development of diabetes and atherosclerosis complications. Dihydrocapsaicin (DHC) induction significantly decreased the atherosclerotic plaque formation in apoE$^{-/-}$ mice. Treatment of HepG2 cells with 0, 25, 50, and 100 μM DHC for 24 h effectively decreased apoM expression at both protein and mRNA level in HepG2 cells in a dosage- and time-dependent way. DHC

treatment negatively influences the Foxa2 expression but positively affects the LXRα expression in HepG2 cells. In addition, overexpression of Foxa2 markedly recompensed the inhibition effect induced by DHC on apoM expression. Treatment of C57BL/6 mice liver with DHC had significantly lower expression of apoM and Foxa2 while had higher expression of LXRα [49].

3.4 Antiobesity

Brown fat-specific thermogenic uncoupling protein-1 and bone morphogenetic protein-8b expression in white adipose tissue (WAT) are stimulated by capsaicin. Browning of WAT is triggered by capsaicin as it promotes expression and activity of sirtuin-1 through TRPV1 channel-based raise of intracellular Ca^{2+} and protein kinase II and AMP-stimulated kinase phosphorylation. Meanwhile, it also improved PPARγ 1 coactivator α expression along with increased metabolic and ambulatory activity. Another possible mechanism involved in WAT browning may include capsaicin-based stimulation of sirtuin-1-dependent deacetylation of PPARγ and the transcription factor PRDM-16 and assisted PPARγ-PRDM-16 interaction [50].

Brown adipose tissue (BAT) is involved in energy balance and body fatness regulation because this is the site of sympathetically activated adaptive nonshivering thermogenesis. Acute cold exposure activates the BAT in humans which in turn causes cold-induced increase in whole-body energy expenditure. BAT metabolic activity was found to be lower in old and obese peoples. BAT serves as energy dissipating activity in the body therefore negatively correlated with body fatness and is considered as an effective proactive approach against body fat accumulation. Repeated exposure to cold induces stimulatory effects through the activation of transient receptor potential (TRP) channels, which are chemosensitive receptors for various naturally occurring herbal and food ingredients. Capsaicin and its analog capsinoids, demonstrative agonists of TRPV1, mimic the effects of cold to decrease body fatness through the activation and recruitment of BAT [51].

Capsainin treatment of bone marrow mesenchymal stem cells (BMSCs) for 6 days followed by 2 days of adipogenic induction demonstrated a significant decrease in the cell viability and proliferation in BMSCs. The capsaicin-treated cells were collected at 2nd, 4th, and 6th days for analysis. After capsaicin exposure, dose and time-dependent reduction in cell viability and proliferation was observed in BMSCs. Interestingly, capsaicin increased the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in order to induce cell cycle arrest at G0-G1 along with increased apoptosis. Capsaicin exposure effectively reduced the initiation of adipogenic differentiation, lipogenesis and maturation with associated repression of PPARγ, C-enhancer-binding proteins (C/EBPα), fatty acid binding protein-4 (FABP4), and stearoyl-CoA desaturase-1 (SCD-1). Capsaicin-based stimulation for ROS and RNS synthesis hinders the adipogenic mesenchymal stem cells discrepancy through different possible mechanisms such as inhibit proliferation, induce apoptosis, or cell cycle arrest [52].

Previous studies reported the involvement of TRPV1 pathway in weight management via enhanced intracellular Ca^{2+} concentration, although the possible mechanism by which dietary capsaicin involved in controlling obesity is not known. Connexin43 (Cx43) molecules take part in adipocyte differentiation by facilitating Ca^{2+} transfer between coupled cells. Role of TRPV1-mediated changes in Cx43-facilitated adipocyte-adipocyte interaction is not completely understood. In a study, effort was made to explore either Cx43 take part in TRPV1-facilitated adipocyte lipid break down in cultured 3 T3-L1 preadipocytes and visceral adipose tissues from humans, wild-type (WT) and TRPV1-deficient ($TRPV1^{-/-}$) mice or not. Findings indicated that capsaicin treatment results in the TRPV1 and Cx43 coexpression along with increased Ca^{2+} influx in 3 T3-L1 preadipocytes and endorsed lipid breakdown in cell as revealed by Oil-red O staining. No effect was observed when capsazepine, a TRPV1 antagonist, and 18 alpha-glycyrrhetinic acid (18α-GA), a gap-junction inhibitor, were administered. Regular intake of capsaicin cause weight reduction of perirenal, mesenteric, and testicular adipose tissues in WT mice fed a high-fat diet. Capsaicin improved the expression levels of cell adhesion molecule (p-CaM), connexin 43 (Cx43), calmodulin-mediated protein kinase II (CaMK-II), PPARδ, and hormone-sensitive lipase (HSL) in mesenteric adipose tissues from WT mice fed a high-fat diet, db/db mice, as well as obese humans; however, these effects were not observed in $TRPV1^{-/-}$ mice. Continuing capsaicin consumption reduced the serum lipids level and body weights of WT mice, but not effect high-fat fed $TRPV1^{-/-}$ mice. These findings inveterate that capsaicin-mediated TRPV1 stimulation improved Ca^{2+} invasion in Cx43-assisted adipocyte-adipocyte interaction which in turn endorses lipolysis both in vitro and in vivo. Cx43 is upregulated by capsaicin intake through activation of TRPV1 to improve visceral fat remodeling [53].

In mice, capsinoids inhibited fat buildup in vivo and in vitro. Liver, a chief site for lipid breakdown, was exposed to capsinoids, and the result showed a significant decrease in HMG-CoA reductase, CPT-1, FAT/CD36, and GLUT4 levels which in turn increase lipid breakdown in both adipose tissues and liver. Moreover, Oil red O staining also demonstrated that capsinoids inhibit fat buildup in the adipocytes [54].

Tan et al. [55] develop capsaicin-chitosan microspheres (CCMSs) through ion-cross-linking and spray drying and exploit its antiobesity suitability in rats (obese). CCMSs impact on body mass, Lee's index, fat, and serum lipids levels were examined. Furthermore, mRNA expression of PPARα, PPARγ, leptin, UCP2, GPR120, FTO, and adiponectin in the liver was assessed by quantitative real-time polymerase chain reaction (PCR) while protein expression of adiponectin, leptin, PPARα, UCP2, and hepatic lipase in serum was assessed by enzyme-linked immunosorbent assay. CCMSs were prepared with 85.17% entrapment efficacy and 8.87% mean drug loading capability. CCMSs demonstrated to be more efficient in controlling body weight, body fat, body mass index (BMI), organ index, fat/body mass ratio, and serum lipids when compared with Orlistat, capsaicin, and chitosan microspheres. The CCMSs downregulated the leptin expression but improved the expression of UCP2, PPARγ, PPARα, and adiponectin. Results showed that total cholesterol, fasting glucose serum levels, and triglyceride levels were also decreased

by capsaicin treatment. Immunoblot exploration and reverse transcription-polymerase chain reaction (RT-PCR) indicated higher expression of adiponectin and other adipokines containing PPAR-α, PPAR-γ, visfatin, and adipsin, but decreased tumor necrosis factor-α and IL-6 expression [56].

Hydrogen peroxide (H_2O_2) at 300–1000 μM level favorably and effectively stimulated capsaicin-mediated high threshold afferents but not low threshold stretch-sensitive afferents, which were only stimulated by expressively greater H_2O_2 levels. The TRPV1 apponents, capsaicin motivated 86% of high threshold afferents. The transient receptor potential cation channel (TRPA1) antagonist, HC-030031, but not the TRPV1 antagonist, capsazepine or the TRPM8 antagonist, M8-B, significantly inhibited the H_2O_2-facilitated stimulation of high threshold afferents. Dimethylthiourea and deferoxamine not altered the H_2O_2 effect on high threshold afferents. The results illustrate that H_2O_2-induced enduring instigation of the most of capsaicin-sensitive high threshold afferents, while not effect threshold stretch-sensitive afferents, when used in the concentration range determined in swelling or reperfusion after ischemia [57].

Study was planned with BALB/c mice and they were subjected to ethanol (5 g/kg body weight) for 6 month to induce oxidative stress. Mice were distributed in four groups and provided with curcumin (0.016%) or capsaicin (0.014%) containing diets with or without ethanol. During this observation period, behavioral disorder was observed in one mouse fed on an alcohol-treated normal diet. Capsaicin treatment significantly decreased the amount of malondialdehyde and phosphatidylcholine hydroperoxide levels in the brain tissue extract but catalase and superoxide dismutase activity remain unaffected [58].

Different cellular processes especially inflammatory injury and antioxidant homeostasis depend on Heme oxygenase-1 (HO-1), i.e., rate-limiting enzyme in the heme metabolism. Use of capsaicin monitor serum creatinine and blood urea nitrogen (BUN) concentrations along with tissue histology improves cisplatin-induced renal dysfunction. Moreover, capsaicin treatment decreases the expression of inflammatory mediators and oxidative stress markers for renal injury. It also persuades HO-1 expression in HK-2 cells and kidney tissues. Shielding properties of capsaicin were entirely revoked by either knockdown in HK-2 cells or treatment with the HO inhibitor HO-1 or ZnPP IX [59].

Capsaicin hinders almost 2.5–9% and 5–20% of complex-I activity and 8–75% of complex-III activity in Bx-panreatic cancer (PC-3) and AsPC-1 cells respectively, which was mainly caused by superoxide dismutase, catalase, and EUK-134. While, in normal HPDE-6 cells, capsaicin treatment not effect complex-I or complex-III activities. Capsaicin treatment effectively reduced the adenosine triphosphate (ATP) concentration in BxPC-3 and AsPC-1 cells and mitigated by catalase or EUK-134 (synthetic superoxide dismutase). Oxidation of mitochondria-specific cardio lipid was considerably higher in capsaicin-treated cells. BxPC-3 derived ρ(0) cells lack mitochondrial DNA and were found to be entirely resilient to capsaicin facilitated ROS synthesis and apoptosis. Findings tell that cytochrome c release, as well as caspase-9 and caspase-3 cleavage caused by mitochondrial membrane potential disruption were effectively obstructed by EUK-134 and catalase in BxPC-3 cells.

The capsaicin treatment reduces glutathione level and decreases the enzymatic performance and expression. Transient transfection results in overexpression of catalase which provides protection against capsaicin-induced ROS production and apoptosis. Besides, reduced in SOD activity with an increase in GSSG/GSH levels was detected in capsaicin (2.5 mg/kg) treated mice tumors [60].

Capsicum oleoresin (75 mg/kg bw/day) is found to be deleteriously linked with serum cholesterol and triglycerides levels in hypercholesterolemic gerbils [61]. The red pepper or its active component capsaicin showed a significant reduction in liver cholesterol and is responsible for enhanced fecal excretion of both free cholesterol and bile acids in female albino rats. This hypocholesterolemic effect of capsaicin is likely to be responsible for the presence of common vanillyl moiety [62].

Reduced weight gain and ameliorated hypertrophy of the liver and adipose tissues were found in male mice treated with high-fat diet, red paprika, and capsanthin. Red paprika and capsanthin treatment also improved serum lipid profile and adipokine secretion, and ameliorated hepatic steatosis by hindering fatty acid oxidation and gluconeogenesis. In epididymal fatty tissue, red paprika and capsanthin inhibited adipogenesis and decreased lipid droplet size [63].

3.5 Antiaging

Redox status of the red blood cell is characterized by lower glutathione (GSH)/oxidized glutathione (GSSG) ratio. In all age groups, GSH level is significantly influenced by capsaicin treatment which in turn causes a major shift in GSH to GSSG ratio and changes the cell redox status. The results assertively prove the antioxidant efficacy of capsaicin and also highlight its ability to modulate the redox status of red blood cells. This result proposes that food components that serve as antioxidant increases GSH level and possibly exhibit effective and proactive contribution against age-related ailments [64, 65].

The glutamate (Glu) induced neurotoxic effect negatively affects the cell viability but capsaicin treatment improved the situation. Use of glutamate (Glu) with capsaicin effectively decreased the ROS synthesis and apoptotic neuronal death by a combined treatment with both phytochemicals. The lower mRNA concentration of cytoplasmic glutathione peroxidase, Cu/Zn and Mn superoxide dismutases, Bcl-x(L) and elevated mRNA contents of interleukin-1β, and tumor necrosis factor-α were successfully reinstated by after treatment with capsaicin and/or resveratrol [66].

Antioxidant activity in the brain and blood of kainic acid (KA) facilitated status epilepticus model (30 mg/kg) was increased by using capsaicin (0.33 mg/kg or 1 mg/kg). Moreover, capsaicin treatment effectively decreased the KA-mediated increase in cytokines IL-1β and TNF-α level in the brain. Results confirmed that combined use of KA and capsaicin (1 mg/kg) because more reduction in apoptotic cell death in the cornu ammonis sections of the hippocampus when compared with their individual use [67]. Acetone (80%) extracted free polyphenols in red *Capsicum annuum var. aviculare* (Tepin) induced lipid peroxidation in brain and liver as well as exhibited the higher Fe(II) chelating ability, hydroxyl (OH) radical scavenging ability than the

bound polyphenols. These free polyphenols caused a significantly higher inhibition in the malondialdehyde (MDA) production in the brain and liver homogenates in a dose-dependent manner [4].

3.6 Anti-inflammtory Role

Atherosclerosis refers to chronic vascular inflammation characterized by combination of endothelial malfunctioning, leukocyte stimulation, lipid accrual, excessive generation of inflammatory mediators, and ROS. Capsaicin plays protective role for human umbilical vein endothelial cells (HUVECs) against oxLDL-facilitated dysfunction. Apo B disintegration along with associated diene formation of the Cu-regulated LDL oxidation is responsible for antioxidant potential of capsaicin. In HUVECs, capsaicin causes collapse of mitochondrial membrane potential and obstructs oxLDL induced ROS synthesis, and caspase-3 stimulation. It also avoids foam cell synthesis in macrophage RAW 264.7 cells [32, 53, 68].

Escherichia coli–derived lipopolysaccharides (EC–LPS) were used at the level of 1 μg/ml to induce inflammation via stimulation of primary peripheral blood mononuclear cells (PBMCs) and U-937 macrophages. Magnetic bead kit analysis showed that nonivamide decreased the EC-LPS-induced release of IL-6 and TNF-α in PBMCs and U-937 macrophages. This anti-inflammatory mechanism may involve MAPK pathway but was independent of NF-κB pathway. In addition, use of trigeminally active compound and an antagonist of TRPV1 or TRPA1 in combination for treating U-937 eliminated the anti-inflammatory activity. Results confirmed that nonivamide exhibited similar anti-inflammatory activity as observed in capsaicin and t-pellitorine. In U-937 macrophages, the tested compounds demonstrated an antiprovocative effect by preventing the EC–LPS-induced activation of the MAPK pathway. In addition, the TRP channel activation performs a vital role in anti-inflammatory capacity of capsaicin and nonivamide [69].

Capsaicin limits lipopolysaccharide (LPS)-facilitated IL-1β, IL-6, and TNF-α formation in a time- and dose-dependent manner. Furthermore, it increases LXRα expression via PPARγ route. Control of LXRα stimulation by siRNA reduced the inhibitory effect of capsaicin on LPS-induced IL-1β, IL-6, and TNF-α synthesis. In addition, LXRα siRNA repealed the inhibitory effect of capsaicin on p65, nuclear factor (NF-κB) protein expression. So, anti-inflammatory effects of capsaicin are LXRα-based, as LXRα link the capsaicin facilitated PPARγ stimulation and NF-κB inactivation in LPS-motivated inflammatory reaction [70] (Table 3).

3.7 Capsaicin in Urological Disorders

Neurogenic bladder is a urological ailment that utterly disturbs the comfort of patient's life and is common in patients with severe sclerosis, spinal cord injury, and other neurological pathologies. Capsaicin has also been evaluated as substitute treatment of neurogenic bladder disorder [71]. Neurogenic bladder is characterized

Table 3 Health perspectives of *capsicum annum*

Disorders	Mechanisms	References
Anticancer	Induced cell cycle detention at G0/G1 phase Enhanced FOXO3a expression	[29, 35]
	Altered histone acetylation	[39]
	Inhibited the colon cancer cells proliferation Decreased the formation and secretion of IL-1β, TNF-α, IL-10, IFN-γ, and IL-1ra	[37]
	Stimulated the secretion of IFN-γ Induced apoptosis, and downregulated the tNOX expression	[40]
	Suppressed the activation of ERK Reduced the paxillin and FAK phosphorylation	[41]
	Enhanced regulation of Dhh/Ptch2/Zeb2 members of the Hedgehog signaling pathway, improve CD24, VEGFA and TIMP1 and lower CD44 and ALCAM mRNA expression	[36]
	Reduced the proliferation of renal carcinoma cells Attenuated transient receptor potential vanilloid type 1 (TRPV1) representative antagonist capsazepine (CPZ) Stimulated P-38 and JNK MAPK pathways	[38]
Cardiovascular	Reduced the triacyclglycerols, total cholesterol, non-high-density lipoprotein cholesterol, and in plasma Stimulated cholesterol 7α-hydroxylase expression but negatively influenced hepatic X receptor alpha	[53]
Antidiabetic	Mediated activation of TRPV1-expressing neurons hyperglycemia perspective	[44, 45]
	Reduced reduces mitochondrial Bcl-2 protein production Increased cytochrome c levels	[47]
	Reduced triglyceride and fasting glucose amounts Increased adiponectin gene/protein and its receptor (AdipoR2) expression Activated hepatic AMP-activated protein kinase	[48]
Antiobesity	Improved bone morphogenetic protein-8b and brown fat-specific thermogenic uncoupling protein-1 expression Triggered browning of WAT	[50]
	Repressed initiation of adipocytic differentiation, lipogenesis, and maturation Repressed PPARγ, C/EBPα, FABP4, and SCD-1	[52]
	Inhibited fat accumulation and significant decrease in HMG-CoA reductase, CPT-1, FAT/CD36 and GLUT4 levels	[54]
	Decreased the levels of malondialdehyde and phosphatidylcholine hydroperoxide levels Enhanced the concentrations of catalase and superoxide dismutase activity	[58]
Antiaging	Decreased the KA-facilitated rise in cytokines IL-1β level and TNF-α declined apoptotic cell death	[67]
Anti-inflammatory	Suppressed ROS production	[32, 53]
	Inhibited the EC-LPS-induced activation of the MAPK pathway	[69]

by two severe conditions namely neurogenic detrusor overactive (NDO) and detrusor hyperreflexia that results in urgency and rise in urinary frequency, and sometime more severe complications [71, 72]. Overactive bladder is a clinical disorder that looks like neurogenic bladder [73], but its causes are not linked with neurological or urogenital sicknesses [74, 75].

Results of the previous findings reported that use of an alcoholic solvent may cause annoyance and pelvic discomfort in more than half patients, thereby decreased the capsaicin effectiveness [76]. The capsaicin or resiniferatoxin (RTX) effect in urinary tract is related to the action on TRPV1 receptors in sensory fibers and urothelial cells [77]. In vitro study was conducted by using bladder urothelial cells from non-neurogenic overactive bladder patients as well as from healthy volunteers. Results showed significant improvement in TRPV1 expression and activation along with higher capsaicin sensitivity in nonneurogenic overactive bladder patients [29, 78]. Possible mechanism by which capsaicin exerts its beneficial effect on the bladder activity is its ability to target and activate the TRPV1 receptors in the C-fibers. The use of both capsaicin and RTX is still not a routine clinical practice clinical use of RTX, and capsaicin is not very common but it may serve as a potential substitute for neurogenic bladder patients who are nonrespondent to conventional treatment with oral antimuscarinic drugs [71].

4 Conclusion

Capsicum annuum is well known all over the world owing to its taste along with characteristic smell and taste. It is also a promising source of carotenoids, capsaicinoids, flavonoids, and vitamin precursors. There is also an emerging trend to use of *capsicum annum* polyphenols in food-based products to curtail the various human degenerative disorders due to its antioxidant and free radical scavenging properties. This fruit has been proven very effective as antioxidant, anticancer, cardioprotective role, oxidative stress prevention, antiobesity, antidiabetic, and antimicrobial activities. Capsaicin is another bioactive compound which is present in higher concentrations in this fruit as compared to other bioactive moieties. The composition of *capsicum annum* polyphenols is significantly varied with genotype, soil type, temperature, maturity stages, and processing conditions. Capsaicin markedly suppressed the production and secretion of TNF-α, IL-1β, IFN-γ, IL-10, and IL-1ra in human cancer cell lines. Additionally, capsaicin lowered the proliferation of renal carcinoma cells; induced apoptotic cell death; attenuated transient receptor potential vanilloid type 1 (TRPV1); upregulated the proapoptotic genes including c-myc, FADD, Bax, and cleaved-caspase-3, -8, and -9; and downregulated the Bcl-2 gene. Capsaicin has been proven effectual to reduce the higher glucose levels, cholesterol and triglycerides concentrations as well as significantly decreased the CPT-1, HMG-CoA reductase, FAT/CD36, and GLUT4 concentration. These compounds from *capsicum annum* markedly caused reduction in level of the cytokines IL-1β and TNF-α in the brain. Moreover, capsaicin also suppresses the

lipopolysaccharide (LPS)-facilitated IL-1β, IL-6, and TNF-α formation. Further, it also enhanced the expression through PPARγ pathway. The current book chapter summarizes the therapeutic role of *Capsicum annuum* polyphenols against human syndromes.

Acknowledgments The authors are thankful to Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. The project was partially supported by Higher Education Commission, Pakistan, under Pak-US Science and Technology Cooperation Program Phase IV with a project entitled "Establishment of Functional and Nutraceutical Food Research Section at the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan."

References

1. Blanco-Ríos AK, Medina-Juarez LA, González-Aguilar GA, Gamez-Meza N (2013) Antioxidant activity of the phenolic and oily fractions of different sweet bell peppers. J Mex Chem Soc 57:137–143
2. Igbokwe GE, Aniakor GC, Anagonye CO (2013) Determination of β-carotene & vitamin C content of fresh green pepper (*Capsicum annum*), fresh red pepper (*Capsicum annum*) and fresh tomatoes (*Solanum lycopersicum*) fruits. Bioscientist 1:89–93
3. Kehie M, Kumaria S, Tandon P (2014) Manipulation of culture strategies to enhance capsaicin biosynthesis in suspension and immobilized cell cultures of Capsicum Chinense Jacq. cv. Naga King Chili. Bioprocess Biosyst Eng 37:1055–1063
4. Oboh G, Rocha TBJ (2007) Distribution and antioxidant activity of polyphenols in ripe and unripe tree pepper (*Capsicum pubescens*). J Food Biochem 31:456–473
5. Hwang JT, Kim SH, Park HS, Kwon DY, Kim MS (2013) Capsaicin stimulates glucose uptake in C2C12 muscle cells via the reactive oxygen species (ROS)/AMPK/p38 MAPK pathway. Biochem Biophys Res Commun 439(1):66–70. https://doi.org/10.1016/j.bbrc.2013.08.027
6. Büttow MW, Barbieri RL, Neitzke RS, Heiden G, Carvalho FIF (2010) Diversidade genética entre acessos de pimentas e pimentões da Embrapa Clima Temperado. Ciência Rural 40(6):1264–1269. https://doi.org/10.1590/S0103-84782010000600004
7. Alvarez-Parrilla E, De LA, Amarowicz R, Shahidi F (2012) Protective effect of fresh and processed Jalapeño and Serrano peppers against food lipid and human LDL cholesterol oxidation. Food Chem 133(3):827–834. https://doi.org/10.1016/j. foodchem.2012.01.100
8. Shetty AA, Magadum S, Managanvi K (2013) Vegetables as sources of antioxidants. J Food Nutr Disord 2(1):1–5. https://doi.org/10.4172/2324-9323.1000104. PMid:25328903
9. Moraes LP, DaPaz MF, Sanjines-Argandoña EJ, Silva LR, Zago TD (2013) Compostos fenólicos e atividade antioxidante de molho de pimenta "Dedo-de-Moça" fermentado. Biochem Biotechnol Rep 1(2):33–38. Retrieved from http://www.uel.br/revistas/uel/index.php/bbr/article/view/14551/12349
10. Mateos RM, Jiménez A, Román P, Romojaro F, Bacarizo S, Leterrier M, Gómez M, Sevilla F, Del Río LA, Corpas FJ, Palma JM (2013) Antioxidant systems from pepper (*Capsicum annuum* L.): involvement in the response to temperature changes in ripe fruits. Int J Mol Sci 14(5):9556–9580. https://doi.org/10.3390/ijms14059556. PMid:23644886
11. Prasad NBC, Shrivastava R, Ravishankar GA (2005) Capsaicin as multifaceted drug from *Capsicum* spp. Evid Based Intern Med 2:147–166
12. Kim YJ, Kim YAE, Yokozawa T (2009) Protection against oxidative stress, inflammation, and apoptosis of high-glucoseexposed proximal tubular epithelial cells by astaxanthin. J Agric Food Chem 57(19):8793–8797

13. Fathima SN (2015) A systemic review on phytochemistry and pharmacological activities of *Capsicum annuum*. Int J Pharm Pharm Res 4(3):51–68
14. Bae H, Jayaprakasha GK, Jifon J, Patil BS (2012) Extraction efficiency and validation of an HPLC method for flavonoid analysis in peppers. Food Chem 130(3):751–758
15. Sgroppo SC, Pereyra MV (2009) Using mild heat treatment to improve the bioactive related compounds on fresh-cut green bell peppers. Int J Food Sci Technol 44:1793–1801
16. Lu J, Papp LV, Fang J, Rodriguez NS, Zhivotovsky B, Holmgren A (2006) Inhibition of mammalian thioredoxin reductase by some flavonoids: implications for myricetin and quercetin anticancer activity. Cancer Res 66(8):4410–4418
17. Tonin FG, Jager AV, Micke GA, Farah JPS, Tavares MFM (2005) Optimization of the separation of flavonoids using solvent-modified micellar electrokinetic chromatography. Electrophoresis 26(17):3387–3396
18. Wahyuni Y, Ballester AR, Sudarmonowati E, Bino RJ, Bovy AG (2011) Metabolite biodiversity in pepper (Capsicum) fruits of thirty-two diverse accessions: variation in health-related compounds and implications for breeding. Phytochemistry 72(11–12):1358–1370
19. Materska M, Perucka I (2005) Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). J Agric Food Chem 53(5):1750–1756
20. Kris-Etherton PM, Lefevre M, Beecher GR, Gross MD, Keen CI, Etherton TD (2004) Bioactive compounds in nutrition and health-research methodologies for establishing biological function: the antioxidant and anti-inflammatory effects of flavonoid on atherosclerosis. Annu Rev Nutr 24:511–538
21. Howard LR, Talcott ST, Brenes CH, Villalon B (2000) Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum Species*) as influenced by maturity. J Agric Food Chem 48:1713–1720
22. Zhuang Y, Chen L, Sun L, Cao J (2012) Bioactive characteristics and antioxidant activities of nine peppers. J Funct Foods 4:331–338
23. Kouassi KC, Koffi-Nevry R, Guillaume LY, Yéssé ZN, Koussémon M, Kablan T, Athanase KK (2012) Profiles of bioactive compounds of some pepper fruit (*Capsicum L.*) varieties grown in Côte D'ivoire. Innovat Rom Food Biotechnol 11:23–31
24. Shotorbani N, Jamei R, Heidari R (2013) Antioxidant activities of two sweet pepper *Capsicum annuum* L. varieties phenolics extracts and the effects of thermal treatment. Avicenna J Phytomed 3:25–34
25. Matsufuji H, Nakamuro H, Chino M, Mitsuharo T (1998) Antioxidant activity of capsanthin and the fatty acid esters in paprika (*Capsicum annuum*). J Agric Food Chem 46:3462–3472
26. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD (2004) Free radicals and antioxidants in human health: current status and future prospects. J Assoc Phys India 52:794–804
27. Ha SH, Kim JB, Park JS, Lee SW, Cho KJ (2007) A comparison of the carotenoid accumulation in capsicum varieties that show different ripening colours: deletion of the capsanthin–-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. J Exp Bot 58:3135–3144
28. Srinivas CH, Sai Pavan Kumar CHN, China RB, Jayathirtha RV, Naidu VG (2009) First stereoselective total synthesis and anticancer activity of new amide alkaloids of roots of pepper. Bioorg Med Chem Lett 19:5915–5918
29. Qian K, Wang G, Cao R, Liu T, Qian G, Guan X, Guo Z, Xiao Y, Wang X (2016) Capsaicin suppresses cell proliferation, induces cell cycle arrest and ROS production in bladder cancer cells through FOXO3a-mediated pathways. Molecules 21(10):1406
30. Vendrely V, Peuchant E, Buscail E, Moranvillier I, Rousseau B, Bedel A, Brillac A, de Verneuil H, Moreau-Gaudry F, Dabernat S (2017) Resveratrol and capsaicin used together as food complements reduce tumor growth and rescue full efficiency of low dose gemcitabine in a pancreatic cancer model. Cancer Lett 390:91. pii: S0304-3835(17)30017-4
31. Hu YW, Ma X, Huang JL, Mao XR, Yang JY, Zhao JY, Li SF, Qiu YR, Yang J, Zheng L, Wang Q (2013) Dihydrocapsaicin attenuates plaque formation through a PPARγ/LXRα

pathway in apoE$^{-/-}$ mice fed a high-fat/high-cholesterol diet. PLoS One 8(6):e66876. https://doi.org/10.1371/journal.pone.0066876
32. Persson MS, Fu Y, Bhattacharya A, Goh SL, van Middelkoop M, Bierma-Zeinstra SM, Walsh D, Doherty M, Zhang WO, OA Trial Bank Consortium (2016) Relative efficacy of topical non-steroidal anti-inflammatory drugs and topical capsaicin in osteoarthritis: protocol for an individual patient data meta-analysis. Syst Rev 5(1):165. https://doi.org/10.1186/s13643-016-0348-8
33. Lee JH, Kim C, Baek SH, Ko JH, Lee SG, Yang WM, Um JY, Sethi G, Ahn KS (2016) Capsaezepine inhibits JAK/STAT3 signaling, tumor growth, and cell survival in prostate cancer. Oncotarget. https://doi.org/10.18632/oncotarget.10775
34. Nishino H, Tokuda H, Satomi Y, Masuda M, Bu P, Onozuka M, Yamaguchi S, Okuda Y, Takayasu J, Tsuruta J et al (1999) Cancer prevention by carotenoids. Pure Appl Chem 71:2273–2278
35. Vendrely V, Peuchant E, Buscail E, Moranvillier I, Rousseau B, Bedel A, Brillac A, de Verneuil H, Moreau-Gaudry F, Dabernat S (2017) Resveratrol and capsaicin used together as food complements reduce tumor growth and rescue full efficiency of low dose gemcitabine in a pancreatic cancer model. Cancer Lett. https://doi.org/10.1016/j.canlet.2017.01.002. pii: S0304-3835(17)30017-4. Epub ahead of print
36. Amantini C, Morelli MB, Nabissi M, Cardinali C, Santoni M, Gismondi A, Santoni G (2016) Capsaicin triggers autophagic cell survival which drives epithelial mesenchymal transition and chemoresistance in bladder cancer cells in an Hedgehog-dependent manner. Oncotarget. https://doi.org/10.18632/oncotarget.10326. Epub ahead of print
37. Bessler H, Djaldetti M (2017) Capsaicin modulates the immune cross talk between human Mononuclears and cells from two colon carcinoma lines. Nutr Cancer 69(1):14–20. https://doi.org/10.1080/01635581.2017.1247893. Epub 2016 Nov 30
38. Liu T, Wang G, Tao H, Yang Z, Wang Y, Meng Z, Cao R, Xiao Y, Wang X, Zhou J (2016) Capsaicin mediates caspases activation and induces apoptosis through P38 and JNK MAPK pathways in human renal carcinoma. BMC Cancer 16(1):790
39. Wang F, Zhao J, Liu D, Zhao T, Lu Z, Zhu L, Cao L, Yang J, Jin J, Cai Y (2016) Capsaicin reactivates hMOF in gastric cancer cells and induces cell growth inhibition. Cancer Biol Ther 17:1117
40. Jin T, Wu H, Wang Y, Peng H (2016) Capsaicin induces immunogenic cell death in human osteosarcoma cells. Exp Ther Med 12(2):765–770
41. Lin MH, Lee YH, Cheng HL, Chen HY, Jhuang FH, Chueh PJ (2016) Capsaicin inhibits multiple bladder cancer cell phenotypes by inhibiting tumor-associated NADH oxidase (tNOX) and Sirtuin1 (SIRT1). Molecules 21(7). https://doi.org/10.3390/Moleculesules21070849. pii: E849
42. Lee JH, Kim C, Baek SH, Ko JH, Lee SG, Yang WM, Um JY, Sethi G, Ahn KS (2016) Capsazepine inhibits JAK/STAT3 signaling, tumor growth, and cell survival in prostate cancer. Oncotarget. https://doi.org/10.18632/oncotarget.10775
43. Sun F, Xiong S, Zhu Z (2016) Dietary capsaicin protects Cardiometabolic organs from dysfunction. Forum Nutr 8(5). https://doi.org/10.3390/nu8050174. pii: E174
44. McCarty MF, DiNicolantonio JJ, O'Keefe JH (2015) Capsaicin may have important potential for promoting vascular and metabolic health. Open Heart 2(1):e000262. https://doi.org/10.1136/openhrt-2015-000262
45. Duzhyy DE, Viatchenko-Karpinski VY, Khomula EV, Voitenko NV, Belan PV (2015) Upregulation of T-type Ca^{2+} channels in long-term diabetes determines increased excitability of a specific type of capsaicin-insensitive DRG neurons. Mol Pain 11:29. https://doi.org/10.1186/s12990-015-0028-z
46. Yuan LJ, Qin Y, Wang L, Zeng Y, Chang H, Wang J, Wang B, Wan J, Chen SH, Zhang QY, Zhu JD, Zhou Y, Mi MT (2016) Capsaicin-containing chili improved postprandial hyperglycemia, hyperinsulinemia, and fasting lipid disorders in women with gestational diabetes mellitus and lowered the incidence of large-for-gestational-age newborns. Clin Nutr 35(2):388–393. https://doi.org/10.1016/j.clnu.2015.02.011. Epub 2015 Mar 2

47. Skrzypski M, Sassek M, Abdelmessih S, Mergler S, Grötzinger C, Metzke D, Wojciechowicz T, Nowak KW, Strowski MZ (2014) Capsaicin induces cytotoxicity in pancreatic neuroendocrine tumor cells via mitochondrial action. Cell Signal 26(1):41–48. https://doi.org/10.1016/j.cellsig.2013.09.014. Epub 2013 Sep 27
48. Kang JH, Tsuyoshi G, Le Ngoc H, Kim HM, Tu TH, Noh HJ, Kim CS, Choe SY, Kawada T, Yoo H, Yu R (2011) Dietary capsaicin attenuates metabolic dysregulation in genetically obese diabetic mice. J Med Food 14(3):310–315. https://doi.org/10.1089/jmf.2010.1367
49. Zhao WX, Hong ZF, Yin ZY, Xie CR, Xu YP, Chi XQ, Zhang S, Wang XM (2014) Capsaicin enhances the drug sensitivity of cholangiocarcinoma through the inhibition of chemotherapeutic-induced autophagy. PLoS One 10(5):e0121538. https://doi.org/10.1371/journal.pone.0121538. eCollection 2014
50. Baskaran P, Krishnan V, Ren J, Thyagarajan B (2016) Capsaicin induces browning of white adipose tissue and counters obesity by activating TRPV1 channel-dependent mechanisms. Br J Pharmacol 173(15):2369–2389. https://doi.org/10.1111/bph.13514
51. Saito M (2015) Capsaicin and related food ingredients reducing body fat through the activation of TRP and Brown fat thermogenesis. Adv Food Nutr Res 76:1–28. https://doi.org/10.1016/bs.afnr.2015.07.002.
52. Ibrahim M, Jang M, Park M, Gobianand K, You S, Yeon SH, Park S, Kim MJ, Lee HJ (2015) Capsaicin inhibits the adipogenic differentiation of bone marrow mesenchymal stem cells by regulating cell proliferation, apoptosis, oxidative and nitrosative stress. Food Funct 6(7):2165–2178. https://doi.org/10.1039/c4fo01069h.
53. Chen J, Li L, Li Y, Liang X, Sun Q, Yu H, Zhong J, Ni Y, Chen J, Zhao Z, Gao P, Wang B, Liu D, Zhu Z, Yan Z (2015) Activation of TRPV1 channel by dietary capsaicin improves visceral fat remodeling through connexin43-mediated Ca^{2+} influx. Cardiovasc Diabetol 14:22. https://doi.org/10.1186/s12933-015-0183-6.
54. Hong ZF, Zhao WX, Yin ZY, Xie CR, Xu YP, Chi XQ, Zhang S, Wang XM (2015) Capsaicin enhances the drug sensitivity of cholangiocarcinoma through the inhibition of chemotherapeutic-induced autophagy. PLoS One 10(5):e0121538. https://doi.org/10.1371/journal.pone.0121538. eCollection 2015
55. Tan S, Gao B, Tao Y, Guo J, Su ZQ (2014) Antiobese effects of capsaicin-chitosan microsphere (CCMS) in obese rats induced by high fat diet. J Agric Food Chem 62(8):1866–1874. https://doi.org/10.1021/jf4040628
56. Lee GR, Shin MK, Yoon DJ, Kim AR, Yu R, Park NH, Han IS (2013) Topical application of capsaicin reduces visceral adipose fat by affecting adipokine levels in high-fat diet-induced obese mice. Obesity (Silver Spring) 21(1):115–122. https://doi.org/10.1002/oby.20246.
57. Nicholas S, Yuan SY, Brookes SJ, Spencer NJ, Zagorodnyuk VP (2017) Hydrogen peroxide preferentially activates capsaicin-sensitive high threshold afferents via TRPA1 channels in the guinea pig bladder. Br J Pharmacol 174(2):126–138
58. Pyun CW, Kim JH, Han KH, Hong GE, Lee CH (2014) In vivo protective effects of dietary curcumin and capsaicin against alcohol-induced oxidative stress. Biofactors 40(5):494–500. https://doi.org/10.1002/biof.1172
59. Jung SH, Kim HJ, Oh GS, Shen A, Lee S, Choe SK, Park R, So HS (2014) Capsaicin ameliorates cisplatin-induced renal injury through induction of heme oxygenase-1. Mol Cells 37(3):234–240. https://doi.org/10.14348/molcells.2014.2322
60. Pramanik KC, Boreddy SR, Srivastava SK (2011) Role of mitochondrial electron transport chain complexes in capsaicin mediated oxidative stress leading to apoptosis in pancreatic cancer cells. PLoS One 6(5):e20151. https://doi.org/10.1371/journal.pone.0020151
61. Gupta RS, Dixit VP, Dobhal MP (2002) Hypocholesterolaemic effect of the oleoresin of *Capsicum annum* L. in gerbils (*Meriones hurrianae* Jerdon). Phytother Res 16:273–275
62. Sambaiah K, Satyanarayana MN (1980) Hypocholesterolemic effect of red pepper & capsaicin. Indian J Exp Biol 18:898–899

63. Kim JS, Ha Y, Kim S, Lee SJ, Ahn J (2017) Red paprika (*Capsicum annuum* L.) and its main carotenoid capsanthin ameliorate impaired lipid metabolism in the liver and adipose tissue of high-fat diet-induced obese mice. J Funct Foods 31:131–140
64. Kumar P, Chand S, Chandra P, Maurya PK (2015) Influence of dietary capsaicin on Redox status in red blood cells during human aging. Adv Pharm Bull 5(4):583–586. https://doi.org/10.15171/apb.2015.078
65. Jiang X, Jia LW, Li XH, Cheng XS, Xie JZ, Ma ZW, Xu WJ, Liu Y, Yao Y, Du LL, Zhou XW (2013) Capsaicin ameliorates stress-induced Alzheimer's disease-like pathological and cognitive impairments in rats. J Alzheimers Dis 35(1):91–105. https://doi.org/10.3233/JAD-121837
66. Lee JG, Yon JM, Lin C, Jung AY, Jung KY, Nam SY (2012) Combined treatment with capsaicin and resveratrol enhances neuroprotection against glutamate-induced toxicity in mouse cerebral cortical neurons. Food Chem Toxicol 50(11):3877–3885. https://doi.org/10.1016/j.fct.2012.08.040
67. Lee TH, Lee JG, Yon JM, Oh KW, Baek IJ, Nahm SS, Lee BJ, Yun YW, Nam SY (2011) Capsaicin prevents kainic acid-induced epileptogenesis in mice. Neurochem Int 58(6):634–640. https://doi.org/10.1016/j.neuint.2011.01.027.
68. Bert J, Mahowald ML, Frizelle S, Dorman CW, Funkenbusch SC and Krug HE (2016). The effect of treatment with Resiniferatoxin and capsaicin on dynamic weight bearing measures and evoked pain responses in a chronic inflammatory arthritis murine model. Intern Med Rev (Wash DC) 16(6). pii: 89
69. Walker J, Ley JP, Schwerzler J, Lieder B, Beltran L, Ziemba PM, Hatt H, Hans J, Widder S, Krammer GE, Somoza V (2017) Nonivamide, a capsaicin analogue, exhibits anti-inflammatory properties in peripheral blood mononuclear cells and U-937 macrophages. Mol Nutr Food Res 1600474
70. Tang J, Luo K, Li Y, Chen Q, Tang D, Wang D, Xiao J (2015) Induced inflammatory cytokine production by upregulation of LXRα. Int Immunopharmacol 28(1):264–269. https://doi.org/10.1016/j.intimp.2015.06.007.
71. Foster HE, Lake AG (2014) Use of Vanilloids in urologic disorders. In: Abdel-Salam EOM (ed) Capsaicin as a therapeutic molecules. Springer, Basel, pp 307–317
72. Haab F (2014) Chapter 1: the conditions of neurogenic detrusor overactivity and overactive bladder. Neurourol Urodyn 33(Suppl S3):S2–S5
73. Wouters AT, Casagrande RA, Wouters F, Watanabe TT, Boabaid FM, Cruz CE, Driemeier D (2013) An outbreak of aflatoxin poisoning in dogs associated with aflatoxin B1-contaminated maize products. J Vet Diagn Investig 25:282–287
74. Wadie BS (2015) Management of refractory OAB in the non-neurogenic patient. Curr Urol Rep 15:438
75. Yamaguchi O, Nishizawa O, Takeda M, Yokoyama O, Homma Y, Kakizaki H, Obara K, Gotoh M, Igawa Y, Seki N (2009) Clinical guidelines for overactive bladder. Int J Urol 16:126–142
76. MacDonald R, Monga M, Fink HA, Wilt TJ (2008) Neurotoxin treatments for urinary incontinence in subjects with spinal cord injury or multiple sclerosis: a systematic review of effectiveness and adverse effects. J Spinal Cord Med 31:157–165
77. Everaerts W, Gevaert T, Nilius B, De Ridder D (2008) On the origin of bladder sensing: trips in urology. Neurourol Urodyn 27:264–273
78. Li M, Sun Y, Simard JM, Chai TC (2011) Increased transient receptor potential vanilloid type 1 (TRPV1) signaling in idiopathic overactive bladder urothelial cells. Neurourol Urodyn 30:606–611

Sesame: Bioactive Compounds and Health Benefits

8

Niti Pathak, Asani Bhaduri, and Ashwani K. Rai

Contents

Abstract

Sesame is a valuable oilseed crop that contains various nutritionally rich bioactive compounds including lignans, tocopherol homologues, phytosterols, etc. Lignans are the product of oxidative coupling of β-hydroxyphenylpropane. Sesame has a

N. Pathak
Department of Botany, Deshbandhu College, University of Delhi, Delhi, India
e-mail: nitiest@gmail.com

A. Bhaduri
Cluster Innovation Centre, University of Delhi, Delhi, India
e-mail: asanii.bhaduri@gmail.com

A. K. Rai (✉)
Department of Botany, Banaras Hindu University, Varanasi, India
e-mail: akrai.bhu@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_59

combination of glycosylated lignans and oil-dispersed lignans. Based on their medicinal and pharmacological properties, the most important lignans are sesamin, sesamol, sesamolin, and sesaminol. Tocopherols (vitamin E compounds) are the lipid-soluble free radicals and constitute a major part of human diet. In sesame seeds, α-, γ-, and δ-tocopherols are found as tocopherol homologues. In addition to lignans and tocopherols, sesame is an important source of phytosterols, phytates, polyunsaturated fatty acids, and bioactive peptides. However, utilization potential of many of these compounds has not yet been fully understood. This chapter delves into the presence of multifarious bioactive components in sesame seeds, their biosynthetic pathway, and functional importance.

Keywords
Bioactive compounds · Sesame · Sesamin · Sesamolin · Sesamol · Pinoresinol · Tocopherol · Phytosterols

Abbreviations

CYP81Q1	Sesamin synthase
DIR1	Dirigent protein
DMPQ	2,3-Dimethyl-5-phytyl-1,4-hydroquinol
VTE1	Tocopherol cyclase
γ-TMT	γ-Tocopherol methyltransferase

1 Introduction

Sesame (*Sesamum indicum*, family Pedaliaceae) is considered as one of the earliest domesticated crops and oilseed plants known to mankind with its multifarious uses. It is found in the tropics and subtropics but is most common in the narrower belt closer to the equator, mostly north of it [1]. Basically, sesame is a crop of the developing countries in more southern latitudes. For a long time, it is being used in religious rituals in India, Egypt, and Persian region [2, 3]. Sesame is regarded as "queen of oilseeds" because of its oil quality [4, 5], sterols, and antioxidative agents, i.e., methylenedioxyphenyl compounds, sesamin, sesamolin, and tocopherols that act as nutraceuticals and impart resistance to oil against oxidative deterioration. Further, the composition of dry decorticated sesame seed per 100 g includes edible portion, i.e., water (3.75 g), energy (2640 kJ; 631 kcal), protein (20.45 g), fat (61.21 g), carbohydrate (11.73 g), dietary fiber (11.6 g), high amounts of Ca (60 mg), Mg (345 mg), P (667 mg), K (370 mg), Fe (6.36 mg), Zn (6.73 mg), vitamin A (66 IU), thiamin (0.70 mg), riboflavin (0.09 mg), niacin (5.80 mg), folate (115 μg), alpha-tocopherol (1.68 mg), and no ascorbic acid [6]. The presence of oxalic acid in sesame seeds makes it little bitter in taste.

The sesame oil is comprised of 83–90% unsaturated fatty acids that contain glycerides of oleic acid (36–54%) and linoleic acid (38–49%). Other components are saturated fatty acids (myristic acid, 0.1% or less; palmitic acid, 8–12%; stearic acid, 3.5–7%; arachidonic acid, 0.5–1%). The unsaponifiable matter (1.2%) includes tocopherols and the lignans sesamin (0.1–0.6%), sesamolin (0.25–0.3%), sesamol,

Fig. 1 Seed color variation in two sesame species cultivated in India

and sesaminol, which give the oil its resistance to rancidity. Extracted sesame cake varies in color from light yellow to grayish black, depending on the dominant seed coat color. Its chemical composition also varies according to cultivar, method of oil extraction, and the presence of testa. The sesame cake has ample amount of calcium and phosphate; protein content ranges from 35% to 47% but is deficient in lysine.

Sesame seed color show variation in different species which could partly be attributed to changes in composition of bioactive phenolics i.e., lignans and tocopherols (Fig. 1). The present chapter provides detailed and updated information on the bioactive components present in sesame seeds, their importance, and health benefits. In particular, sesame lignans and tocopherols have been focused at length: the biosynthetic pathway, the gene regulation, and the biotechnological approaches to enhance the concentration of lignans and tocopherols in sesame crop.

2 Bioactive Compounds in Sesame

Bioactive compounds are beneficial components in food and are accountable for disease-preventing properties. They include a range of chemical compounds including phenolics, carotenoids, phytosterols, and polyunsaturated fatty acids. These compounds are often utilized as antioxidants and other purposes such as inhibiting cholesterol absorption, blocking the activity of bacterial toxins, etc. Our recent review on value addition in sesame crop has elaborated the production of value-added products such as sesame oil and meal, thereby enhancing its economic importance [7]. The target candidates for this study are bioactive compounds such as lignans, tocopherols, phytates, phytosterols, and polyunsaturated fatty acids (Fig. 2).

2.1 Phenolics

Natural phenolic compounds are secondary metabolites, which are widely distributed in the plant kingdom. Plant phenols are noted for their role in prevention of

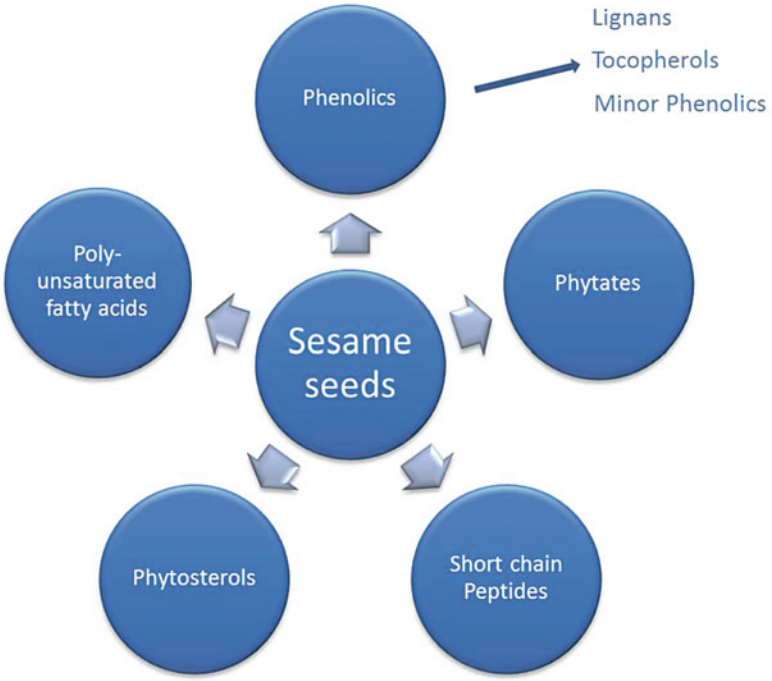


Fig. 2 Distribution of bioactive components in sesame seeds

various ailments associated with oxidative stress such as cardiovascular and neurodegenerative diseases and cancer [8]. Phenolics are characterized by the presence of aromatic ring (at least one) coupled with a few hydroxyl groups. The antioxidative potential of phenolics is very high as these compounds can produce stable radical intermediates utilizing electrons. Due to their antioxidative potential, they play an important role in the stabilization of edible oils and protection from off-flavor formation [8]. Recent findings suggest that phenolics could play an important role in conditions associated with oxidative stress [9, 10]. The antioxidant property of sesame seed and its oil along with various health properties is attributed to the presence of lignans such as sesamin, sesamolin, sesaminol, sesangolin, 2-episalatin, and tocopherol isomers [11]. Sesame oil is extremely resistant to oxidative rancidity due to the presence of chemically related compounds sesamol and sesamol dimer.

2.1.1 Lignans: Chemistry and Biosynthesis

The common feature of many natural products is recognized as a C_6C_3 unit, i.e., a propylbenzene or phenylpropanoid skeleton [12]. In a review of natural resins, Haworth was first to suggest (1936) [13] that the class of compounds derived from two C_6C_3 units having β,β′ linkage (8–8′ bond) should be called as lignans. Lignan is actually a constituent of lignin, a generic name for the compound resulting from two p-hydroxyphenylpropane molecules. Sesame seed contains two major groups of

lignans: (1) oil-soluble lignans (sesamin, sesamolin, sesaminol, sesamolinol, and pinoresinol) and (2) glycosylated water-soluble lignans (sesaminol triglucoside, pinoresinol triglucoside, sesaminol monoglucoside, pinoresinol monoglucoside, and two isomers of pinoresinol diglucoside and sesaminol diglucoside) [14–16]. The antioxidative property of sesame seed is associated with lignan components present in the oil and are unique to sesame. The lignans, sesamin, and sesamolin and their derivatives prevent oxidation of the oil and give it a long shelf life and stability [17]. Lignans and tocopherols are also reported to act in coordination resulting in enhanced vitamin E activity [18]. Namiki [19] suggested that the bioactive compounds present in sesame seed cannot individually describe the high oxidative stability of roasted sesame oil, but the cumulative effect of all the components of sesame oil protects the roasted sesame oil from oxidative deterioration.

The oil fraction of most oilseeds comprises mainly of triacylglycerols (95–99%) acting as a dispersing media for a wide range of lipophilic and amphiphilic secondary metabolites, together known as the unsaponifiable fraction of the seed. These unsaponifiable portions can be extracted with lipophilic solvents after saponification of acyl lipids [20] and are of importance as the presence of the lignans may be taken as marker for genuineness of the oil. Other constituents, tocopherols and tocotrienols, are strong antioxidants and provide stability to the oil. Also, lignans, especially sesamin, have currently been recognized for possessing interesting physiological bioactivities against chronic diseases.

Sesaminol, a water-soluble glycoside along with fat-soluble sesamin and sesamolin, constitutes the major lignans of sesame seeds. The isomer of sesamin called episesamin is generated in the process of refining sesame oil. The functional methylenedioxyphenyl group of most of these lignans especially sesamin, sesamolin, and sesangolin initiates the activities [21, 22], and these molecules in turn utilize their effect via inhibition of liver microsome oxidases [23].

Sesamin Biosynthesis

Phenylalanine and tyrosine are precursors for various plant substances such as tannins, polymeric lignin, and lignans. Phenylalanine is converted to cinnamic acid by the enzyme phenylalanine ammonia lyase (PAL). This is followed by a series of hydroxylation, methylation, and reduction leading to production of coumaric acid, caffeic acid, ferulic acid, and eventually E-coniferyl alcohol [24]. However, it has also been claimed that [^{14}C]-labeled tyrosine is incorporated into sesamin when administered to the cell suspension cultures of *Sesamum indicum* [25]. E-coniferyl alcohol has also been shown to undergo stereoselective coupling to synthesize (+)-pinoresinol in *Sesamum indicum* seeds (Fig. 3). (+)-Pinoresinol is then metabolized further, and methylenedioxyphenyl group is added in maturing sesame seeds which result in production of (+)-piperitol and (+)-sesamin and (+)-sesamolin [26]. A 78 kDa dirigent protein is known to assist in a stereoselective bimolecular coupling to produce (+)-pinoresinol [27]. Lignan synthesis is developmentally regulated and depends upon the stage of seed maturity. The most mature seeds of 8 weeks efficiently convert (+)-pinoresinol into (+)-piperitol and (+)-sesamin, while younger seeds have higher conversion to (+)-sesamolin [28].

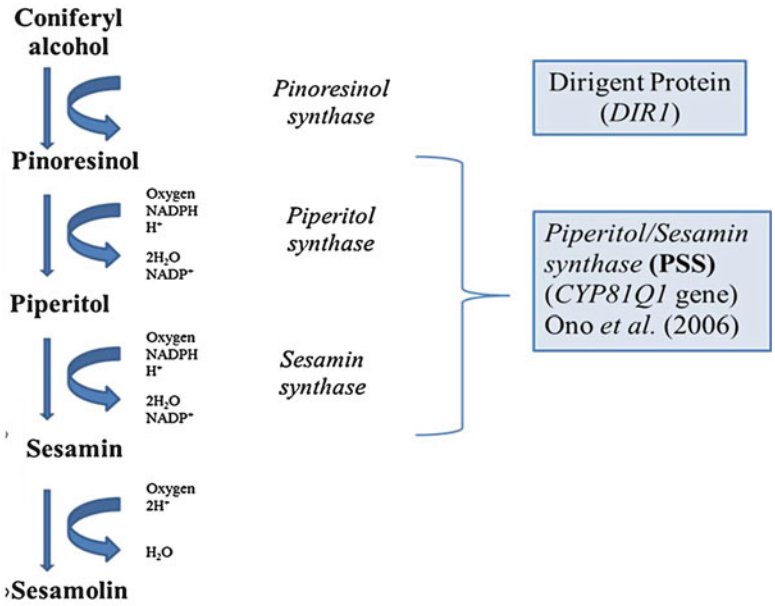


Fig. 3 Diagrammatic representation of sesamin biosynthetic pathway depicting conversion of coniferyl alcohol to pinoresinol with unstable intermediate piperitol followed by conversion to sesamin. Sesamin is further converted to sesamolin. Boxes indicate action of enzymes

Recently, it is shown that the synthesis of sesamin from pinoresinol is catalyzed by *CYP81Q1* in two steps [29].

2.1.2 Tocopherol Chemistry and Biosynthesis

Tocochromanols have both the hydrophobic and hydrophilic elements – these biomolecules generally have a lipophilic isoprenoid side chain linked to the membrane lipids and a polar chromanol ring toward the membrane surface. Tocochromanols inhibit membrane lipid peroxidation and scavenge reactive oxygen species. It is a well-known fact that antioxidants neutralize free radicals, thereby preventing DNA damage. Being scavenger of reactive oxygen species, tocopherols minimize free radical attack and interrupt lipid peroxidation. In this way, these molecules protect cell membranes, enable lipid repair and replacement, and are useful in preventing cancer and heart diseases [30]. Tocopherols are known to play a role in plant metabolism, for instance, sugar transport from leaves to phloem [31].

Tocopherols are an important group of plant phenolics that have antioxidative activity and nutritional values [32]. They belong to a family of molecules that have a chromanol ring (chroman ring with an alcoholic hydroxyl group) and a 12-carbon aliphatic side chain containing two methyl groups in the middle and two more methyl groups at the end. Plants synthesize eight different vitamin E forms, including α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols [33]. All tocopherols and tocotrienols consist of a chromanol ring and varying number of methyl

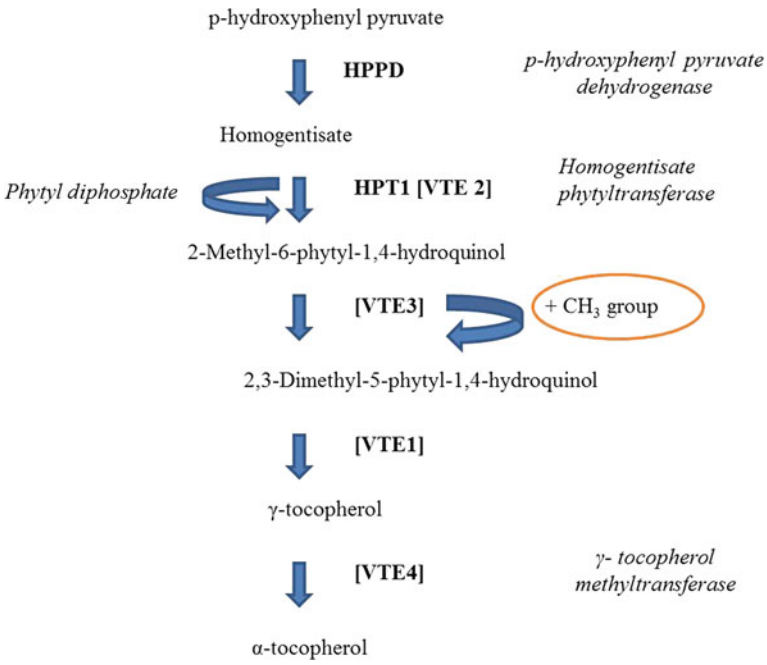


Fig. 4 Diagrammatic representation of tocopherol biosynthetic pathway depicting conversion of p-hydroxyphenylpyruvate to homogentisate, which gets converted to methylated hydroquinol and finally to tocopherol homologues

groups on the chromanol ring. Metabolic fate and biological activities of tocols depend upon their structural features. The entire isoforms act as lipid antioxidants, and α-tocopherol has the highest vitamin E activity [34, 35]. The difference between tocopherols and tocotrienols is that tocopherols have a saturated tail, while tocotrienols have an unsaturated tail.

Higher plants like dicots have tocopherols in almost all parts including roots, stems, leaves, flowers, fruits, and seeds [36, 37]. However, the total tocopherol content and different forms of tocopherols in these tissues differ considerably. Among the tocopherols, α-tocopherol is the predominant form in photosynthetic tissues such as stems and leaves. In most seed crops, α-tocopherol is present only in a minor form, and γ- and δ-tocopherols tend to predominate [38].

Tocopherol Biosynthesis

The hydroquinone ring of tocopherol is derived from the shikimate pathway of aromatic amino acid synthesis (Fig. 4). Biosynthesis of homogentisate, the precursor for tocopherol, tocotrienol, and plastoquinone, is catalyzed by p-hydroxyphenylpyruvate dioxygenase (*HPPD*) [39]. After attachment of the hydrophobic side chain by homogentisate phytyl transferase (*HPT1/VTE2*) [40, 41] and on methylation (*VTE3*) [42, 43], 2,3-dimethyl-5-phytyl-1,4-hydroquinol (DMPQ) is formed, which is converted to γ-tocopherol by tocopherol cyclase (*VTE1*) [44, 45].

Tocopherol cyclase is considered as a key enzyme in tocopherol biosynthesis. Final methylation by γ-tocopherol methyltransferase (γ-TMT, *VTE4*) results in the production of α-tocopherol.

2.1.3 Sesame Seed: Lignan and Tocopherol Bioactivities

Sesamin and sesamolin were initially considered as the major lignans in sesame seeds [46]. Sesaminol, another major lignan, was identified later from sesame seeds [47]. These seed components possess unique properties, and regular human consumption helps in lowering blood lipids [48] and arachidonic acid levels [49]. The sesame seed lignans are also known to reduce cholesterol level by inhibiting its absorption and synthesis simultaneously [50]. These lignans are anticarcinogenic [51] and anti-inflammatory [52] and are known to increase hepatic fatty acid oxidation [53]. They have immunomodulatory activities [54] and possess antihypertensive [55, 56] and neuroprotective effects against hypoxia or brain damage [57].

In addition to lignans, multiple tocopherol homologues [α-tocopherol (αT), δ-tocopherol (δT), and γ-tocopherol (γT), tocotrienols] are also present in sesame seeds, which possess antioxidative and health-promoting properties. Sesame lignans are found to exhibit synergistic effect with tocopherols on vitamin E activity and act as specific inhibitor of fatty acid metabolism in humans [58]. These nutritional properties of the sesame seeds contributed by the principal factors lignans and tocopherols have promoted their use in the daily diet worldwide.

Sesame lignans have antioxidant and tocopherol-sparing activities [59–62]. They are reported to reduce cholesterol level [48, 50, 63] and exhibit antihypertensive [64] and anti-inflammatory activities [65] as well as affect lipid metabolism by enhancing gene expression and hepatic enzyme (acyl CoA oxidase, carnitine palmitoyltransferase, bifunctional enzyme, and 3-ketoacyl-CoA-thiolase) activities involved in fatty acid oxidation [66, 67]. On the other hand, lignans reduce the activities of enzymes involved in lipogenesis (acetyl-CoA carboxylase, fatty acid synthase, ATP citrate lyase, glucose-6-phosphate dehydrogenase, and pyruvate kinase) by altering the gene expression [66]. Hence, sesame plays a crucial role in minimizing the vulnerability and increasing the safety against atherosclerosis, cancer, and cardiovascular diseases [48, 64, 68].

Thus, sesame has both preventive and therapeutic values in a variety of chronic diseases, owing to its rich lignan content and its antioxidative, anticholesterolemic, and antihypertensive properties. Sesame lignans, especially sesamin, are absorbed in human body, undergo enterohepatic circulation, and are converted into strong antioxidative metabolites. Intestinal bacteria metabolize them into other bioactive compounds such as mammalian lignans enterolactone and enterodiol compounds, which the role in breast, prostate, and colon cancers, bone health, and cardiovascular diseases is understudy [69–71].

In traditional Chinese and Indian systems of medicine like Ayurveda, sesame occupies an important position with diverse pharmaceutical application. In China, sesame oil is used in treatment of toothaches and gum diseases [72]. In India, it is being used as an antibacterial mouthwash to relieve anxiety and insomnia and in the treatment of blurred vision, dizziness, and headache [72]. Sesame oil is also noted for burn-healing effect [73]. Moist-exposed burn ointment (MEBO) a purely herbal

formulation from China contains sesame oil as an active ingredient along with β-sitosterol, berberine, and other plant extracts in trace amount [74] and is usually used in managing the burns of the face, neck, and hand [73].

The main antioxidant activity of α-tocopherol is to break the radical chain in membranes and lipoproteins [75]. Its antioxidant potential along with various functions at the molecular level mitigates the possibility of cardiovascular diseases and cancers [76, 77]. Other tocols that are present in lesser amount can also perform the antioxidative and biological activities. Gamma-tocopherol (γ-T), for instance, is more potent in decreasing platelet aggregation and LDL oxidation and delaying intra-arterial thrombus formation than that of α-tocopherol [78, 79]. Tocotrienols inhibit cholesterol biosynthesis [80] and are found effective in reducing the tendency of breast cancer [81]. Thus, tocopherols have high antioxidant, antitumor, and hypocholesterolemic potential. γ-Tocopherol is the major tocopherol in sesame, whereas α- and δ-tocopherols are present in very small amounts. It has been reported that γ-tocopherol is a more effective antioxidant compared to other tocopherols [82] but has poor vitamin E activity in biological systems [76].

Tocopherols terminate the recyclable chain reaction of polyunsaturated fatty acid (PUFA) radicals produced by oxidation of lipid [83]. The lipid peroxy radicals that are scavenged by tocopherols are converted into tocopheroxyl radicals, which with the help of ascorbate and other antioxidants are recycled back as the corresponding tocopherol [84]. In this manner, a tocopherol molecule can undergo in multiple lipid peroxidation chain-breaking events before it is finally degraded.

2.2 Minor Phenolics

In addition to tocopherols and lignans, sesame contains other phenolics like naphthoquinone and phenolic acids in trace amounts [85–88]. Sesamol, which is known as a strong free radical scavenger [89], is also present in sesame oil. It is a free phenol with methylenediphenoxy group.

2.3 Phytosterols

Phytosterols (sterols and stanols) are plant triterpenes with preventive functions in many diseases especially cancer. They have been shown to possess antioxidant [90], anti-inflammatory [91], and antibacterial properties [92]. Similar in structure to cholesterol (phytosterols have extra methyl group at C-24 position), these plant sterols, when digested, compete with cholesterol for small intestine absorption leading to lowering of the cholesterol level in blood [93]. The recommended functional foods usually contain phytosterols extracted from plant sources, or at times processed foods have phytosterols as supplement and are sold as cholesterol-lowering foods. Although corn and legumes are used to extract phytosterols, it is the sesame seeds that have the highest (400–413 mg 100 g^{-1}) amount of phytosterols [94].

In a recent study, Gharby et al. [95] noted that β-sitosterol constitutes a major portion of phytosterols in sesame seed and oil, and campesterol and stigmasterol are the other important sterols present. Compared to other phytosterols, β-sitosterol has been studied more extensively for its beneficial and physiological effect on human being. β-Sitosterol lowers cholesterol level [96], enhances immunity, and has anti-inflammatory properties [97]. The other major component is campesterol, which accounts for about 17.8% of the total sterols. Stigmasterol and Δ5-avenasterol measure about 6.4% and 10.2%, respectively, in sesame oil. Minor sterols present are Δ7-stigmasterol and Δ7-avenasterol. The total sterol content in sesame seed oil is approximately 540 mg/100 g oil.

2.4 Phytates

Phytic acid is a bioactive compound with wide distribution in plant foods. Due to its molecular structure, phytic acid has affinity to polyvalent cations such as minerals and trace elements. Phytic acid is one of the most important sources of phosphorus in plant seeds, and sesame is no exception. In fact, sesame seeds are richer in phytate than the commonly known legumes. In oil seeds such as sunflower, soybean, sesame, linseed, and rapeseed, the phytic acid content ranges from 1% to 5.4% compared to 0.2–2.9% in legumes. A defatted sesame meal has much higher phytate concentration than that of soybean meal [98]. Graf and Dintzis [99] have measured 5.36% phytic acid content in sesame seeds. Often, phytates are termed as anti-nutrient for preventing mineral absorption from meal, but it is also seen that phytates have anticancerous and hypocholesterolemic activities [100, 101].

2.5 Polyunsaturated Fatty Acids

Fatty acids are carboxylic acids with long-chain hydrocarbon side groups derived from or contained as esterified molecular form in lipids (fat, oil, or wax) of microbes, animals, and plants usually ranging from 1 to 30 carbon atoms in length attached to a terminal carboxyl group [102]. Polyunsaturated fatty acids (PUFAs) are long-chain fatty acids containing two or more double bonds introduced by specific desaturase enzymes. Over the years, vegetable oils rich in various PUFAs have emerged as potential dietary elements for normal growth and development of human beings with considerable biomedical significance. LC-x-3-PUFAs are important for human health in maintaining the cellular membrane by regulating cholesterol synthesis, transportation [103], and eicosanoid synthesis [104].

Sesame is a high-energy food containing approximately 50% oil. The fatty acid composition of sesame oil is highly desirable with about 80–85% unsaturated acids and only 15–20% of saturated acids. Sesame oil consists mainly of linoleic (35–50%) and oleic (35–50%) acids, with small amount of palmitic (7–12%) and stearic (3.5–6%) acids, but with only traces of linolenic acid [105, 106]. Studies indicate that a high intake of n-6 fatty acids shifts the physiologic state to one that is

pro-thrombotic and pro-aggregatory, characterized by increase in blood viscosity, vasospasm, and vasoconstriction and decrease in bleeding time. The n-3 fatty acids, however, have anti-inflammatory, antithrombic, hypolipidemic, and vasodilatory properties [103]. Sesame in combination with soybean oil increases the vitamin E activity along with nutritive value of the lipid [19, 107].

Sesame varieties differ greatly in fatty acid contents of their seeds, and this understanding could lead to crop engineering with an aim to develop better quality edible oil in future [108]. Indian sesame germplasms have lower level of saturated fatty acids compared to other sesame varieties with palmitic acid being the prominent one. C18:1 and C18:2 are the major unsaturated fatty acids present in Indian varieties [109]. In unsaturated fatty acids, while the content of C18:1 and C18:2 is quite high in sesame oil (38–49% and 17–43%, respectively), the C18:3 content is below par (<1%) [110]. A critical analysis of different cultivars is the need of hour, if we are assertive to improve the nutritional quality of oil by engineering the fatty acid biosynthetic pathway.

2.6 Short-Chain Peptides, Protein Hydrolysates, and Their Functional Properties

The bioactive polypeptides are amino acid chains joined by amide or peptide bonds, with molecular weight not exceeding beyond 20 kDa. These are protein fragments that have independent function in various biochemical, physiological, or cellular processes. While some of these peptides are formed naturally, it is the artificial protein hydrolysates that are much in use and are structurally and functionally a diverse group of peptides. Proteins can be digested by proteases, or specific fragment can be created in bio-fermenters utilizing microbes [111]. Sesame food preparation is also an important source of protein [112]. Protein hydrolysates are widely in use as nutritional supplement, functional ingredient, food flavor enhancer, pharmaceuticals, and cosmetics [113, 114]. They display hormone- or drug-like activities and based on the mode of action can be classified as antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral binding, and antioxidative [115]. Use of papain for producing sesame protein hydrolysates having better functional properties, high storability, and emulsifying properties than that of the original sesame protein lysate has been studied by Bandyopadhyay and Ghosh [116].

3 Quantitative Insight in Lignan and Tocopherol Content: Interspecific and Intraspecific Variation

Sesame crops are of high nutritional value owing to the presence of antioxidants lignans (sesamin, sesamolin, and sesamol) and tocopherols (α-, γ-, and δ-forms). Separation of tocopherol homologues in sesame by RPLC on triacontyl (C_{30}) stationary phase has been successfully optimized. The reproducibility of the procedure is in the range 1.7–3.9%, and recovery ranged from 91% to 99% [117]. Lignans and tocopherol homologue contents in a wide collection of 143 sesame lines (wild species,

landraces, introgressed lines, and cultivars) collected from diverse agroecological zones of India have been determined by reverse-phase HPLC (RP-HPLC) [118]. Screening of sesame germplasm revealed an exploitable level of variation in major bioactive compounds. High levels of sesamin and γ-tocopherol suggested for the efficient introduction of these lines in the trait enhancement of other oilseed crops.

4 Biotechnological Approaches for Sesame

Sesame crop is relatively superior in oil quantity and quality compared to many major oil crops. The oil content varies from 35% to 60%, but mostly it is about 50% of seed weight [119]. Therefore, genetic engineering of sesame directed toward creation of sesame oil having diverse composition of lignans and tocopherols with high nutritional value is an important biotechnological aspect. With high genetic diversity, sesame becomes a promising oilseed crop for performing genetic manipulations to obtain high yield and quality oil [120].

4.1 Genome Sequencing of Sesame Crop: Unraveling the Oil Biosynthetic Pathway

Higher oil content compared to other oilseed crops, combined with small genome related to oil quality enhancement, makes sesame an invaluable model plant for studying oil biosynthesis. Wang et al. [121] have done de novo assembling of the genome, where a set of 12 transcriptomes and 29 resequenced accessions provided a large resource for exploring the mechanisms underlying different oil content between sesame and soybean, as well as among the sesame accessions. The results reveal the presence of whole genome duplication and absence of the Toll/interleukin-1 receptor domain in resistance genes. Genes and oil biosynthetic pathways responsible for high oil content were determined employing comparative genomic and transcriptomic analyses. That has indicated for the expansion of type 1 lipid transfer genes by tandem duplication, the contraction of lipid degradation genes, and the differential expression of essential genes in the triacylglycerol biosynthesis pathway in the early stage of seed development [121].

Resequencing data study in 29 sesame accessions from 12 countries suggests that the high genetic diversity of lipid-related genes is linked with the wide variation in oil content. Additionally, the results shed light on the pivotal stage of seed development, oil accumulation, and potential key genes for sesamin production, an important lignan with numerous health benefits. Recently, two more sesame landraces and the chloroplast genome have been sequenced [121, 122] enriching the sesame genome dataset. A recent genome-wide association study has identified *SiNST1* as the candidate gene for lignin and cellulose biosynthesis, and this gene could indirectly be associated with sesamin and sesamolin content [123]. Thankfully, a few

open access platform like Sinbase is also being maintained where all these datasets are managed for further analysis and utilization by sesame researchers [121].

4.2 Functional Gene Expression: "Lignan Biosynthetic Genes"

High-throughput sequencing technology has provided ample data on DNA sequences for the genomes of many plant species. Expressed sequence tags (EST) of many crop species have been generated, and thousands of sequences have been annotated as putative functional genes using powerful bioinformatics tools. Impact on breeding programs could be reached with this approach, because quality of crop plants is a direct function of their metabolite content [124] and quality of plant tissues determines their commercial value with respect to flavor, fragrance, shelf life, physical attributes, etc. [125].

Comparative analysis of expressed sequence tags from *Sesamum indicum* and *Arabidopsis thaliana* developing seeds has been done by Suh et al. [126]. The group could identify the genes involved in accumulation of seed storage products and in the biosynthesis of lignans, sesamin, and sesamolin. Their study could also identify the identical and different gene expression profiles during sesame and *Arabidopsis* seed development and the genes specific to sesame seeds [126]. Sirato-Yasumoto et al. [67] reported that sesamin content in sesame seeds was controlled by polygenes, because F2 populations originating from reciprocal crosses between high sesamin and normal sesamin varieties showed a continuous distribution in sesamin content, and correlation coefficients between F2 and F3 generations were positive and highly significant for sesamin content.

Ono et al. [29] reported that *CYP81Q1* is a single gene encoding (+)-piperitol/(+)-*sesamin synthase* in the *Sesamum indicum* genome, suggesting that *CYP81Q1* is a single enzyme, which catalyzes the formation of (+)-sesamin from (+)-pinoresinol. The *CYP81Q1* homologue (*CYP81Q3*) showed no activity of (+)-sesamin biosynthesis, due to (+)-sesamin deficiency in this species, whereas *S. radiatum* showed dual activity of the enzyme. Further, expression profile of *CYP81Q1* gene was temporally consistent with the accumulation pattern of (+)-sesamin during seed development. Hata et al. [127] detected sesamin (using ultra-performance liquid chromatography-fluorescence detection) in sesame leaves of two Japanese sesame varieties "Gomazou" and "Kin-goma," which differed in sesamin content of the seed, and probed genotypic differences. The higher sesamin content of "Gomazou" leaves correlated well with that of seeds and the expression of the sesamin biosynthetic gene *CYP81Q1*, indicating that genotypic difference of *CYP81Q1* gene expression affected leaf sesamin contents.

To comprehend sesame domestication, we examined the expression of sesamin synthase (*CYP81Q1*) during capsule maturation in three wild *Sesamum* spp. and four sesame cultivars [128]. Among the cultivars, only *S. indicum* (CO-1) exhibited transcript abundance of sesamin synthase and high sesamin content similar to *S. malabaricum*, whereas other cultivars had low expression, indicating that sesamin synthase was not favored during domestication.

5 Conclusions

Sesame is no more an "orphan" crop – the widespread collection of varieties and landraces coupled with extensive research in every aspect has made the crop as the perfect model system amidst other oilseeds. The superior quality of sesame seed oil containing a variety of lignans and tocochromanols merits a higher place among the oilseed crops being consumed worldwide. The diversity of sesame cultivars and their characterization has given sesame researchers enough impetus to create genetically engineered sesame varieties with high yield of secondary metabolites. Sesame seeds are microcapsules for health promotion and disease prevention in humans and an sustained effort in this area of oilseed research would be of immense value to the plant breeders as well as consumers.

Acknowledgments Ashwani K Rai gratefully acknowledges the National Academy of Sciences, India, for awarding NASI-Senior Scientist Platinum Jubilee Fellowship. Niti Pathak wishes to thank Dr. K V Bhat, NBPGR for the work carried out in his lab.

References

1. Ashri A (2007) Sesame (*Sesamum indium* L.) In: Singh RJ (ed) Genetic resources, chromosome engineering, and crop improvement, Oilseed crops, vol 4. CRC Press, Boca Raton, pp 231–289
2. Joshi AB (1961) Sesamum. Indian Central Oilseed Committee, Hyderabad, pp 1–109
3. Weiss EA (1971) Sesame, castor and safflower, barnes and noble, World crop series. Leonard Hill, New York, pp 311–525
4. Bedigian D, Seihler DS, Harlan JR (1985) Sesamin, sesamolin and the origin of sesame. Biochem Syst Ecol 13:133–139
5. Bedigian D, Harlan JR (1986) Evidence for cultivation of sesame in the ancient world. Econ Bot 40:137–154
6. USDA (2015) USDA national nutrient database for standard reference, release 18. U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory, Beltsville. http://www.nal.usda.gov/fnic/foodcomp
7. Pathak N, Rai AK, Ratna K, Bhat KV (2014) Value addition in sesame: a perspective on bioactive components for enhancing utility and profitability. Pharmacogn Rev 8(16):147–155. https://doi.org/10.4103/0973-7847.134249
8. Dimitrios B (2006) Sources of natural phenol antioxidants. Trends Food Sci Technol 17:505–512
9. Manach C, Williamson G, Morand C, Scalbert A, Remesy C (2005) Bioavailability and bioefficacy of polyphenols in humans I- review of 97 bioavailability studies. Am J Clin Nutr 81:230S–242S
10. Temple NJ (2000) Antioxidants and disease: more questions than answers. Nutr Res 20:449–559
11. Kamal-Eldin A, Appelquist LA, Yousif G (1994) Lignan analysis in seed oils from four sesamum species: comparison of different chromatographic methods. J Am Oil Chem Soc 71:141–145
12. Robinson R (1927) The relationship of some complex natural products to the simple sugars and amino acids. Durham Univ Philos Soc 8:14–59
13. Haworth RD (1936) Natural resins. Annu Rep Progr Chem 33:266–279
14. Katsuzaki H, Osawa T, Kawakishi S (1994) Chemistry and antioxidative activity of lignan glucosides in sesame seed. ACS Symp Ser 574:275–280

15. Katsuzaki H, Osawa T, Kawashiki S (1994) Chemistry and antioxidative activity of lignan glucosides in sesame seed, Chapter 28. In: Food phytochemicals for cancer prevention, ACS symposium series, vol 547. American Chemical Society, Washington, DC, pp 275–280
16. Moazzami AA, Andersson RE, Kamal-Eldin A (2006) HPLC analysis of sesaminol glucosides in sesame seeds. J Agric Food Chem 54:633–638. https://doi.org/10.1021/jf051541g
17. Brar G, Ahuja KL (1979) Sesame: its culture, genetics, breeding and biochemistry. Annu Rev Plant Sci 1:245–313
18. Yamashita K, Iizuka Y, Imai T, Namiki M (1995) Sesame seed and its lignans produce marked enhancement of vitamin E activity in rats fed a low alpha- tocopherol diet. Lipids 30:1019–1028
19. Namiki M (1995) The chemistry and physiological functions of sesame. Food Rev Int 11:281–329
20. Kamal-Eldin A (2005) Minor components in vegetable oils. In: Shahidi F (ed) Baileys industrial fats and oils. Chapter 12, edible oil and fat products: speciality oils and oil products. Wiley, Sussex
21. Haller HL, Mc Govran ER, Goodhue LD, Sullivan WN (1942) The synergistic action of sesamin with pyrethrum insecticides. J Org Chem 7(2):183–184
22. Jones WA, Beroza M, Decker ED (1962) Isolation and structure of sesangolin: a constituent of *Sesamum angolense*. J Org Chem 27:3232–3235
23. Cassida JE, Engel JL, Essac EG, Kamienski FX, Kuwatsuka S (1966) Methylene-14C-dioxyphenyl compounds: metabolism in relation to their synergistic action. Science 153:1130–1133
24. Mathews CK, Van Holde KE, Ahern KG (2000) Biochemistry, 3rd edn, Benjamin/Cummings, an imprint of Addison Wesley Longman, pp 700–704
25. Jain SC, Khanna P (1973) Production of sterols from *Sesamum indicum* L. tissue culture. Indian J Pharm 35:163–164
26. Kato MJ, Chu A, Davin LB, Lewis NG (1998) Biosynthesis of antioxidant lignans in *Sesamum indicum* seeds. Phytochemistry 47(4):583–591
27. Davin LB, Wang HB, Crowell AL, Bedgar DL, Martin DM, Sarkanen S, Lewis NG (1997) Stereoselective bimolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center. Science 275:362–366
28. Jiao Y, Davin LB, Lewis NG (1998) Furanofuran lignan metabolism as a function of seed maturation in *Sesamum indicum*: methylenedioxy bridge formation. Phytochemistry 49:387–394
29. Ono E, Nakai M, Fukui Y, Tomimori N, Fukuchi-Mizutani M, Saito M, Satake H, Tanaka T, Katsuta M, Umezawa T, Tanaka Y (2006) Formation of two methylenedioxy bridges by a *Sesamum CYP81Q* protein yielding a furofuran lignan, (+)-sesamin. Proc Natl Acad Sci USA 103(26):10116–10121
30. Yoshida Y, Niki E, Noguchi N (2003) Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. Chem Phys Lipids 123(1):63–75
31. Hofius D, Sonnewald U (2003) Vitamin E biosynthesis: biochemistry meets cell biology. Trends Plant Sci 8(1):6–8
32. Brigelius-Flohe R, Traber MG (1999) Vitamin E: function and metabolism. FASEB J 13(10): 1145–1155
33. Colombo ML (2010) An update on vitamin E, tocopherol and tocotrienol- perspectives. Molecules 15(4):2103–2113. https://doi.org/10.3390/molecules15042103
34. Bramley PM, Elmadfa I, Kafatos A, Kelly FJ, Manios Y, Rexborough HE, Schuch W, Sheehy PJA, Wagner KH (2000) Vitamin E. J Sci Food Agric 80:913–938
35. Herbers K (2003) Vitamin production in transgenic plants. J Plant Physiol 160:821–829. https://doi.org/10.1078/0176-1617-01024
36. Franzen JJ, Bausch D, Glatze D, Wagner E (1991) Distribution of vitamin E in spruce seedling and mature tree organs, and within the genus. Phytochemistry 30:147–151
37. Hassapidou MN, Manoukas AG (1993) Tocopherol and tocotrienol compositions of raw table olive fruit. J Sci Food Agric 61(2):277–280
38. DellaPenna (2005) Progress in the dissection and manipulation of vitamin E synthesis. Trends Plant Sci 10:574–579. https://doi.org/10.1016/j.tplants.2005.10.007

39. Norris SR, Shen X, DellaPenna D (1998) Complementation of the *Arabidopsis* pds1 mutation with the gene encoding p-hydroxyphenylpyruvate dioxygenase. Plant Physiol 117:1317–1323
40. Collakova E, DellaPenna D (2001) Isolation and functional analysis of homogentisate phytyltransferase from *Synechocystis* sp. PCC 6803 and *Arabidopsis*. Plant Physiol 127:1113–1124
41. Savidge B, Weiss JD, Wong YHH, Lassner MW, Mitsky TA, Shewmaker CK, Beittenmiller D, Valentin HE (2002) Isolation and characterization of homogentisate phytyltransferase genes from *Synechocystis* sp. PCC 6803 and *Arabidopsis*. Plant Physiol 129:321–322
42. Cheng Z, Sattler S, Maeda H, Sakuragi Y, Bryant DA, Dellapenna D (2003) Highly divergent methyltransferases catalyze a conserved reaction in tocopherol and plastoquinone synthesis in cyanobacteria and photosynthetic eukaryotes. Plant Cell 15:2343–2356
43. Van Eenennaam AL, Lincoln K, Durett TP, Valentin HE, Shewmaker CK, Thorne GM, Jiang J, Baszis SR, Levering CK, Aasen ED, Hao M, Stein JC (2003) Engineering vitamin E content: from *Arabidopsis* mutant to soy oil. Plant Cell 15(12):3007–3019
44. Porfirova S, Bergmüller E, Tropf S, Lemke R, Dörmann P (2002) Isolation of an *Arabidopsis* mutant lacking vitamin E and identification of a cyclase essential for all tocopherol biosynthesis. Proc Natl Acad Sci U S A 99:12495–12500
45. Sattler SE, Cajon EB, Coughlin SJ, DellaPenna D (2003) Characterization of tocopherol cyclases from higher plants and cyanobacteria: evolutionary implications for tocopherol synthesis and function. Plant Physiol 132:2184–2195
46. Budowski P, Markley KS (1951) The chemical and physiological properties of sesame oil. Chem Rev 48:125–151
47. Osawa T, Nagata M, Namiki M, Fukuda Y (1985) Sesamolinol, a novel antioxidant isolated from sesame seeds. Agric Biol Chem 49:3351–3352
48. Hirata F, Fujita K, Ishikura Y, Hosoda K, Ishikawa T, Nakamura H (1996) Hypercholesterolemic effect of sesame lignan in human. Atherosclerosis 122:135–136
49. Shimizu S, Akimoto K, Shinmen Y, Kawashima H, Sugano M, Yamada H (1991) Sesamin is a potent and specific inhibitor of delta-5-desaturase in polyunsaturated fatty acid biosynthesis. Lipids 26:512–516
50. Hirose N, Inoue T, Nishihara K, Sugano M, Akimoto K, Shimizu S, Yamada S (1991) Inhibition of cholesterol absorption and synthesis in rats by sesamin. J Lipid Res 32:629–638
51. Yokota T, Matsuzaki Y, Koyama M, Hitomi T, Kawanaka M, Enoki-Konish M, Okuyama Y, Takayasu J, Nishino H, Nishikawa A, Osawa T, Sakai T (2007) Sesamin, a lignan of sesame, down-regulates cyclin D1 protein expression in human tumor cells. Cancer Sci 98(9): 1447–1453. https://doi.org/10.1111/j.1349-7006.2007.00560.x
52. Hsu DZ (2005) Effect of sesame oil on oxidative-stress-associated renal injury in endotoxemic rats: involvement of nitric oxide and proinflammatory cytokines. Shock 24:276–280
53. Ashakumary L, Rouyer I, Takahashi Y, Ide T, Fukuda N, Aoyama T, Hashimoto T, Mizugaki M, Sugano M (1999) Sesamin, a sesame lignan, is a potent inducer of hepatic fatty acid oxidation in the rat. Metabolism 48:1303–1313
54. Nonaka M, Yamashita K, Izuka Y, Namiki M (1997) Effects of sesaminol and sesamin on eicosanoid production and immunoglobulin level in rats given ethanol. Biosci Biotechnol Biochem 61:836–839
55. Lee CC, Chen PR, Lin S, Tsai SC, Wang BW, Chen WW (2004) Sesamin induces nitric oxide and decreases endothelin-1 production in HUVECs: possible implications for its antihypertensive effect. J Hypertens 22:2329–2338
56. Nakano D, Kurumazuka D, Nagai Y, Nishiyama A, Kiso Y, Matsumura Y (2008) Dietary sesamin suppresses aortic NADPH oxidase in DOCA salt hypertensive rats. Clin Exp Pharmacol Physiol 35(3):324–326. https://doi.org/10.1111/j.1440-1681.2007.04817.x
57. Cheng FC, Jinn TR, Hou RC, Tzen JTC (2006) Neuroprotective effects of sesamin and sesamolin on gerbil brain in cerebral ischemia. Int J Biomed Sci 2(3):284–288
58. Hemalatha S, Ghafoorunissa (2004) Lignans and tocopherols in Indian sesame cultivars. J Am Oil Chem Soc 81:467–470

59. Abe C, Ikeda S, Yamashina K (2005) Dietary sesame seeds elevate α-tocopherol concentration in rat brain. J Nutr Sci Vitaminol 51:223–230
60. Kamal-Eldin A, Pettersson D, Appelqvist LÅ (1995) Sesamin (a compound from sesame oil) increases tocopherol levels in rats fed ad libitum. Lipids 30:499–505
61. Wu WH, Kang YP, Wang NH, Jou HJ, Wang TA (2006) Sesame ingestion affects sex hormones, antioxidant status, and blood lipids in postmenopausal women. J Nutr 136(5): 1270–1275
62. Mak DHF, Po YC, Kam MK (2011) Antioxidant and anti-carcinogenic potentials of sesame lignans. In: Bedigian D (ed) Sesame the genus sesamum. CRC Press, Boca Raton
63. Sandra MS, Lilian UT (2011) Sesame seeds and its lignans: metabolism and bioactivities. In: Bedigian D (ed) Sesame the genus Sesamum. CRC Press, Boca Raton
64. Matsumara Y, Kita S, Tanida Y, Taguchi S, Morimoto S, Akimoto K, Tanaka T (1998) Antihypertensive effect of sesamin, protection against development and maintenance of hypertension in stroke-prone spontaneously hypertensive rats. Biol Pharm Bull 21:469–473
65. Chavali SR, Zhong WW, Forse RA (1998) Dietary α-linolenic acid increases TNF-α, and decreases IL-6, IL-10 in response to LPS: effect of sesamin on the Δ-5 desaturation of ω6 and ω3 fatty acids in mice. Prostaglandins Leukot Essent Fat Acids 58(3):185–191
66. Lim JS, Adachi Y, Takahashi Y, Ide T (2007) Comparative analysis of sesame lignans (sesamin and sesamolin) in affecting hepatic fatty acid metabolism in rats. Br J Nutr 97(1):85–95. https://doi.org/10.1017/S0007114507252699
67. Sirato-Yasumoto S, Katsuta M, Okuyama Y, Takahashi Y, Ide T (2001) Effect of sesame seeds rich in sesamin and sesamolin on fatty acid oxidation in rat liver. J Agric Food Chem 49:2647–2651
68. Hirose N, Doi F, Ueki T, Akazawa K, Chijiiwa K (1992) Suppressive effect of sesamin against 7, 12-dimethylbenz[a]-anthracene induced rat mammary carcinogenesis. Anticancer Res 12:1259–1265
69. Coulman KD, Liu Z, Quan HW, Michaelides J, Thompson LU (2005) Whole sesame seed is as rich a source of mammalian lignan precursors as whole flaxseed. Nutr Cancer 52:156–165. https://doi.org/10.1207/s15327914nc5202_6
70. Liu Z, Saarinen NM, Thompson LU (2006) Sesamin is one of the major precursors of mammalian lignans in sesame seed (*Sesamum indicum*) as observed in vitro and in rats. J Nutr 136:906–912
71. Penalvo JL, Heinonen SM, Aura AM, Adlercreutz H (2005) Dietary sesamin is converted to enterolactone in humans. J Nutr 135:1056–1062
72. Annussek G (2001) Sesame oil in: gale encyclopedia of alternative medicine. Gale Group and Looksmart, Detroit
73. Ang ES, Lee ST, Gan CS, See PG, Chan YH, Nag LH, Machin D (2001) Evaluating the role of alternative therapy in burn wound management: randomized trial comparing moist exposed burn ointment with conventional methods in the management of patients with second- degree burns. Med Gen Med 3:2–7
74. Yong YL (1999) Analysis of MEBO cream, Report no. 99033191. Institute of Science and Forensic Medicine, Department of Scientific Services, Health Science Division, Singapore
75. Kamal-Eldin A, Appelqvist LÅ (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31:671–701
76. Burton GW, Traber MG (1990) Vitamin E in antioxidant activity biokinetics and bioavailability. Annu Rev Nutr 10:375–382. https://doi.org/10.1146/annurev.nu.10.070190.002041
77. Burton GW (1994) Vitamin E: molecular and biological function. Proc Nutr Soc 53(2):251–262
78. Li D, Saldeen T, Romeo F, Mehta JL (1999) Relative effects of alpha- and gamma-tocopherol on low-density lipoprotein oxidation and superoxide dismutase and nitric oxide synthase activity and protein expression in rats. J Cardiovasc Pharmacol Ther 4:219–226
79. Saldeen T, Engström K, Jokela R, Wallin R (1999) Natural antioxidants and anticarcinogens in nutrition, health and disease. In: Importance of in vitro stability for in vivo effects of fish oils. The Royal Society of Chemistry, Cambridge, UK, Special Publication 240, pp 326–330

80. Qureshi AA, Bradlow BA, Brace L, Manganello J, Peterson DM, Pearce BC, Wright JJK, Gapor A, Elson CE (1995) Response of hypercholesterolemic subjects to administration of tocotrienols. Lipids 30(12):1171–1177
81. Schwenke DC (2002) Does lack of tocopherols and tocotrienols put women at increased risk of breast cancer? J Nutr Biochem 13(1):2–20
82. Olcott HS, Emerson OH (1937) Antioxidants and the autoxidation of fats, IX, the antioxidant properties of the tocopherols. J Am Oil Chem Soc 59(6):1008–1009
83. Girotti AW (1998) Lipid hydroperoxide generation, turnover, and effector action in biological systems. J Lipid Res 39:1529–1542
84. Liebler DC (1993) The role of metabolism in the antioxidant functions of vitamin E. Crit Rev Toxicol 23:147–169. https://doi.org/10.3109/10408449309117115
85. Dabrowski KJ, Sosulski F (1984) Quantification of free and hydrolizable phenolic acids in seeds by capillary gas liquid chromatography. J Agric Food Chem 32(1):123–127
86. Feroj-Hasan AFM, Begu S, Furumoto T, Fukui H (2000) A new chlorinated red napthaquinone from roots of *Sesamum indicum*. Biosci Biotechnol Biochem 64:873–874. https://doi.org/10.1271/bbb.64.873
87. Lyon CK (1972) Sesame, present knowledge of composition and use. J Am Oil Chem Soc 49:245–249
88. Shimoda T, Takabayashi J, Ashira W, Takafuji (1997) Response of predatory insect *Scolothrips takahashi* towards herbivore induced plant volatiles under laboratory and field conditions. J Chem Ecol 23:2033–2048
89. Salunkhe DK, Chavan JK, Adsule RN, Kadam SS (1991) World oilseeds: chemistry, technology and utilization. Springer, New York, pp 1–554
90. Van Rensburg SJ, Daniels WM, Van Zyl JM, Taljaard JJ (2000) A comparative study of the effects of cholesterol, beta-sitosterol, beta-sitosterol glucoside, dehydroepiandrosterone sulphate and melatonin on in vitro lipid peroxidation. Metab Brain Dis 15:257–265
91. Bouic PJ (2002) Sterols and sterolins: new drugs for the immune system? Drug Discov Today 7:775–778
92. Zhao W, Miao X, Jia S, Pan Y, Huang Y (2005) Isolation and characterization of microsatellite loci from the mulberry *Morus* L. Plant Sci 168:519–525
93. Moreau RA, Whitaker BD, Hicks Kevin B (2002) Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Prog Lipid Res 41:457–500
94. Mohamed HM, Awatif II (1998) The use of sesame oil unsaponifiable matter as a natural antioxidant. Food Chem 62:269–276
95. Gharby S, Harhar H, Bouzoubaa Z, Asdadi A, El Yadini A, Charrouf Z (2015) Chemical characterization and oxidative stability of seed and oil of sesame grown in Morocco. J Saudi Soc Agric Sci 16:105–111. https://doi.org/10.1016/j.jssas.2015.03.004
96. Pegel KH (1997) The importance of sitosterol and sitosterolin in human and animal nutrition. S Afr J Sci 93:263–268
97. Nieman DC (1994) Exercise, infection and immunity. Int J Sports Med 15:131–141. https://doi.org/10.1055/s-2007-1021128
98. de Boland AR, Garner GB, O'Dell BL (1975) Identification and properties of "phytate" in cereal grains and oilseed products. J Agric Food Chem 23:1186–1189
99. Graf E, Dintzis FR (1982) High-performance liquid chromatographic method for the determination of phytate. Anal Biochem 119:413–417
100. Urbano G, López-Jurado M, Aranda P, Vidal-Valverde C, Tenorio E, Porres J (2000) The role of phytic acid in legumes: antinutrient or beneficial function? J Physiol Biochem 56:283–294
101. Kuroda Y, Shamsuddin AM (1995) Inositol phosphates have novel anticancer function. J Nutr 125:725S–732S
102. Gunstone F, Harwood JL, Padley FB (1994) The lipid handbook, 2nd edn. Chapman and Hall, London, pp 47–208

103. Simopoulos AP (1999) Essential fatty acids in health and chronic disease. Am J Clin Nutr 70(3 Suppl):560S–569S
104. Kankaanpaa P, Sutas Y, Salminen S, Lichtenstein A, Isolauri E (1999) Dietary fatty acids and allergy. Ann Med 31:282–287
105. Kamal-Eldin A, Appelqvist LÅ (1994) Variation in the composition of sterols, tocopherols and lignans in seed oils from four *Sesamum* species. J Am Oil Chem Soc 71:149–156
106. Spencer GF, Herb SF, Gormisky PJ (1976) Fatty acid composition as a basis for identification of commercial fats and oils. J Am Oil Chem Soc 53:94–96
107. Shahidi F, Tan Z (2011) Physiological effects of sesame bioactive and antioxidant compounds. In: Bedigian D (ed) Sesame the genus sesamum. CRC Press, Boca Raton
108. Uzun B, Arslan C, Furat S (2008) Variation in fatty acid compositions, oil content and oil yield in germplasm collection of sesame (*Sesamum indicum* L.) J Am Oil Chem Soc 85:1135–1142
109. Mondal N, Bhat KV, Srivastava PS (2010) Variation in fatty acid composition in Indian germplasm of sesame. J Am Oil Chem Soc 87(11):1263–1269
110. Bhunia RK, Chakraborty A, Kaur R et al (2015) Analysis of fatty acid and lignan composition of Indian germplasm of sesame in terms of their nutritional merits. J Am Oil Chem Soc 92:65–76
111. Aluko R (2012) Bioactive peptides. In: Functional foods and nutraceuticals, Food science text series. Springer, New York, pp 37–61
112. Dench JE, Rivas N, Caygill JC (1981) Selected functional properties of sesame (*Sesamum indicum* L.). Flour and two protein isolates. J Sci Food Agric 32:557–564. https://doi.org/10.1002/jsfa.2740320606
113. Frokjaer S (1994) Use of hydrolysates for protein supplementation. Food Technol 48:86–88
114. Giese J (1994) Proteins as ingredients: types, functions, applications. Food Technol 48:50–60
115. Sánchez A, Vázquez A (2017) Bioactive peptides: a review. Food Qual Saf 1(1):29–46. https://doi.org/10.1093/fqs/fyx006
116. Bandyopadhyay K, Ghosh S (2002) Preparation and characterization of papain-modified sesame (*Sesamum indicum* L.) protein isolates. J Agric Food Chem 50(23):6854–6857
117. Saha S, Walia S, Kundu A, Pathak N (2013) Effect of mobile phase on resolution of the isomers and homologues of tocopherols on a triacontyl stationary phase. Anal Bioanal Chem 405:9285–9295. https://doi.org/10.1007/s00216-013-7336-9
118. Pathak N, Rai AK, Saha S, Walia SK, Sen SK, Bhat KV (2014) Quantitative dissection of antioxidative bioactive components in cultivated and wild sesame germplasm reveals potentially exploitable wide genetic variability. J Crop Sci Biotechnol 17(3):127–139
119. Ashri A, Downey RK, Robbelen G (1989) *Brassica* species. In: Ashri A, Robbelen G, Downey RK (eds) Oil crops of the world. McGraw-Hill, New York, pp 339–382
120. Pathak N, Rai AK, Kumari R, Thapa A, Bhat KV (2014) Sesame crop: an underexploited oilseed holds tremendous potential for enhanced food value. Agric Sci 5(6):519–529. https://doi.org/10.4236/as.2014.56054
121. Wang L, Yu S, Tong C, Zhao Y, Liu Y, Song C, Zhang Y, Zhang X, Wang Y, Hua W, Li D, Li D, Li F, Yu J, Xu C, Han X, Huang S, Tai S, Wang J, Xu X, Li Y, Liu S, Varshney RK, Wang J, Zhang X (2014) Genome sequencing of the high oil crop sesame provides insight into oil biosynthesis. Genome Biol 15:R39. https://doi.org/10.1186/gb-2014-15-2-r39
122. Wei X, Zhu X, Yu J, Wang L, Zhang Y, Li D, Zhou R, Zhang X (2016) Identification of sesame genomic variations from genome comparison of landrace and variety. Front Plant Sci 7:1169. https://doi.org/10.3389/fpls.2016.01169
123. Wei X et al (2015) Genetic discovery for oil production and quality in sesame. Nat Commun 6:8609. https://doi.org/10.1038/ncomms9609
124. Memelink J (2004) Tailoring the plant metabolome without a loose stitch. Trends Plant Sci 7:305–307. https://doi.org/10.1016/j.tplants.2005.05.006
125. Hall C, Tulbek MC, Xu Y (2006) Flaxseed. Adv Food Nutr Res 51:1–97
126. Suh MC, Kim MJ, Hur CG, Bae JM, Park YI, Chung CH, Kang CW, Ohlrogge JB (2003) Comparative analysis of expressed sequence tags from *Sesamum indicum* and *Arabidopsis thaliana* developing seeds. Plant Mol Biol 52(6):1107–1123

127. Hata N, Hayashi Y, Okazawa A, Ono E, Satake H, Kobayashi A (2010) Comparison of sesamin contents and *CYP81Q1* gene expressions in aboveground vegetative organs between two Japanese sesame (*Sesamum indicum* L.) varieties differing in seed sesamin contents. Plant Sci 178(6):510–516
128. Pathak N, Bhaduri A, Bhat KV, Rai AK (2015) Tracking sesamin synthase gene expression through seed maturity in wild and cultivated sesame species – a domestication footprint. Plant Biol 17(5):1039–1046. https://doi.org/10.1111/plb.12327

Nutritional Quality of *Mangifera* Species

9

Luis M. Anaya-Esparza and Efigenia Montalvo-González

Contents

Abstract

Mango is known as the king of the fruits; its nutritional importance, unique flavor, and delicious taste impart this status as super fruit. Furthermore, it is commercially cultivated in different tropical and subtropical areas in the world. *Mangifera indica* is the most important fruit of this genus; over 60 different species of edible mangoes are grown worldwide; however, the most of them are not marketable

L. M. Anaya-Esparza
Laboratorio de Microbiología de Alimentos, Departamento de Ciencias Pecuarias y Agrícolas, Universidad de Guadalajara, Centro Universitario de los Altos, Tepatitlán de Morelos, Jalisco, Mexico

Laboratorio Integral de Investigación en Alimentos, Instituto Tecnológico de Tepic, Tepic, Nayarit, Mexico
e-mail: l_m_ae@hotmail.com

E. Montalvo-González (✉)
Laboratorio Integral de Investigación en Alimentos, Instituto Tecnológico de Tepic, Tepic, Nayarit, Mexico
e-mail: efimontalvo@gmail.com; emontalvo@ittepic.edu.mx

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_70

and underutilized and commonly are denominated as wild mango species, but they exhibit higher nutritional values. Mango species present morphological, physiological, sensorial, and nutritional differences among them. However, most of *Mangifera* species are characterized by their strong aroma, intense peel coloration, attractive fragrance, and delicious taste. Also, mango fruit is considered very healthy, good source of energy, and easily digestible.

Mango pulp and peel are a good source of carbohydrates, dietary fiber, vitamins (B, C, and E), minerals (Ca, P, Fe, Na, and K), and bioactive compounds (polyphenolic compounds, flavonoids, mangiferin, lupeol, and carotenoids). Most of *Mangifera* species have good nutrimental quality; their consumption may contribute nutrition. In general, the commercially (*M. indica*) and wild mango species can be considered for many purposes including for processing and for consumption because mango may be considered an excellent source for improving nutrition. However, enhanced knowledge of the status of such species and information on their health benefits is critical in efforts to promote these valuables mango species.

Keywords

Mangifera species · Wild mango · Nutritional quality · Bioactive compounds · Health benefits

Abbreviations

AA	Ascorbic acid
DF	Dietary fiber
DRI	Dietary reference intake
DW	Dry weight
IDF	Insoluble dietary fiber
SDF	Soluble dietary fiber
TA	Titratable acidity
TDF	Total dietary fiber
TSP	Total soluble polyphenols
TSS	Total soluble solids

1 Introduction

Mango fruit has been recognized as the "king of the fruits," having socioeconomic importance in many countries and/or localities. Mango fruit is cultivated commercially in more than 87 countries around of the world, but also, mango ranks among major fruits worldwide [1, 2]. Furthermore, mango is a rich source of bioactive compounds and essential macro- and micronutrients; it is a powerful nutritive fruit, containing most of the essential substances needed by the human health. Its nutritional importance, unique flavor, and delicious taste impart this status as super fruit [3].

Mangifera is a genus that belongs to the Anacardiaceae family in the order of Sapindales, which is native to the Southeast Asia, in particular originated in India, Indo-Myanmar border, and Bangladesh [4]. Kostermans and Bompard [5] listed 58

species in the genus *Mangifera*, but today, this number is considerably increased [4]; majority of which were distributed in Asia. Currently, mango is cultivated in different tropical and subtropical areas worldwide [6, 7], and it has several species, varieties, and cultivars [8]. Around the world there are more than 150 mango varieties, and the main producers are India, China, Thailand, Indonesia, and Mexico [9]. Previous data are about the best-known species, *Mangifera indica*, a commercially important member of this genus, where the most representative varieties worldwide are "Tommy-Kent," "Tommy Atkins," "Haden," "Keitt," and "Ataulfo" varieties [10]. However, there are another consumable, but not marketable wild mango species in the genus *Mangifera* such as *M. casturi*, *M. zeylanica*, *M. odorata*, *M. lalijiwa*, *M. caesia*, *M. foetida*, *M. laurina*, and *M. pajang*, among others [11]; they represent a great potential for food, industrial, and pharmaceutical use. Unfortunately, the wild mangoes are vulnerable and in danger of extension [12–16].

2 Description of *M. species* Fruits

Mango is a tropical and exotic fruit with many species, varieties, and cultivars [1]. According to Hidayat et al. [6], the classification of *Mangifera* species is still labile; it is because of the complexity of the *Mangifera* genus. Furthermore, several mango fruit characterizations including pulp color (light, yellow, gold, and orange), pulp texture (soft, intermediate, or strong), fiber quantity in pulp, juiciness of pulp (slightly juicy, juicy, or very juicy), pulp aroma (absent, mild, intermediate, or strong), and eating quality (poor, good, very good, or excellent) have been developed [17]. For this reason, mangoes are grouped under two broad categories as wild and cultivated [18]. The fruit description of some edible *Mangifera* species is presented below.

Mangifera indica fruit is more or less compressed, oblong or sub-uniform, fibrous, highly variable in size, shape, weight, and peel coloration and has fleshy drupe with sweet juice and large and compressed seed. In the same way, fruits of *M. andamanica* are oval shaped and small in size and weight (11–15 g); their flesh is yellow, fibrous, and juicy with sweet taste (22 °Brix) [19]. Likewise, *M. caesia* fruit is an ovoid-oblong drupe, necked at base, and medium sized (12–20 cm), with whitish, yellowish, greenish, or pale brownish, smooth, thin skin (1 mm); the pulp is white, soft, juicy, and fibrous, with a peculiar sourish taste; it can be sweet, acidic, or both and has strong smell at maturity [20, 21]. Additionally, the fruits from *M. odorata* are obliquely ellipsoid-oblong or oblong drupe, are medium sized (10–13 cm), and have an average weight of about 200–320 g with a thin skin (3–4 mm) but also are characterized by their strong odor; the pulp is yellow to orange yellow, firm, fibrous, and juicy and has sweet to acidic, sweet taste (21 °Brix). The fruit peel has greenish purple color to canary yellow at maturity [19, 20, 22, 23].

With respect to *M. sylvatica*, the fruit is obliquely ovate, small (8–10 cm long; 27 g), and compressed; the pulp is yellow in color, has a sweet and sour taste, is very aromatic, and is almost fiberless. Their skin is very thin but has a big kernel (40% of its weight). The fruits look very much like that of the common mango (*M. indica*) [24–26]. *M. foetida* fruit is obliquely ovoid-oblong or almost globose drupe, small to

medium in size, dirty dark olive green or yellowish green in color with brown lenticels, fibrous, juicy, savory, and with strong smell and has skin of 5 mm thick and pale yellowish-white flesh when immature that turns into yellow or golden yellow when ripe [20, 21]. *M. laurina* fruit is a drupe-like small mango, obliquely oblong, small in size (5–7 cm), and medium green turning greenish yellow to yellow in color at maturity; flesh is yellow, watery, sweet, with a strong resinous taste, juicy, very acidic, and fibrous [20, 22, 23]. On the other hand, *M. pajang* fruit is a big drupe (2–3 kg), brownish, globose to broad-ovoid, 15–20 cm across, and roughish. Flesh is yellow, fibrous, acidic to acid-sweet, and middy fragrant [20, 21].

Furthermore, the fruits of *M. zeylanica* have an average weight of 150 g; they have a thin skin and are watery, sweet, and pleasant in flavor [22]. Also, the fruit of *M. casturi* is produced in large racemes of ten or more; fruits are small (50–84 g) compared to other mango species; immature fruits are green, and when ripe the color changes to brown or purple black, and it has a shiny surface. The flesh is orange with fiber; its taste is unique (slightly sweet) similar to lychee fruit [21, 22]. The fruits from *M. lalijiwa* exhibited a medium size of fruits (250 g) with green skin; the flesh is white pale yellow with particular brown honey pockets in the flesh. Fruits are very sweet and aromatic with a distinguish honey flavor [21].

In the last years, some research has demonstrated the relationship of some wild mango species, for example, *M. odorata* and *M. foetida* also have close relationship based on internal transcribed spacer nuclear ribosomal DNA [4]. Some authors have mentioned that *M. odorata* is a hybrid result from *M. indica* and *M. foetida* [27]. Also, a close relationship between *M. laurina* and *M. sylvatica* to *M. indica* was previously reported [8].

Mango species exhibited morphological, physiological, and sensorial differences among them. In general, the mango fruit is a simple, large, more or less compressed, fleshy, and resinous drupe. It varies in size, shape, color, taste, and nutritional value. However, most of the *Mangifera* species are characterized by their strong aroma, intense peel coloration, attractive fragrance, and delicious taste.

3 Traditional Uses and Health Importance

Mango fruits can be eaten immature green, mature green, or ripe [20] and usually are consumed fresh with or without peel; however, due to the excellent organoleptic attributes that it exhibits, the pulp is used as an ingredient to elaborate food and beverage products such as juices, nectars, puree, jam, jelly, yogurt, wine, and others [2]. On the other hand, in traditional folk medicine, mango pulp is consumed as an antiparasitic, laxative, and stomachic, among others [1, 28]. According to the World Health Organization, traditional medicine system based on the use of plants is an important source of health care [29]. In recent years, secondary metabolites with known pharmaceutical activities have been extensively investigated as a source of medical agents [30, 31]. In particular, *Mangifera* species exhibited several biological compounds as mangiferin, mangiferic acid, mangiferol, ellagic acid, steroids, alkaloids, terpenoids, saponins, tannins, and others [31, 32]. In this context, the intake of

Table 1 Physicochemical parameters of different flesh *Mangifera* species at green mature stage (GSM) and consumed mature stage (CMS)

Parameter	Maturity stage	pH	AT (% citric acid)	TSS (°Brix)
M. indica cv. Tommy-Kent	GSM	3.48	4.26	10.7
	CMS	4.07	2.39	15.1
M. indica cv. Tommy Atkins	GSM	3.62	4.12	10.9
	CMS	3.91	2.08	16.7
M. zeylanica	GSM	3.53	2.19	11.5
	CMS	4.06	1.26	17.7
M. casturi	GSM	3.11	5.02	7.2
	CMS	4.12	2.40	20.4
M. lalijiwa	GSM	3.23	5.49	9.4
	CMS	4.37	1.67	17.2
M. odorata	GSM	3.90	2.83	6.7
	CMS	4.12	2.49	10.6

Source: Barbosa-Gámez et al. [3]

mango pulp promotes strengthening of the body's defenses, mainly due to the presence of bioactive compounds in fruit pulp and peel as discussed below.

4 Postharvest Quality Parameters of *Mangifera* Species

It has been demonstrated that the majority changes related to the quality in mango fruit occur during its ripening [33, 34]. Barbosa-Gámez et al. [3] evaluated the changes in physiochemical parameters (pH, titratable acidity, and total soluble solids) of the pulp of *M. casturi*, *M. lalijiwa*, *M. odorata*, *M. zeylanica*, and *M. indica* cv. Tommy-Kent and Tommy Atkins, which are harvested in two maturity stages (green mature stage and consumed mature stage). In general, changes in pH, titratable acidity (TA), and total soluble solids (TSS) were observed in both maturity stages as shown in Table 1. In all *M. species* studied, an increase in pH and TSS content was reported when green to consumed mature stage, while TA decreases. Furthermore, numerous physical and physiological changes of mango ripening involving surface color, shape, size, shoulder growth, specific gravity, and firmness have been correlated with the fruit maturity stage [1].

5 Nutrimental Quality in *Mangifera* Species

Fruits play an important role in human nutrition by providing additional sources of energy and bioactive compounds, and their consumption is recommended by many health organizations [35]. Mango is a popular and economically important fruit worldwide mainly for its excellent organoleptic properties and nutritional value [32]. According to Masibo and He [36], mango fruit is unique because each of its parts

(pulp, peel, and kernel) is utilizable. In the literature, mango is described as a fruit with high amounts of water (72–86%) and carbohydrate (9–25%) and low protein (0.9–5.1%) and lipid (0.2–2.7%) content as presented in Table 2; these values are depending on each *Mangifera* species and can vary with the location of cultivation, variety, and stage of maturity.

In particular, differences in nutrimental contents were observed between *Mangifera* species. *M. zeylanica* exhibited the highest water (86%) and lipid content (2.7%). On the other hand, *M. foetida* exhibited the lowest water (72%) content but the highest carbohydrate (25%) content. With respect to the protein content, *M. lalijiwa* (4.7%), *M. odorata* (4.7%), and *M. casturi* (5.1%) showed similar values. Furthermore, some *Mangifera* species as *M. lalijiwa*, *M. odorata*, *M. casturi*, *M. zeylanica*, and *M. sylvatica* exhibited better nutrimental values compared to *M. indica* cv. "Tommy-Kent" and "Tommy Atkins." The nutrimental quality of the fruit is influenced by species, variety, nutritional status, and environmental conditions during growth of the parent plant [1].

The mango pulp is considered very healthy, good source of energy (63–92 kcal), and easily digestible (Table 2). Furthermore, mango fruit has been considered as a functional food because it is a source of dietary fiber [37]. The importance of dietary fiber (DF) content in fruit and its implications in human nutrition and health as prebiotic and regulating the glucose and lipid levels (cholesterol and triacylglycerols) in blood have been reported previously [38]. The total (TDF), soluble (SDF), and insoluble (IDF) dietary fiber values in some *Mangifera* species pulp at two maturity stages are shown in Table 3. In general, Barbosa-Gámez et al. [3] reported a decrease in total dietary fiber content when unripe to ripe mango fruit in all *Mangifera* species is evaluated. However, all mango species exhibited high amounts of dietary fiber, but *M. lalijiwa* showed the highest content in immature stage (41 g/100 g DW) and *M. zeylanica* in mature stage (20 g/100 g DW).

Vitamins and mineral contribute a major part of nutrimental content of fruits; these compounds are essential nutrients that are required for various biochemical and physiological processes in the body. In the case of mango, significant values of vitamin C, vitamin E, B vitamins (Table 4), calcium, phosphorous, iron, sodium, and potassium (Table 5) were found. Ascorbic acid (AA) or vitamin C is the most important water-soluble antioxidant, usually present and highly bioavailable in tropical fruits [39]. According to the Institute of Medicine's Food and Nutrition Board [40], the dietary reference intake (DRI) for vitamin C is 90 mg/day for males and 75 mg/day for females. In the case of mango, vitamin C content was dependent of each species and varies from 47 to 400 mg per 100 g of edible portion; the highest vitamin C content was found in wild mango species (*M. zeylanica*, *M. pentandra*, and *M. pajang*). Daily consumption of 100 g/day of mango (e.g., *M. indica* by popular) pulp could ensure an intake of 100% of the DRI. Furthermore, an interesting values of vitamin E (3.4–7.8 mg/100 g) and B vitamins as niacin (0.6–329 mg/100 g), niacinamide (8–149 mg/100 g), pyridoxine (4.7–86.2 mg/100 g), riboflavin (0.04–97.6 mg/100 g), and thiamine (0.05–8 mg/100 g) were found in different *Mangifera* species, which are another important vitamin groups for health care [41].

Table 2 Nutrimental composition and energy value (per 100 g of edible portion on fresh weight basis) of different *Mangifera* species

Mango species	Energy (kcal)	Moisture (g)	Ash (g)	Total protein (g)	Total fat (g)	Carbohydrates (g)	Total fiber (g)	References
M. indica cv. Tommy-Kent	66.3	83.5	3.4	4.04	1.5	9.2	–	[3]
M. indica cv. Tommy Atkins	75	85	2.3	3.7	1.8	11.0	–	[3]
M. caesia	47.8–64	81.2–86.5	0.6	1.0	0.2	14.6–11.9	2.4	[20]
M. foetida	76	72.5–78.5	0.8	0.8–1.4	0.2	17.9–25.4	1.8	[20]
M. odorata	69.3–83	79–80	0.7	0.9–1.0	0.1–1.8	15.6–19	1.4	[20]
M. casturi	89.9	85.1	5.3	5.1	1.5	14	–	[3]
M. lalijiwa	89.2	81.2	2.3	4.7	1.6	11.1	–	[3]
M. odorata	63.9	80.1	2.7	4.7	2.3	6.1	–	[3]
M. zeylanica	92.3	86.3	3.6	4.2	2.7	12.8	–	[3]
M. sylvatica	59	85	1.93	5	3.2	1.95	2	[26]

Table 3 Soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF) contents (% dry weight) in pulp of different *Mangifera* species

Mango species	Maturity stage	SDF	IDF	TDF
M. indica cv. Tommy-Kent	GSM	5.6	8.9	14.5
	CMS	5.3	8.1	13.3
M. indica cv. Tommy Atkins	GSM	4.7	11.5	16.2
	CMS	4.2	5.2	9.4
M. zeylanica	GSM	12.5	18.0	30.5
	CMS	11.0	8.6	19.6
M. casturi	GSM	10.3	15.0	25.3
	CMS	6.1	12.5	18.6
M. lalijiwa	GSM	6.9	11	17.9
	CMS	5.1	8.3	13.4
M. odorata	GSM	11.8	29	40.8
	CMS	7.3	8.3	15.6

Source: Barbosa-Gámez et al. [3]

Mango is a major source of carbohydrates, dietary fiber, vitamins, and minerals with high energy value but low in calorie. Fresh as well as processed form of mango fruit is an important part of people's diet. Furthermore, most of *Mangifera* species have good nutrimental quality; their consumption may contribute nutrition in many countries around the world. Also, some of these wild mango species have been domesticated for its use and commercialization.

6 Phytochemicals Present in *Mangifera* Species

In addition to their delicious taste, refreshing flavor, aroma, and nutritional value, mango fruit provides "bioactive compounds" to improve human health [34]. Bioactive compounds are defined as "inherent non-nutrient constituents of food plants with anticipated health promoting/beneficial and/or toxic effects when ingested" [42]. According to Raman et al. [7], the concentration of these compounds depends on many factors (e.g., varieties, maturity stage). In general, mango fruit has bioactive compounds in roots, leaves, bark, seeds, peel, and pulp. The types of compounds include β-carotene, isoflavones, vitamin C, mangiferin, gallic acid, lupeol, kaempferol, quercetin, and α-tocopherol, among others [43–45]. All these compounds have great potential for use in food and pharmaceutical industry (Raman et al. [7]). As mentioned above mango fruit is consumed with or without peel; for this reason we will focus on the bioactive compounds present in edible part (pulp and peel) of mango fruit.

6.1 Phytochemicals Present in Mango Pulp

Mango, like most fruits, is an important source of natural compounds considered as bioactives [34]. In the edible part of mango fruit, compounds as polyphenols,

Table 4 Vitamin content in different *Mangifera* species pulp (mg/100 g DW)

Mango specie	Ascorbic acid[a]	Vitamin E[a]	Niacin[a]	Niacinamide[a]	Pyridoxine[a]	Riboflavin[a]	Thiamine[a]	References
M. indica cv. Tommy-Kent	140	5.19	104.2	28.2	15.1	28.2	5.1	[3, 28]
M. indica cv. Tommy Atkins	64.5	7.8	81.1	46.5	14.6	21.1	4.8	[3]
M. caesia	125	7.4	1.2			0.16	0.05	[20, 46]
M. foetida	122		0.6			0.04	0.09	[20, 45, 46, 48]
M. laurina	135							[46]
M. odorata	47		329.2	149	86.2	97.6	7.2	[3, 46, 48]
M. pajang	403							[48]
M. casturi	100	4.8	87	66	46	25	8	[3]
M. lalijiwa	97.6	3.8	50	8	4.7	7.2	3.1	[3]
M. pentandra	400							[46]
M. longipetiolata	322							[46]
M. zeylanica	400		160	36	30	30	6.8	[3, 46]

[a]Results are expressed as mg per 100 g DW

Table 5 Mineral content in different *Mangifera* species pulp (mg per 100 g DW)

	Minerals				
Mango specie	Ca	P	Fe	Na	K
M. indica	10		0.13		
M. caesia	7	17	0.3	1	120
M. foetida	16	19	0.2	2	361
M. odorata	9	13	0.4	2	187

Source: Lim [20]

phytosterols, isoflavones, and β-carotene, among others, have been identified as shown in Table 6.

Polyphenols are among the most extensive groups of phytochemicals present in fruits. Mango is an excellent source of dietary antioxidants as phenolic compounds. The concentration of phenolic compounds in mango pulp varies with species and/or variety, ranging from 200 to 3000 mg of gallic acid equivalents per 100 g of edible portion (Table 6), which exhibited a great antioxidant capacity [46] (Table 7). According to Palafox-Carlos et al. [34], the major phenolic compounds found in "Ataulfo" mango pulp are chlorogenic, gallic, protocatechuic, and vanillic acid. In addition, the authors mentioned that the gallic acid (39%) showed the highest contribution on antioxidant capacity followed by chlorogenic acid (21%), while major phenolic compounds in *M. indica* cv. Keitt pulp are gallic acid; mono-, tetra-, and penta-galloyl glucoside; hydroxybenzoic acid; and gallotannins [47]. The identified phenolic compounds coincide with those reported by Masibo and He [36] for mango pulp. Furthermore, Masibo and He [36] mentioned that the flavonoids are the most abundant polyphenols in our diet; unfortunately, information about flavonoid content in *Mangifera* species is scarce, and the values reported for some mango species ranged from 100 to 500 mg per 100 g of edible portion. Khoo and Ismail [48] have reported the presence of isoflavones as daidzein and genistein in *M. foetida*, *M. pajang*, and *M. odorata* pulps. These compounds can act as phytoestrogens, which may serve as health-promoting compounds in consumer's diet. *M. odorata* possessed the highest daidzein and genistein content (11.6 mg/100 g), followed by *M. pajang* (9.02 mg/100 g) and *M. foetida* (6.81 mg/100 g). In all cases daidzein content was higher than genistein content. According to the authors, the variability of the isoflavone content in mango fruits may be influenced by several internal and external factors [7]. Recently, López-Cobo et al. [49] in three mango cultivars (Keitt, Osteen, and Sensation) from *Mangifera indica* reported the presence of Alk(en)ylresorcinols and p-coumaric acid.

Mangiferin is a xanthone that exhibits great potential as antioxidant; it is a pharmacological active phytochemical and a natural polyphenolic antioxidant [50]. Mangiferin content of mango pulp ranged from 0.078 to 4 mg per 100 g. Furthermore, another phytochemical, lupeol, a well-known triterpene, is also found in several medicinal plants and fruits, including mango. The lupeol content in mango pulp from different mango species ranged from 0.006 to 0.181 mg per 100 g of edible portion. Some authors have mentioned that mangiferin and lupeol can act as an anti-inflammatory, antidiabetic, and cholesterol-lowering agent [50, 51].

Table 6 Bioactive compounds present in different ripe *Mangifera* species pulp

Mango specie	Total phenolic (mg/100 g)	Total flavonoid (mg/100 g)	Total isoflavones (mg/100 g)	Mangiferin (mg/100 g)	Lupeol (mg/100 g)	Total carotene (mg/100 g)	References
M. indica cv. Tommy-Kent	524[a]			4.4[b]		10.67	[3, 28, 50]
M. indica cv. Tommy Atkins	420[a]					0.006	[3, 61]
M. indica Ataulfo	80–174				0.010	0.030–0.060	[34, 44]
M. indica B Green				0.253	0.006		[43]
M. indica Dashehari				0.217	0.181		[43]
M. indica Chousa				0.078	0.023		[43]
M. caesia	2637	550					[46]
M. foetida	2918	550	6.81			4.81	[45, 46, 48]
M. laurina	144	176					[46]
M. odorata	257	202	11.6			3.95	[3, 46, 48]
M. pajang	7055	256	9.02				[48]
M. casturi	1538[a]						[3]
M. lalijiwa	595[a]						[3]
M. pentandra	676	118					[46]
M. longipetiolata	263	129					[46]
M. zeylanica	676[a]	118					[3, 46]

[a]Total soluble polyphenols
[b]Mango puree

Table 7 Antioxidant capacity by different methods in *Mangifera* species pulp

Mangifera species	ABTS	DPPH (%)	IC_{50} (mg/mL)	FRAP[a]	References
M. indica		73	10.21	0.64	[46]
M. indica cv. Tommy-Kent	39.20[a]	5.10[a]		0.37[a]	[3]
M. indica cv. Tommy Atkins	209.4[a]	1.46[a]		12.1[a]	[3]
M. caesia		92	8.14	0.66	[46]
M. foetida		17	43.22	0.62	[46]
M. laurina		56	13.32	0.64	[46]
M. odorata	88.30[a]	37	20.16	0.52	[3, 46]
M. pajang		19	38	0.49	[46]
M. zeylanica	150[a]	2.91[a]		13.15[a]	[3]
M. lalijiwa	130.8[a]	15.27[a]		6.94[a]	[3]
M. pentandra		56	13.27	0.65	[46]
M. longipetiolata		90	8.33	0.61	[46]
M. casturi	156[a]	13.54[a]		11[a]	[3]

[a]mmol kg^{-1} dry weight

The attractive color of mango pulp is mainly due to the presence of abundant β-carotene, but also, carotenoids are a potent antioxidant with several human health benefits [52]. Carotene content in *Mangifera* species ranged from 0.006 to 10.7 mg per 100 g of edible portion. *M. foetida* and *M. odorata* are *Mangifera* fruits which are considered as underutilized tropical fruits. But, the flesh of these mango species exhibited an important total carotene content as reported by Khoo et al. [45]; furthermore, carotene content of *M. foetida* (4.81 mg/100 g) and *M. odorata* (3.95 mg/100 g) is comparable to other commercial mangoes. Ajila et al. [53] mentioned that the yellow-orange flesh or ripened mango is attributable to the presence of carotenes. In this context, many of the wild mango species exhibited yellow-orange color pulp, indicating the possible presence of carotenes in their pulps [48].

Furthermore, Vilela et al. [54] mentioned that ripe mango pulp from *M. indica* cv. "Tommy Atkins" and other cultivars is a rich source of phytosterols and other lipophilic phytochemical (Table 8). The major groups of lipophilic compounds in mango pulp are sterols (947 mg/100 g DW) and fatty acids (949 mg/100 g DW), followed by the steryl glycosides (201 mg/100 g DW) [54]; some of these lipids have been termed as "bioactive lipids" because of their potential benefits for human health [55].

6.2 Phytochemicals Present in Mango Peel

Mango peel has been recognized as source for obtaining valuable components [56, 57] as phytochemicals (polyphenols, carotenoids) and vitamins (E and C) with different health-promoting properties [53], mainly by its antioxidant activity [34]. Moreover, mango peels are great source of proteins, carbohydrates, and dietary fiber [58–60], which make it suitable to be processed for value-added applications in functional foods

Table 8 Compounds identified in the lipophilic extracts of ripe mango pulp

Compound	*Mangifera indica* cv. Tommy Atkins (mg per 100 g db)
Fatty acids	940
Saturated	324
Dodecanoic acid	4
Tetradecanoic acid	26
Pentadecanoic acid	2
Hexadecanoic acid	228
Heptadecanoic acid	8
Octadecanoic acid	28
Eicosanoic acid	2
Docosanoic acid	8
Tetracosanoic acid	10
Pentacosadiynoic acid	8
Unsaturated	612
Hexadec-9-enoic acid	91
Heptadec-9-enoic acid	16
Octadeca-9,12-dienoic acid	48
Octadeca-9,12,15-trienoic acid	131
cis-Octadeca-9-enoic acid	205
trans-Octadec-9-enoic acid	122
Diacids	1
Long-chain aliphatic alcohols	104
Sterols	947
β-Sitosterol	571
Campesterol	149
Stigmasterol	68
Steryl glucosides	201
Others	195
α-tocopherol	64

Source: Vilela et al. [54]

and nutraceuticals [61] as discussed below. Currently, mango peel flour is used as functional ingredient in food products, mainly in bakery products [62].

Ediriweera et al. [63] reported that major lipophilic compounds identified in *M. zeylanica* peel (chloroform extract) were 1H-cycloprop[e]azulen-7-ol-decahydro-1,1,7-trimethyl-4-methylene, β-sitosterol, 9,12-octadecadienoic acid, caryophyllene oxide, phenol-3-pentadecyl, and α-tocopherol, among others. Also, Kim et al. [47] informed about the presence of unsaturated fatty acids as oleic acid, linoleic acid, and ethyl linoleate in peels from *Mangifera indica* L. cv. "Irwin."

Hassan et al. [60] reported in *Mangifera pajang* K. a content of TDF of 72% (SDF: 33.4% and IDF: 38.8%). Ajila et al. [64] stated mango peel to be a rich source of fiber, and 30–50% SDF and 50–70% IDF can be considered as a well-balanced range for maximum health benefits, due to each fraction that has different

physiological effects [65]. Similar results were reported in mango peels by Ajila and Prasada Rao [66] in "Badami" and "Raspuri" varieties (TDF ranged from 40% to 72% in both cases). Furthermore, DF has been associated with polyphenol compounds and called "antioxidant dietary fiber" [66].

With respect to ascorbic acid, green and ripe mango (*Mangifera indica* var. "Chokanan") peel contained an ascorbic acid of 109 and 52 mg per 100 g (DW), respectively [62]. Previously, Ajila et al. [53] reported an AA content of 34 and 39 mg per 100 g (DW) in raw and ripe peel of mango "Raspuri" and "Badami" cultivars, respectively. Also, Sogi et al. [61] informed an AA value of 75 mg/100 g (DW) in mango (*Mangifera indica* cv. "Tommy Atkins") peel. According to Ayala-Zavala et al. [37], mango peel exhibits great agro-industrial potential for use as functional ingredient or as anti-browning additive in food processing due to high content level of AA in the samples that act as natural antioxidant.

Tocopherol species are present in foods; α-tocopherol is most important to human health [67]. Ajila et al. [56] reported the α-tocopherol content for raw (10.4 mg ATE per 100 g DW) and ripe (23 mg ATE) mango peels from *M. indica* var. Badami. Nonetheless, Abbasi et al. [67] reported a high amount of vitamin E in peels (ranged from 7 to 43 mg per 100 g DW) than in pulp (ranged from 0.87 to 4.12 mg) of nine "Chinese" mango (*M. indica*) varieties, and they mentioned that the variations in results indicate that phytochemical composition in fruits may greatly be affected by genetic diversity within/among the cultivars and other factors as maturity stage and harvesting time. These high values show that the consumption of mango peel can contribute at the dietary needs for intake of vitamin E.

Concerning with total soluble polyphenol (TSP) compounds, García-Magaña et al. [58] reported TSP of 6.8 and 4.2 g GAE (per 100 g db), respectively, in "Ataulfo" and "Tommy Atkins" mangoes. Hassan et al. [60] informed a concentration of 9.8 g GAE (per 100 g DW) in Bambangan (*M. pajang* K.) mango. Furthermore, Sáyago-Ayerdi et al. [68] characterized the hydrolyzable polyphenol profile in the peels of the "Ataulfo" mango; they reported that mango peel may contain series of gallotannins between 5 and 13 units that possess high antioxidant activity. This means that the peel of the mango species can be considered an excellent source of antioxidants. Shieber et al. [50] reported the presence of mangiferin, quercetin, and kaempferol and their related conjugates in mango peel from *M. indica* cv. "Tommy Atkins." Prasad et al. [69] identified six phenolic compounds (pyrogallic acid, gallic acid, catechin, epicatechin, mangiferin, and rutin) from *M. pajang* peels. Blancas-Benitez et al. [70] informed about the presence of chlorogenic acid (82%) and vanillin acid (17%) in "Ataulfo" mango peel (*M. indica*). On the other hand, Barreto et al. [71] studied 16 varieties of mango (*M. indica*) peel, and they reported minimal differences in the profiles between cultivars; however, there was considerable variation in the amounts of the major phenolic compounds. These findings demonstrated that every mango species is genetically different and unique, as has been pointed in this study.

7 Other *Mangifera* Species with Potential for Consumption

Other commercially valued species that produced edible fruits are *Mangifera similis*, *M. quadrifida*, *M. griffithii*, *M. altissima*, *M. gebede*, *M. macrocarpa*, *M. rufocostata*, *M. flava*, *M. applanata*, *M. macrocarpa*, *M. duperreana*, *M. oblongifolia*, *M. kashiana*, *M gracilipes*, *M. sclerophylla*, *M. merilli*, *M. rumphii*, *M. rigida*, *M. quemanga*, and *M. superba*, among others [72]. The ripe fruits are acid-sweet and have a pleasant flavor [20]. Additionally, there are many cultivars or varieties from *M. indica* that exhibit a great potential for their commercialization. According to many authors, *Mangifera* family includes many wild *Mangifera* species [1, 2]. Unfortunately, nutrimental information about these mango species are scarce. Naik et al. [73] opine that it is the large variability that has hindered the production of the commercial varieties on a large scale. However, it is true that this large biodiversity has not been exploited to the full potential [19].

8 Conclusion

Evidence showed that some of the wild *Mangifera* species such as *M. caesia*, *M. foetida*, *M. odorata*, *M. casturi*, *M. lalijiwa*, *M. zeylanica*, *M. sylvatica*, and others are healthy fruits to consume specially from the nutrimental viewpoint and are similar or better nutrimental source than the popular *M. indica*. In general, the commercially (*M. indica*) and wild mango species can be considered for many purposes including for processing and for consumption because mango may be considered an excellent source for improving nutrition. However, enhanced knowledge of the status of such species is necessary for the conservation of these valuable species. Also, this chapter offers a better understanding of the nutrimental and functional potential of these fruit species.

References

1. Tharanathan RN, Yashsa HM, Prabha TN (2006) Mango (*Mangifera indica* L.), "the king of fruits"-an overview. Food Rev Intl 22:95–123
2. Mozhui R, Rongsensashi, Limasenla, Changkija S (2011) Wild edible fruits used by the tribals of Dimapur district of Nagaland, India. Pleione 5(1):56–64
3. Barbosa-Gámez I, Caballero-Montoya KP, Ledesma N, Sayago-Ayerdí SG, García-Magaña ML, Bishop von Wettberg EJ, Montalvo-González E (2017) Changes in the nutritional quality of five *Mangifera* species harvested at two maturity stages. J Sci Food Agric 97(14):4987–4994
4. Suparman S, Pancoro A, Hidayat T (2013) Phylogenetic analysis of *Mangifera* base on RBCL sequences, chloroplast DNA. Sci Hortic 62:235–240
5. Kostermans AJGH, Bompard JM (1993) The mangoes: their botany, nomenclature, horticulture and utilization. Academic, London
6. Hidayat T, Pancoro A, Kusumawaty D, Eiadthong W (2011) Molecular diversification and phylogeny of *Mangifera* (*Anacardiaceae*) in Indonesia and Thailand. Int J Adv Sci Eng Inf Technol 1:88–91

7. Raman A, Burckhardt D, Harris KM (2009) Biology and adaptive radiation in the gall-inducing Cecidomyiidae (*Insecat diptera*) and Calophyidae (*Insecta hemipera*) on *Mangifera indica* (*Anacardiaceae*) in the Indian subcontinent. Trop Zool 22:27–56
8. Eiadthong W, Yonemori K, Kanzaki S, Sugiura A (2000) Amplified fragment length polymorphism analysis for studying genetic relationships among *Mangifera* species in Thailand. J Am Soc Hortic Sci 125(2):160–164
9. FAOSTAT-FAO (2010) Statistical database. Food and Agriculture Organization of the United Nations, Codex Alimentarius Commision. http://faostat3.fao.org/browse/rankings/commodities_by_country_exports/. Accessed 25 Oct 2017
10. Campbell RJ, Ledesma N (2013) The changing face of varieties for the Western Hemisphere, Fairchild Tropical Botanic Garden, The Fairchild Farm, 14885 SW 248 St., Homestead, FL 33032. In: Proceedings of the IX international mango symposium Ed: Ping Lu. Acta Hortic 992, ISHS, pp 55–58
11. Takatemjen, Jamir NS, Deb M (2009) Wild edible fruits of Wokha district of Nagaland, India. Pleione 3(1):59–62
12. IUCN Red List of Threatened Species. Version 2015.3. World Conservation Monitoring Centre 1998. *Mangifera casturi*. http://www.iucnredlist.org. Accessed 25 Oct 2017
13. IUCN Red List of Threatened Species. Version 2015.3. World Conservation Monitoring Centre 1998. *Mangifera lalijiwa*. http://www.iucnredlist.org. Accessed 25 Oct 2017
14. IUCN Red List of Threatened Species. Version 2015.3. World Conservation Monitoring Centre 1998. *Mangifera odorata*. http://www.iucnredlist.org. Accessed 25 Oct 2017
15. IUCN Red List of Threatened Species. Version 2015.3. World Conservation Monitoring Centre 1998. *Mangifera zeylanica*. http://www.iucnredlist.org. Accessed 25 Oct 2017
16. IUCN Red List of Threatened Species TM 2015. Monitored *Mangifera*. http://www.iucnredlist.org/. Accessed 25 Oct 2017
17. Khan AS, Ali S, Khan IA (2015) Morphological and molecular characterization and evaluation of mango germplasm: an overview. Sci Hortic 194:353–366
18. Anjaneyulu V, Radhika P (2000) The triterpenoids and steroids from *Mangifera indica* Linn. Indian J Chem 39:883–893
19. Dinesh MR, Ravishankar KV, Nischita P, Sandya BS, Padmakar B, Ganeshan S, Chitiraichelvan R, Sharma TVR (2015) Exploration, characterization and phylogenetic studies in wild *Mangifera indica* relatives. Am J Plant Sci 6:2151–2160
20. Lim TK (2010) Edible medicinal and non-medicinal plants, vol 1. Springer, New York
21. Ledesma N, Campbell R (2014) Conservation and commercial development of *Mangifera* species (wild mangos) in Florida. Proc Fla State Hortic Soc 127:1–4
22. Campbell RJ (2007) Kastooree (*Mangifera casturi*) as a graft interstoock for wild mangoes. Proc Fla State Hortic Soc 120:15–16
23. Campbell RJ (2007) The potential of new *Mangifera* species in Florida. Proc Fla State Hortic Soc 120:15–16
24. Baul TK, Alam MJ, Nath TK (2016) *M. sylvatica* Roxb., in the forests of South-Eastern Bangladesh: a potential underutilized tree for small-scale forestry. Small Scale For 15:149–158
25. Zaman R, Parvez M, Jakaria Md, Sayeed MA, Islam M (2015) In vitro clot lysis activity of different extracts of *Mangifera sylvatica* Roxb., leaves. Res J Med Plant 9(3):135–140
26. Akter S, McDonald MA, Marriot R (2016) *Mangifera sylvatica* (wild mango): a new cocoa butter alternative. Sci Rep 1–9
27. Kiew R, Teo LL, Gan YY (2003) Assessment of the hybrid status of some *Malesian* plants using amplified fragment length polymorphism. Telopea 10(1):225–232
28. Corrales-Bernal A, Urango LA, Rojano B, Maldonado ME (2014) Efectos *in vitro* e *in vivo* de la pulpa de mango (*Mangifera indica* cv Azúcar) en la carcinogénesis de colon. Arch Latinoam Nutr 64(1):16–23
29. World Health Organization (2000) General guidelines for methodologies on research and evaluation of traditional medicine. World Health Organization, Geneva
30. Arif M, Fareed S (2010) Pharmacognostic investigation and authentication of potentially utilized fruit *Spondias Mangifera* (wild). Int J Pharm Clin Res 2(1):31–35

31. Gupta C, Garg AP, Gupta S (2010) Antimicrobial and phytochemicals studies of fresh ripe pulp and dried unripe pulp of *Mangifera indica* (AMCHUR). Middle-East J Sci Res 5(2):75–80
32. Kim H, Kim H, Mosaddik A, Gyawali R, Ahn KS, Cho SK (2012) Induction of apoptosis by ethanolic extract of mango peel and comparative analysis of the chemical constitutes of mango peel and flesh. Food Chem 133(2):416–422
33. Zaharah SS, Singh Z, Symons GM, Reid JB (2013) Mode of action of abscisic acid in triggering ethylene biosynthesis and softening during ripening in mango fruit. Postharvest Biol Technol 75:37–44
34. Palafox-Carlos H, Yahia EM, González-Aguilar GA (2012) Identification and quantification of major phenolic compounds from mango (*Mangifera indica*, cv. Ataulfo) fruit HPLC-DAD-MS/MS-ESI and their individual contribution to the antioxidant activity during ripening. Food Chem 135:105–111
35. FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization (2008) Report on microbiological hazards in fresh fruits and vegetables. http://www.fao.org/ag/agn/agns/files/FFV_2007_Final.pdf. Accessed 25 Oct 2017
36. Masibo M, He Q (2008) Major mango polyphenols and their potential significance to human health. Compr Rev Food Sci Food Saf 7:309–319
37. Ayala-Zavala JF, Vega-Vega V, Rosas-Domínguez C, Palafox-Carlos H, Villa-Rodríguez JA, Siddiqui M, Dávila-Aviña JE, González-Aguilar GA (2011) Agro-industrial potential of exotic fruit byproducts as a source of food additives. Food Res Int 44:1866–1874
38. Goñi I, Díaz-Rubio ME, Pérez-Jiménez J, Saura-Calixto F (2009) Towards an updated methodology for measurement of dietary fiber, including associated polyphenols, in food and beverages. Food Res Int 42:840–846
39. Chambial S, Dwivedi S, Shukla KS, John PJ, Sharma P (2013) Vitamin C in disease prevention and cure: an overview. Indian J Clin Biochem 28(4):314–328
40. Institute of Medicine (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academies Press, Washington, DC
41. Kennedy DO, Veasey R, Watson A, Dodd F, Jones E, Maggini S, Haskell CF (2010) Effects of high-dose B vitamin complex with vitamin C and minerals on subjective mood and performance in healthy males. Psychopharmacology 211(1):55–68
42. Gry J, Black L, Eriksen FD et al (2007) EuroFIRBASIS: a combined composition and biological activity database for bioactive compounds in plant-based foods. Trends Food Sci Technol 18(8):434–444
43. Jyotshna, Srivastava P, Killadi B, Shanker K (2015) Uni-dimensional double development HPTLC-densitometry method for simultaneous analysis of mangiferin and lupeol content in mango (*Mangifera indica* L.) pulp and peel during storage. Food Chem 176:91–98
44. Ruiz-Montañez G, Ragazzo-Sánchez JA, Calderón-Santoyo M, Velazquez-De La Cruz G, de León JR, Navarro-Ocaña A (2014) Evaluation of extraction methods for preparative scale obtention of mangiferin and lupeol from mango peels (*Mangifera indica* L.) Food Chem 159:267–272
45. Khoo HE, Ismail A, Mohd-Esa N, Idris S (2008) Carotenoid content of underutilized tropical fruits. Plant Foods Hum Nutr 63:170–175
46. Mirtaf AHS, Salma I, Razali M (2016) Natural antioxidant properties of selected wild *Mangifera species* in Malaysia. J Trop Agric Food Sci 44(1):63–72
47. Barnajee N, Kim H, Krenek K, Talcott ST, Mertens-Talcott SU (2015) Mango polyphenols suppressed tumor growth in breast cancer engrafts in mice: role of the PI3K/AKT pathway and associated microRNAs. Nutr Res 35:744–751
48. Khoo HE, Ismail A (2008) Determination of daidzein and genistein contents in *Mangifera* fruits. Malays J Nutr 14(2):189–198
49. López-Cobo A, Verardo V, Diaz-de-Cerio E, Segura-Carretero A, Fernández-Gutiérrez A, Gómez-Caravaca AM (2017) Use of HPLC-and GC-QTOF to determine hydrophilic and lipophilic phenols in mango fruit (*Mangifera indica* L.) and its by-products. Food Res Int 100:423–434

50. Schieber A, Ullrich W, Carle R (2000) Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. Innov Food Sci Emerg Technol 1:161–166
51. Siddique HR, Saleem M (2011) Beneficial health effects of lupeol triterpene: a review of preclinical studies. Life Sci 88(7–8):285–293
52. Krinsky NI (1989) Carotenoids as chemopreventive agents. Prev Med 18:592–602
53. Ajila CM, Bhat SG, Prasada Rao UJS (2007) Valuable components of raw and ripe peels from two Indian mango varieties. Food Chem 102:1006–1011
54. Vilela C, Santos SAO, Oliveira L, Camacho JF, Cordeiro N, Freire CSR, Silvestre AJD (2013) The ripe pulp of *Mangifera indica* L: a rich source of phytosterols and other lipophilic phytochemicals. Food Res Int 54:1535–1540
55. Chen B, McClements DJ, Decker EA (2013) Design of foods with bioactive lipids for improved health. Annu Rev Food Sci Technol 4:35–56
56. Ajila CM, Jaganmohan Rao L, Prasada Rao UJS (2010) Characterization of bioactive compounds from raw and ripe *Mangifera indica* L. peel extracts. Food Chem Toxicol 48(12):3406–3411
57. Ajila CM, Naidu KA, Bhat SG, Prasada Rao UJS (2007) Bioactive compounds and antioxidant potential of mango peel extract. Food Chem 105(3):982–988
58. García-Magaña ML, García HS, Bello-Pérez LA, Sayago-Ayerdi SG, Mata MM (2013) Functional properties and dietary fiber characterization of mango processing by-products (*Mangifera indica* L., cv Ataulfo and Tommy Atkins). Plant Foods Hum Nutr 68(3):254–258
59. Ajila CM, Aalami M, Leelavathi K, Rao UJSP (2010) Mango peel powder: a potential source of antioxidant and dietary fiber in macaroni preparations. Innov Food Sci Emerg Technol 11(1):219–224
60. Hassan FA, Ismail A, Hamid AA, Azlan A, Al-Sheraji SH (2011) Characterisation of fibre-rich powder and antioxidant capacity of *Mangifera pajang* K. fruit peels. Food Chem 126(1):283–288
61. Sogi DS, Siddiq M, Dolan KD (2015) Total phenolics, carotenoids and antioxidant properties of Tommy Atkins mango cubes as affected by drying techniques. LWT-Food Sci Technol 62(1):564–568
62. Aziz NAA, Wong LM, Bhat R, Cheng LH (2012) Evaluation of processed green and ripe mango peel and pulp flours (*Mangifera indica* var. Chokanan) in terms of chemical composition, antioxidant compounds and functional properties. J Sci Food Agric 92(3):557–563
63. Ediriweera MK, Tennekoon KH, Samarakoon SR, Thabrew I, De Silva ED (2017) Induction of apoptosis in MCF-7 breast cancer cells by Sri Lankan endemic mango (*Mangifera zeylanica*) fruit peel through oxidative stress and analysis of its phytochemical constituents. J Food Biochem 41:1–9
64. Ajila CM, Leelavathi K, Prasada Rao UJS (2008) Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. J Cereal Sci 48(2):319–326
65. Gondi M, Basha SA, Bhaskar JJ, Salimath PV, Prasada Rao UJS (2015) Anti-diabetic effect of dietary mango (*Mangifera indica* L.) peel in streptozotocin-induced diabetic rats. J Sci Food Agric 95(5):991–999
66. Ajila CM, Prasada Rao UJS (2013) Mango peel dietary fibre: composition and associated bound phenolics. J Funct Foods 5(1):444–450
67. Abbasi AM, Liu F, Guo X, Fu X, Li T, Liu RH (2017) Phytochemical composition, cellular antioxidant capacity and antiproliferative activity in mango (*Mangifera indica* L.) pulp and peel. Int J Food Sci Technol 52(3):817–826
68. Sáyago-Ayerdi SG, Moreno-Hernández CL, Montalvo-González E, García-Magaña ML, de Oca MMM, Torres JL, Pérez-Jiménez J (2013) Mexican 'Ataulfo' mango (*Mangifera indica* L) as a source of hydrolyzable tannins. Analysis by MALDI-TOF/TOF MS. Food Res Int 51:188–194

69. Prasad KN, Hassan FA, Yang B, Kong KW, Ramanan RN, Azlan A, Ismail A (2011) Response surface optimization for the extraction of phenolic compounds and antioxidant capacities of underutilized *Mangifera pajang* Kosterm peels. Food Chem 128(4):1121–1127
70. Blancas-Benitez FJ, Mercado-Mercado G, Quirós-Sauceda AE, Montalvo-González E, González-Aguilar GA, Sáyago-Ayerdi SG (2015) Bioaccessibility of polyphenols associated with dietary fiber and in vitro kinetics release of polyphenols in Mexican 'Ataulfo' mango (*Mangifera indica* L.) by-products. Food Funct 6(3):859–868
71. Barreto JC, Trevisan MT, Hull WE, Erben G, de Brito ES, Pfundstein B, Wurtele G, Spiegelhalder B, Owen RW (2008) Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.) J Agric Food Chem 56(14):5599–5610
72. Dinesh MR et al (2011) Mangifera. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, tropical and subtropical fruits. Springer, New York
73. Naik KC, Rao CB, Ramani VS (1958) Problems in crop improvement in mango. Indian J Hortic 15:159–116

Part II

Proteins and Their Biological Activity

Plant Proteins from Legumes

10

Catherine Bennetau-Pelissero

Contents

C. Bennetau-Pelissero (✉)
Department of Life Science and Health, University of Bordeaux, Bordeaux, France

Department Feed and Food, Bordeaux Sciences Agro, Gradignan, France
e-mail: catherine.bennetau@u-bordeaux.fr

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_3

Abstract

Legumes are part of the human edible panel since prehistory times but the remains that reached our last centuries were all from a period posterior to fire domestication. In all parts of the world where human civilizations developed, pulses were associated with cereals and the combination of their proteins managed to cover the essential amino-acid requirements of Humans and animals. Legumes gathering more than 19,000 different species, all present high protein content due to specific symbiosis with *rhizobia* and arbuscular *mycorrhizae* present in the soils. These associations are thought to originate from first symbiotic events dating from more than 60 million years before present. They allow the plants to fix nitrogen that is used for protein biosynthesis. The nutritional value of actual pulses is generally higher than that of other crops especially since domestication and the genetic selection processes operated by humans. Beside proteins with suitable amino-acid profiles, legumes also contain digestible carbohydrates and some of them also contain fat. In some cases, these fat include polyunsaturated fatty acids that increase further the nutritional value of the corresponding legumes. However, if such valuable plants managed to survive along geological periods, it is because their evolution with their environmental pressure lead them to develop anti-nutritional substances to protect themselves from their predators. Here will be discussed some of these anti-nutritional substances, the so-called tannins, phytic acid, saponins, phytoestrogens, lipoxygenase, hemagglutinin, trypsin inhibitor, as well as allergens. Because all these substances are basically useful for the crops, it is only during processing that they should be removed. Therefore, a special focus is made on traditional versus modern recipes and industrial food processing. Their respective impacts on basic nutritional components (amino-acids, fats, carbohydrates, vitamins, and minerals) as well as on the anti-nutritional factors listed above are examined. Basically, wet processing which was most frequently developed in the past, associated orf not with fermentation or germination, is also the most efficient in removing all anti-nutritional factors.

Keywords

Legumes · Prehistoric domestication · Proteins · Amino-acid profiles · Anti-nutritional factors · Tannins · Saponins · Phytic Acids · Phytoestrogens · Oligosaccharids · Hemagglutinins · Lipoxygenases · Tripsin inhibitors · Allergens

1 Introduction

Legumes have been used for thousands of years in human nutrition and constitute one of the pillar bases of human civilization. Among other early domesticated pulses are lentils (*Lens culinaris*, L. Medic.), pea (*Pisum sativum*, L.), chickpea (*Cicer arietinum*), broad bean (*Vicia Faba*, L.), common bean (*Phaseolus vulgaris*, L.),

lupine (*Lupineus albus*, L.), peanut (*Arachis hypogaea*, L.), cowpea (*Vigna unguiculata*, L. Walp.), bambara groundnut (*Vigna subterranean*, L. Verdc), or soy (*Glycine max*, L. Merr). In all continents, pulses were domesticated although the archeological traces of these events are not always easy to show. The origin of high protein content and quality in legumes is the symbiotic nitrogen fixation by rhizobia and mycorrhizae in plant root nodules. This leads to increased production of protein and injects nitrogen into agricultural systems. Nitrogen from rhizobia symbiosis also provides residual soil nitrogen to subsequent non legume crops. Therefore, legumes have been used in crop rotations for thousands of years. The proteins of legumes are not only concentrated but also exhibit interesting amino-acid profiles that make them attractive for animals and humans. Therefore, and to allow plant survival to pest and predators, legumes progressively developed an arsenal of biochemical "weapons" to limit their palatability and digestibility. Thus beside excellent theoretical nutritional characteristics, legumes developed anti-nutritional factors such as tannins, phytic acid, saponins, oligosaccharides, hemagglutinins, lipoxygenases, protease inhibitors, or certain polyphenols. These substances help preventing large consumption of plants in raw and forced humans to invent cooking processes and specific recipes including fermentation or germination. These traditional processing practices have recently been renewed thanks to modernization of processing tools and industrialization of the food preparation. These new processes can act only partially on anti-nutritional agents. This may be a cause of increasing allergy prevalence among human consumers. Nowadays, modern recording tools allow to show that in all continents and with all legumes these allergic manifestation due to pulses consumption exist and possibly rise. This review tends to go through all these aspects giving perspective to the issue of plant proteins in human future.

2 History of Legumes Cultivation and Consumption

Domestication of a crop leads to the appearance of novel traits which can be favorable to harvest and to human uses. According to Caracuta et al. [1] experiments conducted on wild modern peas, chickpeas and lentils prove that neither harvesting of wild stands or cultivation of wild legumes results in profitable yields [2–4]. Therefore the added value got from the actual crops, directly comes from domestication. Among other traits, the seed-coat should be thinner and smoother in domesticated stocks to facilitate water penetration and germination [5]. However, using this mutation as a trait of domestication, while exploring antic farming sites, remains controversial, because several cultured legumes including lentils or grass pea do not exhibit great differences with their wild relatives [6, 7]. Oppositely, increase in seed size is considered to be one of the major domestication-traits even though this increase in seed-size does not occur at the early stage of domestication but rather later as a result of crop improvements [1]. Legumes have been associated to cereals as basic foodstuff in the human history for as long as agriculture and settlement started. The remains from excavations of the Neolithic period already, shows associations of wheat varieties and barley to lentils and peas in the Near East region

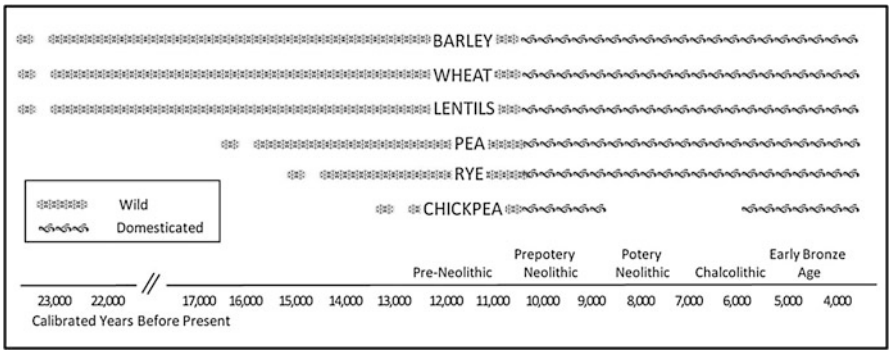


Fig. 1 Diagrammatic representation of the appearance of the main crops during human history as revealed by remains found in ancient human settlements

where cereal cultivation was shown to be first performed [8], then came chickpea (Fig. 1). The present chapter shows that all over the world and in all continents, legumes were domesticated to be part of human protein intake. They were always associated to cereals which allow the best amino-acid combination to fulfill human nutritional requirements.

2.1 Near East and Western Europe

2.1.1 Lentils

According to Zohary and Hopf [8], lentils (*Lens culinaris* L.) are found in remains which were determined to be as old as agriculture itself. Lentils seem to be closely associated with the start of wheat and barley cultivation in the Near East. The presence of small lentil-seeds was shown among remains retrieved from pre-farming zones of North Syria dated from 10,000 to 9,500 Cal BP (Calibrated Before Present). A few traces of the use of lentils are then found in Iraq, Iran, Anatolia, and Jordan. These traces are from the Neolithic period and more precisely from the ninth millennium Cal BP. and consist of small lentil-seeds (2.5–3.0 mm in diameter). This size does not allow to conclude on the wild or cultivated origin of the seeds. Larger amounts of lentil-seeds were also discovered in later phases of the Neolithic settlement in the Near East. According to Zohary and Hopf [8], they were found: in Syria (8,250 to 7,950 Cal BP) [9], in Turkey (7,800 to 7,000 Cal BP) [10], and in Iran (7,500 to 7,000 Cal BP) [11]. At that time, lentil-seeds had already attained 4.2 mm in diameter which is characteristic of a development under domestication. Lentils spread to East and then to Western Europe during the Bonze and the Iron ages, respectively. Because the remains of lentil-seeds in France seems to be less evident in early and late Bronze age than they are in Germany, Hungary, and Switzerland, it is assumed that the use of lentil-seeds spread from East to West during this particular period. However, only the seed-size of lentils can help to diagnose for domestication. Unfortunately this trait

evolves very slowly and is affected by environmental factors, and therefore, it is difficult to ascertain that we would be able to determine the one set of *Lens* domestication in the future.

2.1.2 Peas

Peas of the *Pisum* gender were found to be common in the Neolithic agriculture settlements in Europe. They were found to be closely associated with the wheat and barley products. According to Zohary and Hopf [8], remains of peas were discovered in early Neolithic farming villages of the Near East (9,000 to 8,000 Cal BP). Preserved, since they were carbonized, pea seeds were already present in aceramic jars of north Iraq, southeast Turkey, and in the prepottery B level in Jericho. According to the remains, the use of these legumes most probably spread over the Near East since remains dated from the eighth millennium Cal BP were found in this area. Remains dated from 7,400 to 7,000 Cal BP [12] show the smooth seed coat characteristic of domesticated peas. Genetic approaches show that the actual species *Pisum sativum* (L.) most probably derives from two main wild species *P. elatius* and *P. humile* which is smaller.

2.1.3 Cicer

Chickpea (*Cicer arietinum* L.) as other early European cultivated crop seems to originate from the Near East and deriving from a progenitor *C. reticulatum*. According to Garrard [13], remains of *C. arietinum* were found in Neolithic settlements of the Euphrate area and close to the natural distribution area of its putative progenitor. Later (10,000 Cal BP), it is recorded in Jericho far from its original region, and this is an indication of domestication. Establishment of the domesticated forms and replacement of the wild ancestral populations of European legumes is thought to have occurred in the Near East. This was performed within a relatively short time. Chickpea, however, appears as an exception among all other "founder crops." When all other crops including legumes are germinating in autumn, flowering in late winter/early spring and maturating in early summer, chickpea is a spring sown crop. Although the wild precursor was probably following the general cycle pattern, chickpea was deliberately sown in late winter (February) and this is considered to be a strong trait of domestication. As mentioned in [14], the winter cycle is found in all wild progenitors of the "founder crops" of Near Eastern agriculture without exception because their harvest yield is far better following winter germination. Plants are then able to use the winter rainfall for growing. It appears that chickpea sowing was delayed to avoid the *Ascochyta* disease whose pathogen agent is *Didymella rabiei*. This fungus has the potential to cause total yield loss on chickpea, and its occurrence is high since 9 fields over 10 can be attacked in a given area. In recent tests, only one out of ten actual varieties was able to resist to the fungal attacks when sowed in winter. The disease does not affect the legume sowed in late winter because of early dryness, and this allows maintaining lower (0.95 tons/ha rather than 3.0 tons/ha) but consistent yields.

2.1.4 Faba

Broad bean belongs to the section *Faba* of the genus *Vicia*. They appear today as big beans even though it is though that the progenitor of the actual broad bean was smaller and most probably derives from the *Vicia faba* var. *minor* [15]. Quite recently [1], reported the discovery of large amount of *Faba* seeds in sites located in the Galilee region of Israel. According to carbon duration, the seeds were collected 11,000–10,000 Cal BP and are the oldest *Faba* seeds ever found so far. The most ancient datation corresponds with a period when water supply was good enough for production and harvesting. The authors argue saying that these *Faba* seeds were most probably cultured under human input as early as the following millennium (10,000 Cal BP.) since seeds remains are still of good size indicating good water supply in a country where dryness was gaining. The earliest remains of broad beans found in Western Europe were from the Iberic peninsula and were dated from the late Neolithic times. In contrast, few carbonized remains of broad beans appear in several Bronze Age sites of the East Mediterranean and Aegean. All Bronze Age broad beans have relatively small seed and thus most probably belong to *Vicia faba* var. *minor*.

2.2 America

2.2.1 Phaseolus

The common bean *Phaseolus vulgaris* (L.) presents multiple variations of fresh and dried varieties. Those include string beans, green beans, French beans, kidney beans, or pinto beans. These varieties and others provide a third of daily dietary protein in some cultures of Africa and of Americas. *P. vulgaris* is originating from Mesoamerica and South America. Its domestication is much more recent than the European legumes since it is thought to start about 8,000 years before present time [16]. Plant domestication is often associated with a suite of morphological changes. In the case of common bean, domestication has led to increases in seed and leaf sizes, as well as to changes in growth habit and other features. Moreover, these morphological shifts occurred not once but twice, as common bean was domesticated independently in Mesoamerica (probably in what is now Mexico) and the Andes [17]. Currently, the domesticated gene pool of the species seems to be organized into four Mesoamerican and three Andean races [18, 19]. These two pools were domesticated independently. However, although the four Mesoamerican races Durango, Jalisco, Mesoamerica, and Guatemala differ in ecological adaptations, geographic ranges, morpho-agronomic traits, allozyme alleles, and random amplification of polymorphic DNA (RAPD) markers [18, 19], they probably all derive from a single domestication event. Indeed, they all present the same predominant phaseolin electrophoretic type S, and similar amplification fragment length polymorphism (AFLP) patterns [20, 21]. In parallel there are three Andean races: Nueva Granada, Peru, and Chile, which also differ in morpho-agronomic characters, allozymes, and phaseolin types [19]. This would support multiple domestications, but their geographic ranges overlap, and they seem to be similar in AFLP patterns [18]. This supports a single origin.

2.2.2 Lupineus

The discovery of the origin of lupine in South America is quite recent. According to Atchison et al. [22], *Lupineus mutabilis,* also called tarwi, was domesticated once and not in the putative south-central Andean core area of early agriculture, but rather in northern Peru, most likely in the Cajamarca region. This area is included, or close to, the distribution area of *L. piurensis*. Therefore, it can be assumed that *L. piurensis* is the most likely wild progenitor of the modern lupine *L. mutabilis*. Demographic analyses suggest that tarwi split from *L. piurensis* around 2,600 Cal BP. and suffered a classical domestication bottleneck. The earliest unequivocal archaeological evidence of domesticated tarwi seeds is from the Mantaro Valley, central Peru 1,800 Cal BP. According to the actual theory, lupine then spread North and South from the initial area of origin in Peru. Therefore, the pulse went south to Bolivia and north to Ecuador and Colombia. Lupine arrived then in Europe with Spanish conquistadores, *i.e.,* in the early fifteenth century.

2.2.3 Arachis

According to Bonavia [23], the geographic area of origin and domestication of the peanut would be the Huarmey valley, near the Peruvian coast. Most of the archeological records of fruits of *Arachis hypogaea* date from approximately 3,500–4,500 years BP. However, based on the distribution and biology of wild species and landraces of *Arachis,* the region of origin of the peanut may have been in the south of Bolivia and the northwest of Argentina [24]. According to Grabiele [25], radiocarbon-dated macro-botanical remains were dated from approximately Cal 7,840 year BP. They appeared as wild *Arachis* species or peanut fruits in early domestication stages. They were recovered in buried preceramic sites in the lower western slopes of the Andes in Northern Peru. However, since this region is not considered as a plant domestication center, the first *Arachis* species may have been first cultivated elsewhere in South America earlier than Cal 8,000 year BP. According to Grabiele *et al*. [25], *A. monticola* may be an intermediate tetraploid ancestor from which *A. hypogaea* has arisen upon domestication. In addition, *A monticola* was most probably obtained from the two diploid species: maternal (*A. duranensis*) and paternal (*A. ipaensis*) wild diploid species of *Arachis*.

2.3 Africa

2.3.1 Cowpea

The African *Vigna* studied here are cowpea [*V. unguiculata* (L.) Walp.] and bambara groundnut [*V. subterranea* (L.) Verdc.] [26]. *Vigna unguiculata* is most probably originating from sub-Saharan Africa. It was introduced in America during the seventeenth century by Spanish conquistadores and is now largely cultured and consumed as black eyed pea in USA and Brazil. According to Kongjaimun et al. [27], cowpea was domesticated from wild cowpea in Africa. In 2007, D'Andrea *et al*. [28] reported the discovery of cowpea remains in Kintampo settlements in Ghana. Kintampo is a Later Stone Age (LSA) tradition of West Africa dating to

3,600–3,200 Cal BP [29]. One seed collected in the B6B site in the Horizon 4 was submitted for radiocarbon dating by AMS. The resulting date was of 3,410 ± 60 Cal BP, at 95.5% c.i. (TO11883). This date confirms a Kintampo association and is consistent with other determinations obtained from the site. Although to date, the earliest remains are originating from Western Africa, a Northern or North-Eastern origin has been argued based on the absence of true ecologically wild cowpea in West Africa [30]. However, the paucity of available data from eastern African Countries has precluded a final assessment. Based on the botanical evidences, it is thought that *Vigna unguiculata* then spread in Asia. There, the cultivated cowpea or its weedy relative was subsequently selected for the vegetable crop, yardlong bean [*Vigna unguiculata* subsp. *sesquipedalis*]. The subspecies' main characteristic is the length of its pods which can range between 35 and 60 cm long. Nowadays, cowpea constitutes a major dietary source of protein for many sub-Saharan populations. When mixed with cereals, protein quality is significantly improved [31].

2.3.2 Bambara Groundnut

The main groundnut originating from Africa is Bambara *Vigna subterranean*. It is an important food legume grown widely in semi-arid Africa and closely related to cowpea (*V. unguiculata*). Bambara shares much of its area of cultivation and origins of genetic diversity with cowpea [32]. According to Philippson and Serge Bahuchet [33], the Bambara African name ⁎/jʊgʊ appeared in the Proto Bantu language probably incorporated thank to the immigration of people from Proto Benue Congo origin. This immigration is dated from a climate deterioration that scourged Sahara and Sahel and which is dated 7,100–6,900 Cal BP. Naming a plant does not mean that it was domesticated and cultured but at least recognized as a define plant probably because of human usage. Bambara groundnut appears as two botanical forms; var. *spontanea* which is most likely the wild forms and var. *subterranea* comprising the cultivated forms. *Vigna subterranean spontanea* is restricted to an area from Nigeria to Sudan, with a center of diversity around Cameroon. *Vigna subterranean subterranea* is found in many parts of the tropics and particularly in sub-Saharan Africa. Both the wild and cultivated forms bear 11 pairs of chromosomes [34]. As mentioned here, the use of this legume by human populations of West Africa probably dates from the early proto Bantu period which seems to coincide with Ceramic Late Stone Age but this still remains to be substantiated by archeobotany.

2.4 Asia

2.4.1 Glycine

The main legume in Asia is identified as soybean *Glycine max*. China is the country of origin of soybean. Deng in [35] reports that soybean was found to be present in the Neolithic site of Baligang in the Shijiahe period (Cal 4,500 BP). According to Hymowitz [36], it was domesticated and therefore cultivated in the Eastern half of North China 3,100 Cal BP. More precisely, soybean domestication probably started

in Liaoning province because the wild soybean grows everywhere and the stages of evolution are apparent [37]. *Glycine max* belongs to the subgenus *Soja*, which also contains *G. soja* and *G. gracilis*. *G. soja*, is a wild species of soybean, found in many different environments in many Asian countries [38]. According to cytological, morphological, and molecular traits, *G. soja* is most probably the ancestor of *G. max*. On the contrary, *G. gracilis* is most probably semi-wild form of *G. max*, which phenotypic traits place it as an intermediate in the speciation between *G. max* and *G. soja*. According to Willis [39], *G. max* ancestors produce tiny, hard seeds that are useless for food unless properly prepared. Therefore, the initial use of soya bean ancestors was essentially as green manure in crop culture rotations. Since then, and until 1915, Manchuria in North-Eastern China has been the heart of soybean production in China [40]. The ancient character for soybean (shu) seems to appear on Zhou dynasty bronze vessels dated around 3,020 Cal BP [36]. Confucius documents dated from 2,500 Cal BP mentioned soybean as one of the five staple grains of China. These were foxtail millet (*Setaria italica*), broomcorn millet (*Panicum miliaceum*), rice (*Oryza sativa*), wheat (*Triticum aestivum*), and legumes essentially soybeans (*Glycine max*).

3 Origin of Protein in Legumes

3.1 An Evolution Process

Legumes are particularly rich in proteins since they produce amino acid from ammonia. This NH_3 is supplied by rhizobia symbiotic organisms which produce it from aerial Nitrogen (N_2). Archeobiology established that the symbiosis between legumes (Fabaceae) and nitrogen-fixing bacteria, the so-called rhizobia, appeared Cal. 60 million years BP [41, 42]. This long association probably explains why adaptative processes gave rise to about 19,500 legume species [43]. Molecular and genetic studies suggest that rhizobia bacteria progressively associated to the more widespread and much older endo-mycorrhizal symbiosis [44]. The association between arbuscular mycoorhizal fungi and plants involve recognition factors generated by the fungus and named Myc factors [45]. As for mycorrhizal association, the plant-rhizobia interaction is most generally initiated by a mutual recognition of molecular signals released by both symbiotic partners. It is characterized by varying degrees of specificity pre-determined by the nature and profile of seed/root exudates from the legume, as well as nodulation factors from the rhizobia [46, 47]. The plant molecules involved in the recognition step are flavonoids [48, 49].

3.2 Adaptation for N_2 Fixation

Nowadays, the association between rhizobia bacteria and mycorrhizal fungi give birth to root nodules able to capture the aerial nitrogen and to inject it into the plant metabolism. Therefore, legumes receive the bulk of nitrogen fixed by rhizobia in the form of

ammonia, which is incorporated into organic form before being exported from nodules. The formation of effective root nodules with compatible soil rhizobia allow to reduce atmospheric N_2 into NH_3 for bacterial and plant use. The symbiosis requires the close association of the bacteria and the plant and the nitrogenase enzyme complex that reduces N_2 to NH_3 is oxygen labile. Rhizobia as other soil bacteria are obligate aerobes and require oxygen for respiration and metabolism. Therefore, they should combine two opposite situations under nitrogen-fixing conditions: oxygen for their own metabolic requirements and anaerobic conditions for nitrogen fixing. To achieve this paradox, the plant and the bacteria produce a micro-aerobic environment around nitrogen-fixing rhizobia in nodules. The outer cells of the nodules play as a barrier to gaseous diffusion limit the rate of oxygen into the central infected tissue. The outer cells, their bacteroids, and plant mitochondria consume oxygen as fast as it can enter the nodules. The oxygen is directly targeted to mitochondria and bacteroids via plant hemoglobins the so-called leghemoglobins. This insures a low oxygen ratio in the vicinity of the nitrogenase enzyme complex [50]. If leghemoglobin transcription is prevented (RNAi), this leads to a higher steady-state level of free oxygen locally a lower ATP/ADP ratio and a complete absence of nitrogenase activity [51].

3.3 The Nitrogen Uptake

The symbiosis between legumes and rhizobia, at its most basic level, results in the exchange of reduced carbon from the plant for reduced nitrogen from the bacteria. Thanks to its photosynthesis the plant produces sucrose which is the primary source of reduced carbon for nodule metabolism [52]. In nodules, phosphorenolpyruvate carboxylase and malate dehydrogenase activities divert carbon flux away from glycolysis to form malate. Malate is considered to be the primary source of carbon for rhizobia bacteroids. During nodule development, bacteroids are programmed to fix N_2 and not to assimilate NH_3 into an organic form. The assimilation of NH_3 is left to the plant, and during nodule development, genes such as those encoding glutamine synthase, glutamate synthase, and aspartate amino transferase are induced. These enzymes can incorporate NH_3 into amino acids for export from nodules [53]. Noteworthy, in legumes of tropical origin, like *Glycine max* and *Vigna unguiculata,* nitrogen is exported from nodules into the plant metabolism as ureides [54]. The nitrogen exportation from the bacteroids to the plant mainly occurs via ammonia rather than via any other more elaborated substance, i.e., amino acid. This transfer most probably occurs in a passive way. However, Glutamate, Asparate, or Glutathione may play a role in the active transport of nitrogenic molecules through the symbiotic membrane separating the bacteroids and the plant.

3.4 How to Explain the Efficiency of a Tripartite Symbiosis

A co-evolution process is generally put forward to explain the specificity of the association between legumes and their symbionts [55]. However, according to

Martínez-Romero [56], in some cases there may rather be a constant selection of micro-symbionts by the plant. The symbiont is selected for its large and fast capabilities for genetic change or of acquisition of symbiotic genes [57]. Rapid changes in symbiotic nucleic acid material could have enabled bacteria to adjust and adapt to the diversification burst of legumes that occurred during the earth evolution. Many studies have been conducted in order to decipher the mechanism of the tripartite association between the plant, the bacteria (rhizobium), and the fungus (mycorrhizae). According to Maillet et al. [58], mycorrhizal fungi of the genus *Glomus* and rhizobia secrete very similar lipo-chitooligosaccharide signal molecules, the so-called Nod factors in rhizobia and Myc factors lipo-chitooligosaccharides in arbuscular endo-mycorrhizal fungi. All Nod factors are lipo-chitooligosaccharides with a β-1,4-linked N-acetyl-D-glucosamine backbone of which the nonreducing sugar moiety is substituted with an acyl chain [59]. In legumes, rhizobial Nod factors are detected thanks to two different Lys-M-domain receptors to kinase. These proteins have an extracellular domain containing three Lys-M domains, a transmembrane domain, and an intracellular kinase domain. Mutations in either of the genes encoding for these receptors can block Nod factor induced responses, suggesting that these proteins operate in conjunction [60].

4 Protein Levels Compared to Other Plants

As mentioned previously, legumes are rich in proteins because they can use nitrogen fixed by their symbiotic nodules to produce endogenous proteins. Therefore when compared to other plant families, they tend to contain a higher rates of protein (Fig. 2). Legumes proteins are classified according to their solubility properties in water, salted water, or ethanol/water solutions. Albumins are soluble in water while globulins are soluble in salt water solutions, and prolamins are soluble in ethanol/water solutions.

4.1 Specific Proteins of Legumes

The most abundant proteins in grain legumes are globulins. These are classified as 2S, 7S, and 11S globulins according to their sedimentation coefficients (S) [61]. The two main storage proteins in soybean (*Glycin max*) are glycinin (11S) and β-conglycinin (7S) [62]. They can reach 41% of the total of grain weight. The glycinins are hexamers with molecular masses of 320,000 and 375,000 Da. The subunits are composed of specific acidic polypeptide chain which molecular mass is 40,000 Da linked by disulfide bonds. In lupine (*Lupinus alba*), the storage proteins are conglutin [63]. They have been classified into four groups: α, β, γ, and δ conglutins. The α–conglutin is a hexameric protein containing monomeric unit either acidic: (α) or basic: (β) subunits with molecular weight between 42,000 and 52,000 Da and between 20,000 and 22,000 Da, respectively. The α-conglutin (11S) may account for about 35–37% of the total seed storage proteins in white

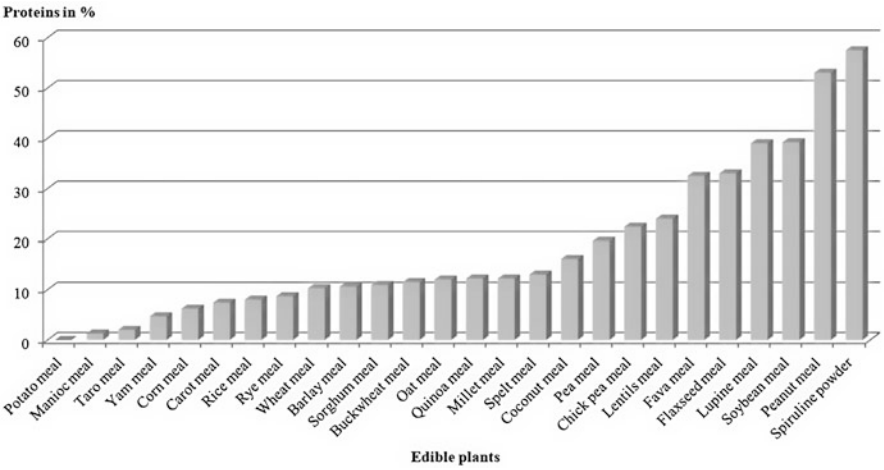


Fig. 2 Protein content of several plants used as human food

lupine seeds [64]. The most abundant lupine globulin is β-conglutin, also named vicilin, which represents about 44–45% of white lupine seed storage proteins [64]. The β-conglutin (7S) is a trimeric protein. Each of its monomer is formed by three polypeptides of low (17000–20,000 Da), medium (25,000–46,000 Da), and high (53,000–64,000 Da) molecular weight. The γ-conglutin accounts for about 5% of the amount of proteins in mature white lupine seeds [64]. The γ-conglutin (7S) is a tetrameric protein of about 50,000 Da composed of two subunits with molecular weight between 17,000 and 29,000 Da linked by disulfide bonds. Finally, δ-conglutin is the least abundant lupine conglutin, representing 3–4% only of total conglutin in white lupine [63]. δ-Conglutin (2S) is a monomeric protein consisting of two subunits of around 4,000 and 9,000 Da, respectively. These subunits are linked by two disulfide bonds. In pea (*Pisum sativum*), the globulin are named vicilin and convicilin (7S) and legumin (11S) [61]. Each group represents 4% and 3%, respectively, of the total weight of pea seeds. According to Barać et al. [65], vicilin appears as a globulin constituted from subunits with molecular weight of 47,300; 35,000; and 28,700 Da. Three minor subunits also appear in SDS-PAGE profiles of Tris-extracts with molecular weights of 37,000; 33,300; and 31,800 Da. Beside, two subunits of convicilin are identified with molecular weight of 77,900 and 72,400 Da. Legumin was identified as a trimer. Each monomer has a molecular weight of about 63,500 Da. These units associate acidic subunits of 40,890 Da and basic subunits of 22,300 and 23,100 Da. In broad bean (*Vicia faba*), the protein content accounts for 25–30% of the total weight, 75% of which are storage globulins legumin (11S) and vicilin (7S). The former is two- to threefold more abundant than the latter. Legumin is then the main storage proteins [66]. As in pea, legumin in *V. faba* is a polypeptide with a molecular weight of 60,000–61,000 Da, which is cleaved in vivo into two components: the α-subunit of 36,200 Da (acidic) and a β-subunit of 22,000 Da (basic). The native legumin molecule is composed of

six α-β units connected by disulfide bonds [66]. *V. faba* legumin in vivo is a hexamer of the basic polypeptide with a molecular weight of 328,000 Da [67]. Vicilin in *Vicia faba* has a molecular weight of 150,000 Da according to Derbyshire et al. [67], which is cleaved into subunits of molecular weight comprises between 55,500 and 33,100 Da (46,000, 43,100 and 33,300). In the common bean (*Phaseolus vulgaris*), the main globulin is called phaseolin (7S) and is a trimer formed from three subunits of 46,000; 49,000; and 50,500 Da [68, 69]. *P. vulgaris* also contains a legumin like globulin with a molecular weight of 340,000 Da. Immuno-determinations of the legume proteins show some cross-reactions between species like between peas and broad beans.

4.2 Absolute Nutritional Value

The nutritional value of different legumes can vary significantly as a result of their peculiar composition [70]. As examples, the quantity and variety of dietary fibers and starch, the protein composition, the rate of several anti-nutritional substances, and the phytochemical content of legumes can influence their dietary value. Legumes usually contain bioactive compounds, including enzyme inhibitors, hemagglutinins (lectins), phytoestrogens, oligosaccharides, saponins, and other phenolic compounds [70]. These substances can play metabolic roles in animals and humans who consume these foods frequently. The consumption of phytochemicals can be either beneficial or adverse and globally require additional investigations. They can also act synergistically or antagonistically with other components of the diet, and their mechanisms of action still have to be deciphered for health and diseases understanding. Some components are also known to improve their digestibility with food processing and this will be discussed later on. The absolute nutritional value of a legume takes into account its nutrient profiles as well as their digestibility.

4.2.1 Amino Acid Profiles

Leguminous proteins are globally low in the essential sulfur containing amino acids, methionine, cystine and cysteine, methionine, as well as in tryptophan (see Table 1) and are therefore considered to be an incomplete source of protein [80]. This is true either for humans or for domestic animal nutrition. Therefore, traditional associations are observed all around the world in human civilizations like *dhal* with rice in India, beans with corn tortillas in Mexico, tofu with rice in Asia, sorghum and cowpeas in Africa, Bambara groundnut and maize kernels in Zimbabwe, or rice and beans in Southern Africa and Latin America.

For domestic animals, the association is frequently corn with soybean or wheat and soybean [81]. For nutritional balance, legumes and cereals are to be consumed in the ratio 35:65 [82]. Although, this chapter is essentially focused on proteins from legumes, the traditional edible species also contain fibers, carbohydrates, and fat in different proportions (Table 2).

Table 1 Amino acid composition of several traditional edible legumes

	Lentils	Pea	Chickpea	Fava	Bean	Lupine	Peanut	Cowpea	Bambara	Soybean	MungBean
	L. culinaris	*P. sativum*	*C. arietinum*	*V. faba*	*P. vulgaris*	*L. mutabilis*	*A. hipogea*	*V. unguiculata*	*V. subterranea*	*G. max*	*V. mungo*
% protein	25.13	26.0	24.4–25.4	32.34	20.99	38.2	22.31**	23	18.8	38.1*	27.5
AA in % protein	Seed	Flour	Seed	Seed	Extruded flour	Flour	Nut	Hydrolysate	Nut	Flour	Seed
Asp	10.76	10.4	10.9–11.5	9.45	11.53	8.41	12.03	11.1	14.61	11.89	13.5
Tre	3.12	3.66	2.7–3.0	3.18	4.43	4.32	3.03	3.45	4.43	5.07	3.15
Ser	4.84	4.37	3.3–3.7	4.5	6.24	5.95	5.96	4.60	6.85	5.42	4.95
Glu	14.20	16.6	17.3–17.8	14.79	14.77	26.12	19.32	18.30	20.95	19.65	21.7
Pro	2.40	5.56	3.8–4.1	3.72	5.53	4.27	4.85	3.98	5.36	4.81	4.23
Gly	3.2	4.43	3.4–3.6	3.81	3.95	3.71	6.92	3.78	4.65	4.37	4.26
Ala	3.92	4.53	4.7–5.2	3.63	3.76	2.81	5.20	4.25	5.14	4.27	4.35
Cys	0.88	0.68	0.4–0.6	0.78	0.95	1.26	1.26	0.75	2.4	1.6	0.75
Val	3.98	5.2	4.1–4.6	3.81	4.81	3.52	4.21	5.98	6.24	3.4	5.20
Met	0.82	0.86	4.1–4.6	0.66	1.24	0.32	1.05	1.58	0.64	0.77	1.92
Ile	3.08	3.8	4.5–4.8	3.45	3.81	3.16	3.1	4.58	5.45	3.96	4.74
Leu	6.68	6.36	8.1–8.5	6.54	7.62	7.41	6.22	7.64	10.21	6.76	8.36
Tyr	2.52	3.05	2.6–2.8	2.91	3.57	4.28	4.27	3.16	3.13	3.53	3.27
Phe	4.36	4.54	5.0–5.3	3.75	5.72	3.25	4.09	6.60	7.69	4.33	5.66
Lys	5.72	8,58	6.7–7.0	5.55	7.10	7.59	3.58	7.28	8.02	9.08	4.19
His	2.40	3.4	2.9–3.2	2.31	3.67	3.06	2.17	3.20	3.86	3.81	2.49
Trp	0.80	0.5	0.8–0.9	0.81	1.19	0.31	0.79	ND	0.6	0.5	0.97
Arg	7.52	13.76	8.0–8.5	9.18	6.00	10.86	9.84	7.30	7.48	7.57	6.33
References	[71]	[72]	[73]	[74]	[75]	[72]	[76]	[77]	[78]	[72]	[79]

* Protein given for seed and AA for flour

** Calculated as the mean of 7 different cultivars

Table 2 Average percentages of different constituents in several crops adapted from [82]

Crop	Carbohydrates	Proteins	Fat	Fibers
Barley	83%	11%	2%	4%
Oats	74%	12%	5%	10%
Rye	82%	13%	2%	3%
Millet	75%	15%	5%	10%
Wheat	78%	14%	2%	3%
Soybean	30%	42%	21%	10%
Chickpeas	58%	25%	5%	12%
Lupines	50%	40%	7%	43%
Lentils	60%	33%	–	11%

4.3 Digestibility (AntiNutritional Factors)

All legumes presenting high protein contents together with other valuable nutrients (polyunsaturated fatty acids, minerals, fibers) have a theoretical high nutritional value. However, if such plants get through the geological times until domestication, it is because despite their nutritional value they manage to limit their destruction by potential predators, by limiting their crude digestibility and appetence. Looking closely to their composition, it can be found that all legumes contain anti-nutritional factors that limit their digestibility and therefore their nutritional interest as crude matter for animal predators in the wild. As we will see further, the consumption of these legumes raise significantly when humans were able to apply basic cooking practices. The main anti-nutritional factors being inventoried here are tannins, phytic acids, oligosaccharides, lipoxygenases, hemaglutinins, anti-protease factors.

4.3.1 Tannins

Tannins (Fig. 3) mainly contained in the seed coats [83] are defined as water-soluble polymeric phenolic compounds exhibiting molecular weights from 500 to 3000 that can combine with proteins, cellulose, gelatin, and pectin to form insoluble complexes [84].

They currently protect the grains against insects, birds, and fungal attacks. The tannins content of legumes can be rather variable between species but also inside the same variety (Table 3).

In addition, it was shown that tannins affect the availability of amino acids, the utilization of protein, and they inhibit the activities of digestive enzymes [87]. Therefore, they tend to reduce the nutritional qualities of plants for their predators [88]. In domestic animals fed sorghum rich in tannins, it was shown that there were significant inverse relationships between tannin content and the mean digestibility of all AA [89]. When fava bean hulls tannins were added to casein diet, the apparent fecal digestibility of total and individual amino acids was decreased in rats [90]. The digestibility of proline, glycine, and glutamic acid were the most affected. It was thought to be due to the interactions of tannins with the proline-rich proteins secreted by the parotid gland since increasing amount of tannin-rich fava bean hulls caused a

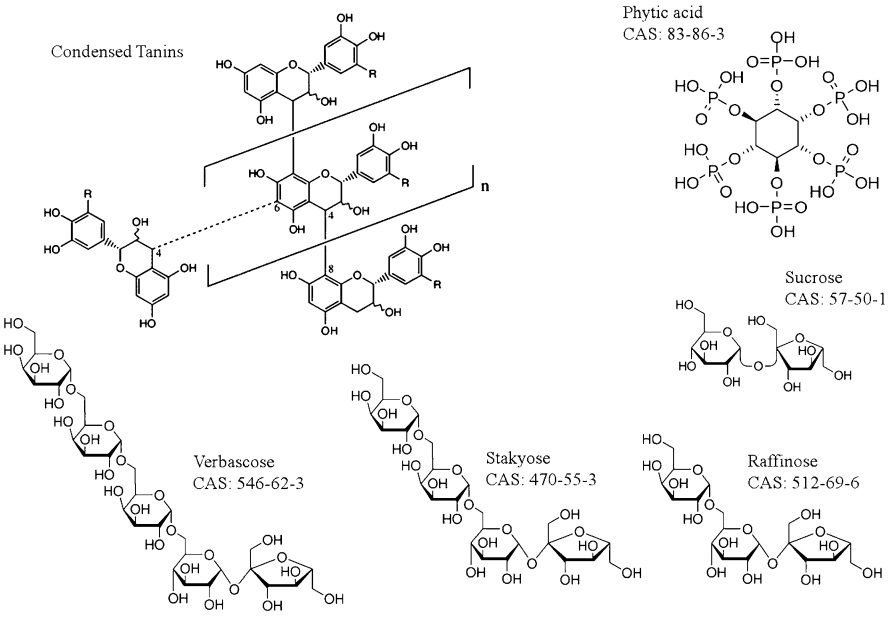


Fig. 3 Chemical structures of some anti-nutritional substances from legumes

Table 3 Tannin content in some legumes

Product	Tannin content (g/kg)	References
Chickpea (*Cicer ariterium*)	0.6–2.7	[85]
Cowpea (*Vigna sinensis*)	1.4–10.2	[85]
Pea (*Pisum sativum*)	0.6–10.5	[85]
Pigeonpea (*Cajanus cajan*)	3.8–17.1	[85]
Dry beans (*Phaseolus vulgaris*)	0.3–12.6	[85]
Kidney beans (*Phaseolus vulgaris*)	5.3–17.55	[86]
Faba bean (*Vicia faba*)	0.5–24.1	[85]
Urd bean (*Vicia mungo*)	8–12	[87]

linear increase in both the relative size of the parotid glands and in the quantity of proline-rich proteins in the rat's gland [90]. In addition, proline-rich proteins are secreted in the saliva and bind dietary tannins in the oral cavity. It was suggested that this phenomenon protects dietary and endogenous protein (digestive enzymes and proteins of the luminal side of the intestinal tract). However, if the salivary secretion is not sufficient, the tannins interactions with digestive enzymes can reduce protein and amino acid digestibility from tannin-containing diets [90, 91].

4.3.2 Phytic Acid

Cereals and legumes contain phytic acid (myoinositol 1,2,3,4,5,6-hexakisdihydrogen phosphate) at levels ranging from 0.4% to 6.4%, w/w. Phytic acid

Table 4 Phytic acid content in several legumes

Legumes	Phytic acid (g/kg)	Phytic acid (g/kg protein)	References
Soyabeans (*Glycine max*)	26	68–76	[93]
Soyabean meal (*Glycine max*)	32–41	62–78	[94]
Common bean (*Phaseolus vulgaris*)	8–11	459–578	[95]
Chick pea (*Cicer arieticum*)	5–12	29–47	[94]
Pigeon pea (*Cajanus cajan*)	7–17	29–72	[94]
Mung bean (*Vigna radiata*)	10–15	45–57	[94]
Urd bean (*Vigna mungo*)	13–15	46–54	[94]
Lentils (*Lens culinaris*)	7	27	[94]

(see Fig. 3) is the most preeminent form of phosphate storage form in most seeds. It accounts for 60–90% of total seed phosphorus [92]. In dicotyledonous seeds, such as legumes, nuts, and oilseeds, phytic acid is found associated with proteins. The amount of phytic acid in legumes can vary greatly (see Table 4).

Phytic acid has been reported to interfere with the action of pepsin on several vegetable and animal proteins *in vitro*. Its anti-proteolytic action probably goes through phytate:protein interaction that forms complexes at low pH [96]. Phytic acid was shown to inhibit trypsin activity in some but not all studies. Phytate was also shown to significantly (up to 25%) inhibit *in vitro* digestion of casein [97]. In addition, fermentation processes of millet, reducing phytate content by 23–26%, improved *in vitro* digestibility by 14–26%. Using microbial phytase, which metabolize phytate, in poultry or swine, improves phosphorous bioavailability and reduces the environmental impact of animal manure. Phytase also increased threonine and valine digestibility [98] and phytate concentration was negatively correlated with the inherent protein and amino acid digestibility of animal feed. From the animal studies, it seems that it is the ileal digestibility that is improved. The effects of phytase supplementation on protein and amino acid digestibility of proteins in several animal species may be explained by the release of protein from the protein-phytate complexes which are natural in feedstuffs. A second effect can be the prevention of formation of protein-phytate complexes directly in the gut. A third effect can be the reduction of the negative effect of phytic acid on pepsin and trypsin activities as well as to the reduction in endogenous amino acid losses [99].

4.3.3 Oligosaccharides

Legumes are classically rich in oligosaccharides of the raffinose family, namely, raffinose, stachyose, and verbascose (see Fig. 3 and Table 5).

According to Devindra *et al.* [106], raffinose family oligosaccharides are galacto-oligosaccharides that can account for more than 50% of the total soluble sugars in some cases. These carbohydrates cannot be hydrolyzed and absorbed, since the human small intestine does not exhibit the appropriate α-galactosidase activity. Therefore, these sugars are digested by the microorganisms present in the large

Table 5 Oligosaccharide content of several legumes

Genus	Species	(sample nb.)	Sucrose	Raffinose	Stachyose	Verbascose	References
Lens	Lentils		12.15	5.63	39.54	11.51	[100]
Pisum	Pea	(n = 3)	30.7 ± 1.9^{cd}	12.3 ± 2.3^{c}	31.2 ± 3.8^{a}	23.9 ± 6.7^{c}	[101]
Cicer	Chickpea	(n = 17)	18.71 ± 0.22	7.35 ± 0.05	15.35 ± 0.07	0.80 ± 0.12	[102]
Vicia	Broad bean	(n = 3)	45.1 ± 1.4^{bd}	6.6 ± 1.8^{bd}	14.2 ± 2.6^{d}	31.8 ± 5.0^{b}	[101]
Phaseolus	Kidney bean	(n = 5)	38.8 ± 5.2^{db}	7.2 ± 3.0^{ad}	48.9 ± 4.5^{e}	2.4 ± 0.5^{a}	[101]
Lupineus	White lupine	(n = 4)	24.10 ± 0.56	11.97 ± 0.41	51.87 ± 2.52	8.77 ± 1.07	[103]
Arachis	Peanuts	(n = 2)	14.22 ± 0.32	0.90 ± 0.015	1.58 ± 0.02	0.42 ± 0.005	[104]
Vigna	Cowpea	(n = 3)	29.3 ± 1.8^{c}	5.0 ± 0.1^{ad}	57.8 ± 1.7^{c}	5.6 ± 1.0^{a}	[101]
Vigna	Bambara	(n = 3)		1.71 ± 0.22	1.19 ± 0.18		[105]
Glycine	Soybean	(n = 6)	59.6 ± 3.1^{a}	7.7 ± 1.6^{a}	30.9 ± 3.9^{a}	1.6 ± 0.4^{a}	[101]
Vigna	Mung bean	(n = 4)	27.8 ± 0.7^{c}	6.1 ± 0.7^{abd}	23.3 ± 1.9^{da}	36.9 ± 1.8^{b}	[101]

Means followed by the same letter within a column are not significantly different at $P < 0.05$.

intestine. This leads to flatus formation [107] because the indigestible raffinose family sugars are fermented anaerobically by the gut flora. This causes intestinal discomfort, nausea, abdominal rumbling, and diarrhea [108]. Therefore, these oligosaccharides which are important for the plant yields can be considered as anti-nutritional agents.

4.3.4 Hemagglutinins

Hemagglutinins are proteins belonging to the lectin family. These molecules are involved in the defense mechanisms of plants via their antifungal activities [109]. They can represent a large fraction of pulse seed proteins especially in beans. These proteins are known to bind carbohydrate moieties which can be present on cell membranes. As such, they can induce cell agglutination. Phytohemagglutinins (PHA-P) are tetrameric structures associating two types of polypeptide chains called PHA-E and PHA-L. These peptides bind preferentially either to erythrocytes or to leukocytes, respectively. Thus, five possible tetrameric isolectins of approximately 120 kDa can be formed, *i.e.,* E_4, E_3L_1, E_2L_2, E_1L_3, and L_4 randomly [110]. A large channel is present in the middle of the tetramer that may protect the protein from proteolytic degradation [111, 112]. The carbohydrate-binding sites of lectins can recognize not only monosaccharides but also oligo- and polysaccharides. Both PHA subunits ("E" and "L") contain an N-glycosylation binding sites. PHA-E is specific for bisected complex-type N-glycans with an outer Gal and bisecting GlcNAc, while PHA-L specifically binds tetra- and triantennary complex-type N-glycans with β6-branching [113, 114]. Close to the carbohydrate binding sites are located the divalent cations Ca^{2+} and Mn^{2+}, which maintain an active stable conformation with a great affinity for sugars [115]. Lectins are resistant to heat and to digestive enzymes, and they also can bind to the surface of enterocytes. This phenomenon can result in toxic reactions because of changes in intestinal permeability [116]. It was shown that PHA can strongly bind to the brush border membrane of the small intestine, and undigested PHA has a dose-dependent effect on hyperblastosis, and tissue growth [117]. High doses of dietary lectins also induce the abnormal development of intestinal microvilli in rats [118] as well as the disruption of enterocytes' plasma membrane repair. This effect usually results in necrotic death of the wounded cells [119]. Blood bioavailability of lectins is poor in normal situation, but it can increase when the intestine barrier is altered.

In their study [120], Putszai and co-workers shown that lectin from *Paseolus vulgaris* were able to decrease casein digestibility in rat. The net protein utilization of crude bean was 11 and that of casein could be reduced in a dose-dependent manner when adding increasing amount of lectin extracted from *Phaseolus vulgaris*. In humans, the oral acute toxicity of *Phaseolus vulgaris* lectins is characterized by nausea, vomiting, bloating, and diarrhea [121]. The legumes' lectins are not always as toxic, and several species express moderate agglutinating effects. Finally, oral administration of certain lectins such as those from *Phaseolus vulgaris* were found to induce immunoglobulin (Ig)E-mediated reactions, and sometimes simultaneously with IgG-mediated reactions [112, 122]. These phenomena take part into the allergic reactions observed in human with several edible pulses. This will be detailed later.

4.3.5 Lipoxygenase

Lipoxygenases are non-heme, iron-containing enzymes widely distributed in plants, fungi, and animals [123]. These enzymes catalyze the dioxygenation of polyunsaturated fatty acids PUFAs into cell signaling agents used as autocrine, paracrine, or endocrine signal molecules. Multiple lipoxygenase genes were identified in plants (at least eight in soybean, *Glycine max*), in animals (at least seven in the mouse), and in humans (five pseudogene characterized) [123]. Their importance is not correlated to their concentration, and cellular effects are now known to be crucial. In plants, lipoxygenases can be found in all organs. In plants, these substances have been shown to have a role in the vegetative growth, wounding, herbivore, and pathogen attack responses and also in mobilization of storage lipids during germination [124]. The role of lipoxygenases in plant defense mechanism was shown to be due to their implications in the synthetic metabolism of signal molecules such as Jasmonic Acid and their methylated parent compounds in sorghum [125], in arabidopsis attacked by aphids [126] or in the pulse *Pisum sativum* [127]. Lipoxygenase (Lox) enzymes play a role in the development of unpleasant flavors in foods containing legumes by oxidation of polyunsaturated fatty acids. This is particularly true for soybean which contains significant levels of these substances [128]. The action of lipoxygenase is thus deleterious to the palatability of legumes rich in fatty acid such as soy or peanut and these enzymes can therefore be considered as anti-nutritional factors.

4.3.6 Saponins

Saponins constitute a class of glycosides found essentially, but not exclusively, in plants. These substances include a steroidal or triterpene aglycone linked to one, two, or three saccharide chains. The carbohydrate chains can vary in size and complexity via ester and/or ether linkages (Fig. 4).

The most common sugars linked to the sapogenin moiety are galactose, arabinose, xylose, and glucose. Saponins express amphiphilic properties thanks to the lipophilic and lipophobic characteristics of the aglycone and carbohydrate moieties, respectively. Saponins occur in numerous edible plants, including legumes (soya, peas, and beans), root crops (potato, yams, asparagus, and alliums), or medicinal herbs (ginger). In grain legumes, saponin contents vary between 0.5% and 5% dry weight, with soybean exhibiting the highest rate (5.6%) [129]. *In vitro*, saponins were shown to inhibit, carrier-mediated galactose transport but not that of L-glucose [130]. Polyethylene glycol 4000, which transfer through the endothelial barrier is known to proceed via an extracellular mechanism, is also increased *in vitro* [130]. This indicates that saponins inhibit active transport and simultaneously increase the general permeability of the enterocyte barrier. This phenomenon is observed *in vitro* at saponins concentrations ranging from 0.3 to 8 mM. Not all saponins exhibit the same potency. Therefore, it appears that some saponins are able to increase the permeability of the small intestinal mucosal cells, facilitating the uptake of substances to which the gut would normally be impermeable. This effect, occurring at rather high concentration, is likely to have *in vivo* consequences when the saponins ingestion is recurrent. These consequences may be deleterious and justify the anti-

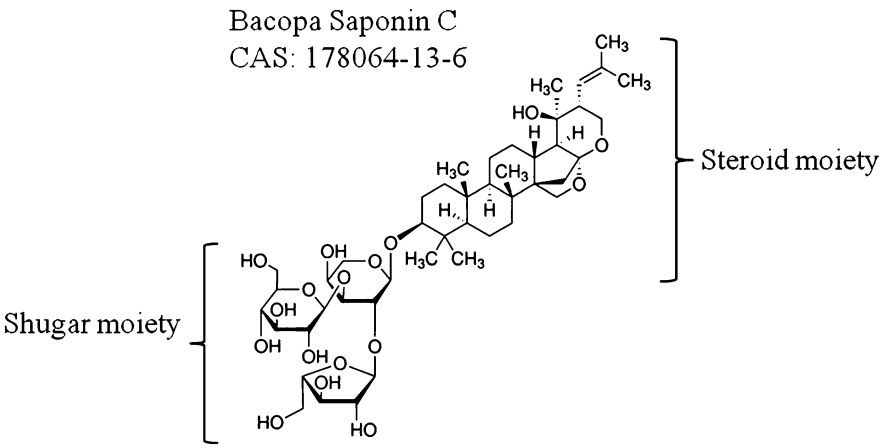


Fig. 4 An example of saponin. Here is Bacopa saponins C with its carbohydrate moiety and its steroidal moiety

nutritional status of saponins. However, saponins also exhibit anti-inflammatory, immunomodulatory and antifungal and antimicrobial activities and are protecting agents for the plants.

4.3.7 Anti-Protease Factors

Proteinase inhibitors are ubiquitously produced since their role is to regulate the proteolytic activity of their target proteinases. They are key factors in living organism interactions, and they appear essential looking at microorganisms and plants interactions, or examining microorganisms and animals interactions. They are also important in the plants and animals interactions. Therefore, many vegetables including legumes, cereals, potatoes, and tomatoes contain protease inhibitors that act on trypsin, chymotrypsin, or carboxypeptidases [92]. Maybe because of its high protein content, soybean is the richest source of dietary trypsin inhibitors and contains the Kunitz inhibitors (see Table 6) and the Bowman-Birk inhibitors [108].

The Kunitz inhibitors have a molecular weight of about 21.5 kDa with two disulfide bridges and act mainly against trypsin. The Bowman-Birk inhibitors have a molecular weight of about 8 kDa formed by 60–90 AA residues and numerous disulfide bonds. They mainly inhibit chymotrypsin and trypsin at independent binding sites. The Bowman-Birk inhibitor (BBI) family is the most widespread group in common bean (*Phaseolus vulgaris*) as well as in other legumes such as soybean (*Glycine max*) and pea (*Pisum sativum*). Variants of these two main types of inhibitors have been characterized with different amino acid sequences, electrophoretic mobility, specificity, and sensitivity to thermal inactivation. The actions of soybean inhibitors were reported to be similar in rat and humans on trypsin and chymotrypsin [129]. According to Lin *et al.* [136], Bowman-Birk inhibitors inhibit trypsin via binding with lysine or arginine at the P1 residue. Soybean, as one of the most concentrated legumes in anti-protease, has been largely studied and therefore the anti-nutritional effects of anti-

Table 6 Values of trypsin inhibitor activities in some legumes

Legumes	Trypsin inhibitor activity (mg/g)	Trypsin inhibitor activity (mg/g protein)	References
Soybean (*Glycine max*)	16.7–27.2	34.7–122.6	[131]
Pea (*Pisum sativum*)	2.7	11.9	[132]
Kidney beans (*Phaseolus vulgaris*)	4.6	13.5–62.3	[86, 133]
Chick pea (*Cicer ariterium*)	1.7–8.5	8–39.5	[134]
Cowpea (*Vigna sinensis*)	1.7	6.4	[135]

protease will be largely illustrated from data obtained on this legume. Many studies reported the deleterious effects of raw soy-protein compared to processed matters on protein digestibility and animal growth [137, 138]. Raw soybean protein preparations can cause pancreas hypertrophy and hyperplasia in susceptible animals since the exposure to soybean trypsin inhibitors results in the increased synthesis and secretion of proteases (such as trypsin, chymotrypsin, and elastase) [139]. Trypsin and chymotrypsin being rich in sulfur-containing amino acids such as methionine and cysteine, the result of this hyper-secretion is a diversion of these essential AA from the synthesis of body tissue proteins. This can result in an alteration of growth in domestic animals since soy protein is also deficient in these amino acids [140]. Direct infusion of the Bowman-Birk inhibitor purified from soybean into the duodenum of men significantly increased the pancreatic secretion of trypsin, chymotrypsin, and elastase. This effect was similar to that observed in rats [108]. Still according to Liener [108], the increased secretion of sulfur-AA-rich proteases would be due to the suppression of the negative feedback regulation of pancreatic secretion via an increased release of cholecystokinin from intestinal mucosa. This release is not only controlled by the protease secretion [141]. Because of their depressive effects on growth and protein digestibility, protease inhibitors from legumes can be considered as anti-nutritional factors. The studies earlier showed that heating and food processing can have beneficial effects on these anti-nutritional substances.

5 Food Processing

As seen above, legumes are at the same time rich in highly nutritive compounds and also in anti-nutritional factors. Their human consumption has been significantly detected during the Pre-neolithic and Neolithic periods when fire was already domesticated. Therefore, these pulses were likely to be cooked before consumption. Indeed, it can be easily demonstrated that the anti-nutritional factors detailed earlier can be partially or totally inactivated by food processing. Traditional heating, boiling, cooking, soaking, simmering, germinating, or fermenting all have been studied and were shown to improve the digestibility of the proteins in legumes. Modern practices including industrial defatting, roasting, extrusion, or

microwave cooking will be analyzed regarding their effect on anti-nutritional factors and bioactive polyphenols.

5.1 Traditional Processing

Legume seeds can be eaten raw as for chickpeas or broad beans when they are still green and at a tender stage (unripe stage) [132]. To improve conservation, legume seeds are traditionally dried to be consumed at distance of the harvest. Most farmers in developing countries naturally dry their mature beans under the sun [142]. Because seed coats contain tannins and other anti-nutritional factors, seeds are often dehulled [142]. Traditional dehulling was performed using a stone grinder and shaking the resulting grains over large flat baskets. More recently threshing, shelling, and grading of legume seeds were mechanically improved using machines [142]. After drying, seeds can be stored for several months before processing. Then two kinds of processes can be separated: the non-fermentation processes and the fermentation processes.

5.1.1 Traditional Non-fermentation Processes

These include sprouting of the fresh seeds and traditional soaking and cooking practices in boiling water. In [143], Khokhar and Chauhan studied domestic methods of processing and cooking the moth bean. Their studies included soaking in plain water, soaking in mineral salt solution, ordinary cooking of soaked seeds, sprouting, and ordinary cooking of sprouts. They described the soaking procedure as follow. After seed cleaning from broken seeds, dust, and other foreign materials, soaking was performed in tap water or mixed salt solution for 12 h at tropical temperature (24 °C). A seed to water ratio of 1:5 (w/v) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice with water and then dried in air. The ordinary cooking procedure was described as follow. Presoaked seeds, after rinsing in water, were put in pans and tap water was added in a 3:1 v:w ratio. Cooking was performed for 60–90 min in water until seeds became soft when felt between fingers. Water was discarded before seeds were dried in air. Khokhar and Chauhan [143] also described the domestic sprouting procedure as follow. The soaked seeds were germinated in clean dishes lined with wet vegetal mesh for 60 h at 25 °C, with frequent watering. Sprouts were then rinsed in water and dried under the sun and cooked till soft like soaked samples mentioned above. For soybean [142], non-fermentation processes include the preparation of soy "milk," soy-cheese (*Tofu*), and soy-sheet (*Yuba*). In all cases, soybeans are cleaned and soaked in water for at least 6 h at room temperature. They can be dehulled before soaking or cooking. For soy-juice, the traditional practice usually includes a precooking step in water lasting 10–30 min. This step usually allows eliminating dust residues with the first water discard. Seeds are then crushed in the water before the final cooking step, lasting additional 10–30 min. Then the water is filtered to collect the juice. The filtered residue (Okara) can be kept for animal feeding. Tofu was traditionally prepared from the juice after cooling. Curdling agents are added to the juice. These can be calcium

chloride, calcium sulfate, magnesium chloride, magnesium sulfate, calcium acetate, or calcium lactate according to Prabhakaran *et al*. [144]. In China, calcium salts mined from mountain quarries have been used for over 2000 years [145]. In Japan, the traditional curdling agent was sea salt that contains small quantity of magnesium chloride ($MgCl_2$). The curd is then drained on a wooden frame with holes in the bottom and coated with fabric. The soybean curd is then pressed using a specific lead to squeeze out the water. This water constitutes the *tofu whey*. According to Maneepun [142], *Yuba* production is a domestic practice only performed at small scale. During thick soy "milk" prolonged cook, a film is formed at the surface. Boiling is prolonged until the sheet of film is thick enough, and then it is removed from the surface of soy "milk" by using a bamboo stick. The wet sheet is placed over a sheet of thick cloth, and air-dried by hanging. *Yuba* is used for wrapping meat and vegetable fillings in Chinese cuisine.

5.1.2 Nutritional Characteristics After Traditional Non-fermentation Processing

According to the study by El Adawi on chickpea [132], boiled chickpea seeds were not significantly different from raw peas in total protein, total amino acid, and total carbohydrate contents. Boiling significantly decreased the non-protein nitrogen, ash, and fat contents due to their diffusion into the cooking water. Crude fiber was significantly increased by boiling and or soaking associated to cooking. Water-soluble vitamins and minerals tend to leak into the water and significantly decrease in chickpeas on boiling [132]. Equally, Mubarak *et al*. [79] tested domestic processes on the anti-nutritional content of mung bean. Among them, they tested traditional cooking steps such as dehulling, soaking, boiling, and germination. They showed that the protein content was only slightly affected by soaking and boiling. All treatments significantly reduced the stachyose and raffinose content of mung bean and germination reduced it to nothing. Meanwhile, starch content was only reduced by germination. When considering the anti-nutritional factors, it appears that trypsin inhibitors and hemagglutinins were destroyed by boiling but only reduced by dehulling, soaking, or germination. Tannins and phytic acids were significantly reduced by all treatments, the most effective being germination. Polyphenols under their glycosylated form also leak into the soaking and cooking water [146] Germination of chickpea seeds resulted in a significant increase in crude protein, non-protein nitrogen, and crude fiber compared to raw seeds, while ash was not significantly affected. Germination also significantly decreased the fat and total carbohydrate contents. To be more precise, raffinose, stachyose, and verbascose were completely eliminated by germination and cooking in water for the latter. The same results were obtained on soybean sprouts [147]. According to El-Adaway [132], trypsin inhibitor activity was significantly decreased (−82%) by boiling. Sprouting was less efficient since it decreased trypsin inhibitor activity by only 34%. Hemagglutinin activity was completely destroyed by cooking and was drastically reduced (77%) by germination. This was also observed by Khalil & Mansour [148] on fava bean seeds. According to El-Adaway [132], tannins (−52%), phytic

acid (−71%), and saponins (−48%) in chickpeas were significantly reduced by simple boiling. Germination was less effective than boiling in reducing tannins and saponins but more effective in reducing phytic acid. This could be due to the phytase activity during germination. Polyphenols in legumes are generally present under their glycosylated forms and as such are soluble in water. Therefore, in all the preparation associating soaking, boiling, or simmering in water, these polyphenol concentrations can be reduced. For isoflavones, the longer the water contact the lower the remaining concentrations [149]. If the soy "milk," *Tofu,* and *Yuba* are considered, the successive soaking, cooking, and simmering in water classically included in their traditional recipes were most likely to reduce their isoflavone concentrations. This is sustained by the study by Liu et al. [150] showing that the vast majority of rural Chinese women were exposed to isoflavone levels lower than 15 mg/day when the actual exposure in modern Asian countries is usually over 20 mg/day and may go up to 120 mg/day. The estrogenic activities of isoflavones were observed at already 45 mg/day in American women [151].

5.1.3 Traditional Fermentation Processes

Legumes were fermented either traditionally or recently using several microbial strains from *Lactobacillus*, *Bacillus*, *Aspergillus*, *Rhyzopus*, *Actinomucor,* and *Saccharomyces* genders [152]. Traditionally fermented soy-food was quite numerous and still include *Natto*, *Miso*, *Tempeh,* fermented *Tofu* (*Sufu*), and soy-sauce (*Si-iu*). *Natto* is produced from soybeans cooked for 2–4 h in renewed water and dried after water discard. Beans are then wrapped in straw and seeded with *Bacillus subtillis natto*. Fermentation is prolonged for 24–48 h. Traditionally in Japan, *Miso* is made from soybean cooked in renewed water for 2 to 4 hours before grinding. Ground soy paste is then mixed with Koji (*Aspergilus oryzae*) developed on rice and/or wheat and other microbial strains together with salt and fermented in anaerobic conditions for 6–8 month. *Miso* tends to resemble the Chinese soy paste (*Tao-cheow*) [142]. *Tao-cheow* is made by incubating soybean paste with *Aspergillus oryzae* or other microbial strain like *Lactobacillus delbrulckii*, *Pediococcus halophilus*, *Saccharomyces* sp. for 3–4 months. After this first stage of *Koji* fermentation, a liquid is formed and separated for production of soy sauce. The resulting paste is packed and pasteurized and can be kept for several years with the best flavor after a year. *Tempeh* is an Indonesian soy-food prepared from soybeans rinsed and precooked 2–3 times in renewed water before being light dried and seeded with *Rhyzopus oligosporus*. Traditionally, precooked seed were packed in banana leaves and the fermentation was performed for 24–48 h at tropical temperature 24–26 °C, with high hygrometry [149]. *Si-iu* production as described in [142] includes two fermentation steps. The first stage is koji production using *Aspergillus orizae* in an aerobic solid-state fermentation for 3–4 months. The second stage is aromi fermentation by mixed cultures of halophilic yeast and lactic acid bacteria. This is an aerobic fermentation in 20–22 percent (w/v) brine solution leading to a bright reddish-brown colored sauce with pleasant aroma and salty taste [142].

5.1.4 Nutritional Characteristics After Traditional Fermentation Processing

Fermentation usually induces phytate hydrolysis because microorganisms possess phytase enzymes, which hydrolyze phytic acid into inositol phosphates [153]. This phenomenon is valuable because myoinositol phosphates with less than five phosphate groups (*i.e.*, IP-1–IP-4) do not inhibit zinc absorption [154], and those with less than three phosphate groups do not affect nonheme iron absorption [155, 156]. Microbial phytases originate either from the microflora present on the surface of legumes or from inoculates used for processing. The phytate reduction can vary and can reach −90% in soya beans, cowpeas, and lima beans. When tannins content are high, the phytase activity can be inhibited, and fermentation is less-effective. Fermentation also improves protein quality and digestibility as well as vitamin B content. Fermentation reduces adverse microbial development and improves food safety. Fermentation also produces small organic acids that improve iron and zinc absorption. This leads to lower pH and increases the activity of endogenous phytase from legume flours [157]. Ibrahim *et al*. [158] also showed in cowpea that fermentation with *Rhizopus oligosporus* dramatically reduced trypsin inhibitor activities. It was the same when fermentation was performed using a lactic acid bacteria *Lactobacillus plantarum* DSM 20205. Note that again in all recipes soya beans were traditionally cooked for long durations (2–4 h) in water and that the water was discarded. Therefore, glycosylated isoflavones were most likely eliminated with the water revealing that the isoflavone exposure probably raised dramatically in recent times in soy consuming countries where industrial processing were developed. Indeed, in industrial Asian countries, the modernization of human soy-food processing occurred 50 years ago, *i.e.*, about two human generations ago.

5.2 Modern Processing

Soy being widely used in industrial countries, either for animal or human feeding, is probably the legume which has been the most intensively processed to adapt to different people tasting and uses. New processes are still extensively developed to fit new demands [145]. As an example, food companies developed soy cakes, soy flakes, soy protein concentrates or isolates, meat substitutes proteins and extenders, and improved the quality of final products. Modern processes include high pressure cooking, autoclave cooking, microwave cooking, and extrusion. Solvent extractions are also modern processing.

5.2.1 Non-Fermented Modern Legume Products

According to Noguchi [145], modern *Tofu* is made from grade soybeans soaked overnight in water. After water discard, boiling water is poured on the beans which are pulverized into a mash. The paste is then ladled into boiling water and allowed to boil gently for about 10 min. Then the mixture is filtered to obtain “soy milk” and the residual material is called *Okara*. In Western countries, the process can be even simpler since after boiling for 1–2 min, softened soya beans are ground in cooking

water before being filtered to collect "soy milk" and *Okara*. In Japan, modern cooking of the beans were developed consisting in quick steaming at high temperature (45 seconds at 180 °C) before crushing and water addition. Subsequent filtration leads to the separation of "soy milk" and *Okara*. A small amount of either calcium sulfate ($CaSO_4$) or magnesium chloride ($MgCl_2$) from gypsum is added to coagulate "soy milk." The curds are then gently removed from the top of the whey and poured into molds lined with cloth. The containers have many draining holes in their bottom to evacuate whey. Other derivatives from "soy milk" or *Tofu* are *Yuba* (soymilk sheets) and *Shimi-tofu*. *Yuba* is made heating thick "soy milk" in pans to evaporate the water. The thin film formed on the surface of "soy milk" is then gently removed and dried. *Shimi-tofu* is prepared from soy protein curd cooled to below 0 °C. During this step, small ice crystals grow, before being thawed to expel excess water. A dried matter is then formed having a sponge-like texture named *Shimi-tofu*.

Beside these modern making of traditional products, new technologies allow producing new types of products based on legumes. The Fig. 5 summarizes the different ways followed to obtain different modern products from soy.

Soy cakes are essentially used for animal feeding. They are obtained from soy oil processing, after the oil extraction by pressing and/or using solvents like hexane. For human feeding, soy meal and oil are the most frequent soy-product, from which most other processed soy-based products derive [142]. New technologies have been introduced in the soybean industry that have and will have significant impact on farming methods, storage, and distribution of legume-based products. The industrial

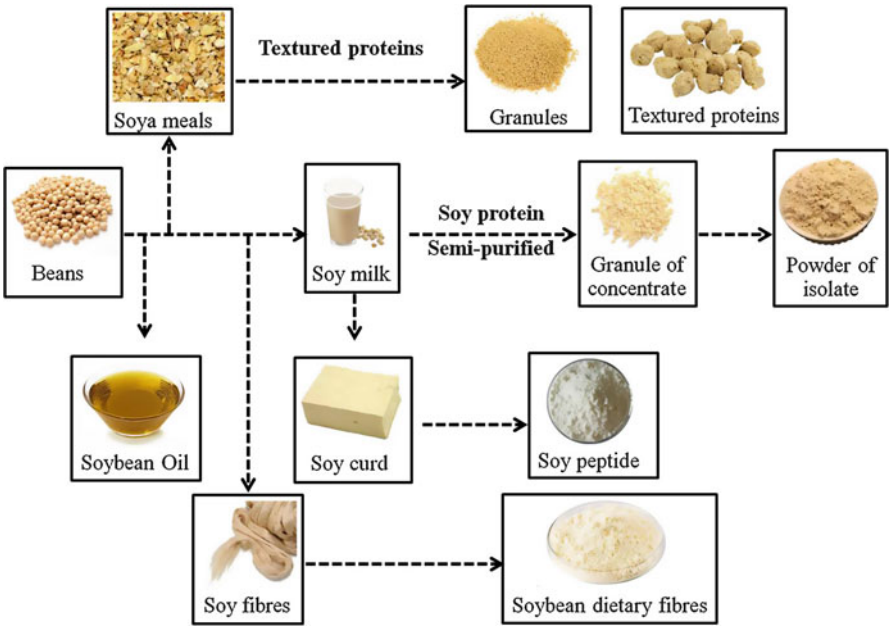


Fig. 5 Different soy-based products and their pathway of production following modern processing

processes always start with cleaning the seeds to remove foreign material. After drying and cracking into pieces, seeds are dehulled. They are heated and rolled into "flakes" before oil extraction. Oil is extracted from soybean flakes using hexane and then degummed and refined for edible and non-edible uses. The protein-rich flakes are toasted, dried, and ground in meal. Soybean meal can be mixed with corn for use in animal feeding. It is also processed into soy flour, soy concentrates, or soy protein isolates. The degumming process of soy oil gives lecithin, which is used in the candy and baking industries. Full fat soy flour from soybean is also used for baking, for soy-based beverages, snack foods, as well as for traditional foods (soy sauce, *Tofu*, and *Miso*). Texturized vegetable protein (TVP) obtained by extrusion of defatted soy flour can be found in different forms (granules, flakes, chunks, or slices). TVP has a long shelf life and can be kept at room temperature for several months. TVP should be re-hydrated with equal quantity of water and can be used as a meat substitute in processed foods (baked goods, meat products, protein drinks, soup bases, and gravies). Microwave and autoclave cooking also are modern processes which are used to prepare canned legumes.

5.2.2 Fermented Modern Legume Products

According to Nogushi [145], modern *Miso* is no more prepared from soya bean boiled for several hours in renewed water but rather from steamed beans. The process still implies long-term fermentation with *Koji* from soya, rice, or wheat and with other microorganisms. Although soy sauce still requires time to be brewed as it is for fine wines and cheeses, modern soy sauce is made from roasted wheat grains crushed and soybeans softened by steaming. A special seed starter is then added to the wheat and soybean mixture and incubated for 3 days. The resulting *Koji* is then combined with brine to form *Moromi*. *Moromi* is then fermented in large tanks until it reaches its full flavor and then pressed into fabrics to extract the raw soy sauce. The latter is then refined and pasteurized before its packaging in bottles.

5.2.3 Nutritional Quality of Legume Under Modern Processing

According to Siulapwa and Mwambungu [159], after oil solvent extraction, soybean seeds moisture is higher than that of row beans and that of extruded full-fat soy seeds. These have lower ash content when compared to raw seeds or to seed extracted with solvent. The extruded product exhibit lower fat and higher protein content than raw seeds and seeds extracted with solvent. Extrusion and solvent extraction reduce crude fibers, Ca^{++}, and phosphorous content. Both processes reduce significantly trypsin inhibitors activities. The processes increase arginine proportion while they decrease methionine. Raw seeds had lower amount of all the nonessential amino acids apart from tyrosine when compared to solvent extracted seeds and extruded seeds. In cowpea (*Vigna unguiculata*), Ibrahim and coworkers [158] showed that long-time soaking (16 h) in bicarbonate solution caused remarkable reduction in the anti-nutritional factors. Pressure cooking was more effective than ordinary cooking to remove phytates. This was confirmed by Khokhar *et al.* [143] on moth bean (*Vigna aconitifolia*) cooked under pressure after soaking in mineral salt solution. Finally, El Adawi [132] showed on chickpea (*Cicer arietinum*)

that microwave cooking reduced non-protein nitrogen, ashes, fibers, and oligosaccharides but had no significant effect on total protein and carbohydrate content including starch. In their study, they also show that this process also significantly reduced trypsin inhibitors and hemagglutinin activities as well as tannins, saponins, and phytic acid. Chickpea autoclaving led to the same reduction of anti-nutritional compounds except for trypsin inhibitors for which it was shown to be more efficient. However, both cooking processes significantly depressed the B vitamins content (Riboflavin, Thiamin, Niacin, and Pyridoxine) sometimes at rates over 50%. Minerals including Na, K, Ca, Mg, P, Mn, Zn, Cu, and Fe tend to leak into the water while boiling. However both microwave and autoclave cooking tend to better preserve their concentrations compared to other practices. These results were remarkably confirmed by Mubarak [79] while working on mung bean seeds (*Phaseolus aureus*). The effect of fermentation was studied in beans (*Phaseolus vulgaris*) [160]. The parameters followed were anti-nutritional factors (α-amylase inhibitor, chymotrypsin inhibitor, cyanogenetic glycosides and lectins) and also fibers (total dietary fiber -TDF-, insoluble -IDF- and soluble -SDF-). Beans were treated by natural and lactic acid fermentation. Autoclaving was added or not after the fermentation process. All treatments decreased the SDF content, while the IDF content were not modified in processed beans. Cellulose content was reduced by treatments and resistant starch increased in processed beans, except after lactic acid fermentation. Fermentation with *Lactobacillus plantarum* increased pectic polysaccharides and Klason lignin. Microorganisms reduced the solubility of dietary fibers. According to Noguchi [145], cooking soybean mash for *Tofu* and "soy milk" in water for at least 10 min before filtering "soy milk" is crucial since anti-nutritional enzymes of the beans are inactivated during boiling. This is confirmed by Chen and co-workers [161] who showed a progressive decrease of trypsin inhibitor activities (Kunitz and Bowman Birk factors) in "soy milk" with or without added salt. In their experiments, 10 min cooking approximately decreased TI activities between 40% and 30% of their initial value. One hour cooking reduces it between 20% and 15% of the initial value. However, not all modern processing of "soy milk" and *Tofu* especially those developed in the West include such step. Consequently, it has been shown in 1997 in Japan [162] that soybean products retain 2.5–12.5% of the initial trypsin inhibitor activity of the whole soybean and that "soy milk" is the food which is the most concentrated. Thus humans are consuming some active trypsin inhibitor in their daily lives. In [163], the phenolic composition of mung beans (*Vigna radiata*) and yellow soybeans (*Glycine max*) were followed under soaking and fermentation using *Lactobacillus plantarum* CECT 748 T. It was shown that soaking induced leaking of conjugated isoflavone for soybeans and increased apigenin derivatives in mung beans. On the other hand, fermentation converted glycosylated isoflavones of soybean into bioactive aglycones and increased the bioactive vitexin in green beans. These data are, in accordance with those from Fernandez-Lopez *et al.* [149], showing that glycosylated isoflavones tend to be progressively removed from soybean matrices during prolonged cooking and simmering in water. These steps were included in soybean traditional recipes. However, nowadays, modern processes tend to replace boiling in water by steaming and

cooking times and contacts of seeds with water were dramatically reduced in modern practices. In addition, extruded soy still contain high amount of isoflavones although they tend to be deglycosylated during the extrusion process [164]. This means that isoflavone exposure of soy-consumers was considerably lower in ancient times than it is nowadays when people consume modern processed soy-products. Because of their estrogenic effects, isoflavones tend to reduce the fertility of the soy consumers (animals and most probably humans) and can therefore be considered as anti-nutritional factors. In addition, the modern environment contains many other endocrine disruptors of anthropoid origin which can act synergistically with isoflavones on reproduction or estrogen-dependent tumor growth [165]. Isoflavone can also exert beneficial effects on several health end-points (bone preservation, prostate cancer protection, breast cancer occurrence, hot flashes relief...) and should be kept for specific health application for specific consumers [149, 166].

6 Allergy

Food allergies are adverse reactions to a harmless food that occur when the immune system reacts to proteins normally present in food without any incidence and that are recognized as foreign substances in some individuals. Then, the immune system triggers a response to neutralize them. Allergic responses vary from person to person and a protein may be allergenic in one individual but not in others. Nowadays, according to Verma *et al*. [167], eight foods or food groups account for over 90% of food allergies (peanuts, soybeans, cow's milk, hen's egg, fish, crustacean, wheat, and tree nuts). According to Maria John *et al*. [168], soybean is listed among the eight first allergenic diets for humans and animals due to the presence of many kinds of allergens whose action can be amplified by the presence of other anti-nutritional factors. Overall, allergenic events recorded after consumption of legumes in decreasing order of frequency may be peanut (*Arachis hypogaea*), soybean (*Glycine max*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), pea (*Pisum sativum*), mung bean (*Vigna radiata*), pigeon pea (*Cajanus cajan*), and lupine (*Lupineus alba*). This takes into account both the allergenic power and the overall consumption at world scale. In Spain in 2015, [169] reported that among 455 adults, the prevalence of legumes allergy was 6.9% (77% women). Legumes involved were: lentil 27% of episodes; bean, 19%; peanut, 16%; soybean, 14%; chickpea, 13%; pea, 8%; and mungo bean, 3%. Anaphylaxis due to food allergies has a prevalence of about 6–8% in children and 4% in adults [167].

6.1 The Proteins of Food Allergies

Food reactions can be classified into three groups: the non-immunologic reactions and the immunologic reactions that can be subdivided again according to the type of reaction involved. The non-immunologic reactions include food intolerance or gluten sensitivity whereas immunologic reactions can either induce immunoglobulin

E (IgE)-mediated symptoms or non-IgE-mediated gastrointestinal symptoms. These phenomena mostly occur early in life, and food allergy frequency usually decreases with age. Legumes usually provoke IgE-mediated reactions. These reactions occur because foreign antigens have been able to stimulate the antigen-presenting cells at the gut level. This means that proteins were able to resist to food processing and then to gastric degradation by local acid and protease. The antigen presenting cells then process the antigens and present them to the Major Histocompatibility Complex (MHC). This activates naive CD_4^+ lymphocytes of the Th_2-type.

These immune cells secrete IL-4 and IL-13, which promote specific IgE secretion by plasma cells. This leads to the IgE antibodies to bind to mast cells. Re-exposure to the same food antigens causes the antigen-IgE antibodies complex to bind to mast cells. This directly provokes mast cells degranulation and release of histamine. Histamine and other mediators (prostaglandin D2, and cysteinyl leucotriene) can provoke vasodilatation, smooth muscle contraction, and mucus secretion that all lead to development of allergenic symptoms [170]. As mentioned earlier, allergenic proteins share common physicochemical and immunological properties. Class 1 food allergens such as legume allergens are stable in gastric fluid; their molecular mass range between 10 and 70 kDa; they are water soluble glycoproteins and can lead to sensitization via gastrointestinal tract [171]. Class 2 food allergens are sensitive to heat and to gastric acid and enzymes, thus sensitization does not occur via oral route. By means of IgE cross reactivity, they can cause allergenic response in people sensitized via inhalant allergens like pollen [172]. The dose of exposure does not seem to be related to either the sensitization process or to the allergenic susceptibility. Less than one microgram levels can induce an allergenic reaction [167] even if some authors report that the minimal level of exposure is over 500 μg of protein but not below this [173]. Glycosylation contributes in the allergenicity of a protein and it seems that the glycan moiety can also interact with IgE antibodies. The structural features of the proteins which are responsible for their allergenic properties are still difficult to predict. In the case of legumes, during digestion, allergens lose conformational form and exist as linear epitopes and sensitized individuals via gastrointestinal tract [67]. To summarize, the legume allergens resist to gastric fluid and proteases, they are heat stable and are glycosylated proteins. They present epitopes that bind to IgE. These can be linear epitopes remaining after food processing and gastric attack, they induce biologically active reactions.

6.2 Main Allergens from Legumes

Several classes of proteins have been involved into legume allergy. These are storage proteins which are considered to be stable to heat and to gut enzymes. Associated to anti-nutritional compounds such as saponins or hemagglutinins, they can cross the gut barrier and can induce immune response. Storage proteins presented earlier in this review are primarily localized in the seed, nut, or kernel. They are classified in Cupins, Prolamins, Pathogenesis Related proteins (PR-proteins), and Profilins.

6.2.1 Cupins Superfamily

The cupins superfamily share two conserved consensus sequence and a β-barrel core domain. Cupins include allergenic seed storage proteins of the vicilin and legumin family. These storage proteins are present in several legumes known for their allergenic properties, *i.e*., soybeans (Gly m5, Gly m6), peanuts (Ara h1, Ara h3), lupine (Lup a), pea (Pis s1), and broad bean (Vign r2, Vign r3).

6.2.2 Prolamin Superfamily

The prolamin superfamily is widely distributed among plants and not only in legumes. Prolamins include cereal seed storage proteins, several important types of allergens from legumes, tree nuts, cereals, fruits, and vegetables. This family includes chickpea allergens (Cic a2S Albumin), which is a 2S protein. It also includes the nonspecific lipid transfer proteins, the cereal alpha-amylase, and some protease inhibitors.

6.2.3 Pathogenesis Related Proteins (PR-Proteins)

PR-proteins stand for more than 10 different types of protein which increase in plants in response to environmental stresses or pathogens. PR-proteins are generally small in size, stable in acidic conditions, and resistant to proteolytic degradation. In legumes, some allergenic proteins were shown to belong to this category such as Vig r1 from mung bean, Ara h8 from peanut, and Gly m4 from soybean.

6.2.4 Profilins

Profilins are small proteins from 12 to 15 KDa. They are found in the cytoplasm of eukaryote cells and therefore they exhibit highly conserved sequences. Plant profilins are involved in a large proportion of cross-reactions between allergenic sources and especially in cross-reactions with pollens. As Class 2 allergens, they are considered to affect 10%–35% people in Europe. Some identified profilin-related legume allergens are Ara h5 from peanut and Gly m3 from soybean.

6.3 The Identified Allergens

In [167], Verma et al. gave a comprehensive table with the allergens from legumes which were known in 2012. In addition, main legumes are classified according to their decreasing importance of allergenic effects (frequency and severity) together with the number of known allergens in Fig. 6. In 2016, Bouakkadia et al. [174] completed the list that was increased to 15 allergens in peanuts, to 14 substances in soybean, and to 4 different compounds in lentils.

6.4 Effect of Food Processes on Allergen Proteins

Verma *et al*. in [175] published a comprehensive review and table showing the impact of different food processes on the inhibition of legume proteins playing as

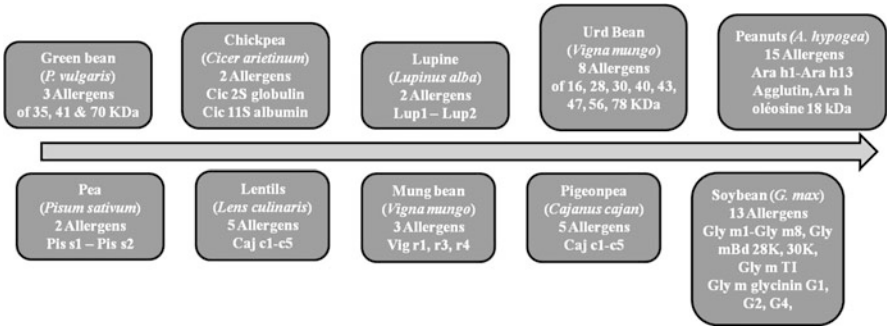


Fig. 6 Classification of the main legumes according to their increasing allergenic properties. The known allergens are mentioned for each pulse

food allergens. They classified the processes into four major categories: roasting, boiling, autoclaving, microwave heating. They reviewed the main allergens from peanuts (*Arachis hypogea*), soybean (*Glycine max*), lentils (*Lens culinaris*), lupine (*Lupinus alba*), pea (*Pisum sativum*), French bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*), and mung bean (*Vigna mungo*). The effect of cooking has to be tested since it is not easy to predict. In some cases, cooking practices lead to elimination of the epitopes and to a reduction of the IgE reactivity. In that case, food processing affects the structural and allergenic properties of allergens by altering their stability or other physicochemical properties. On the opposite, in other cases, there is an increase of immunoreactivity of the antigens which leads to a higher IgE reactivity, a greater release of histamine and other mediators to finally increase the allergenic response. This can be explained since three-dimensional structures of proteins are generally correlated with their activity. Different temperature treatment can induce variable changes in protein structure. As an example, when heated at 70–80 °C, proteins lose their secondary structure whereas at 80–90 °C, new bonds and rearrangements of disulfide bonds can occur. At higher temperature (90–100 °C), protein aggregates can be formed [176]. Over 100 °C, new bonds between lysine residues and other substances may be created leading to the formation of adducts [177]. Protein digestibility and absorption usually increases with heat treatments. However, in some cases, thermal processing may also lead to formation of neoantigens that were not originally present and that can enhance the allergenic response. These neoantigens can result from the Maillard reaction, of protein with sugar residues upon heating. From the review of Verma *et al*. [175], it appears that boiling and autoclaving are overall good ways to reduce or to eliminate allergens although these procedures were not always efficient especially during short treatments on lentils, lupine, or French beans allergens. It should be noted that the traditional recipes which included prolonged cooking or simmering in water were empirically designed to get rid of anti-nutritional factors but can also be considered as efficient on allergens. Finally it can be summarized the following elements: Microwave heating is efficient in reducing soybean allergens but not lupine allergens. Autoclaving was reported to decrease the allergenicity of lentils, pea, chickpea,

lupine, and peanuts. Boiling is efficient in reducing the immune reactions of peanuts, mung bean, chickpea, and soybean antigens but the duration of the treatment is crucial and generally as to be prolonged for better efficiency. Roasting decreases mung bean allergenicity but increases that of peanut. As a consequence, food processing should be taken into account while comparing the prevalence of allergic reactions to various legumes over different world regions.

7 Conclusion

Proteins from legumes are undoubtedly of remarkable nutritional interest although they are better combined with cereal proteins to cover the animal food requirements. These combinations are known to exist since the domestication of crops anywhere human civilizations developed. Therefore, considering the actual expansion of the human population, pulses can be considered as a solution for future sustainable nutrition. Their ability to fix and enrich the soils in nitrogen as well as their capacity to produce protective and signal molecules to prevent or face pest attacks give them advantages when phytochemical treatments are due to be reduced. However, if these invaluable plants reached our modern times, it is because they also manage to protect themselves from their natural predators. Therefore, co-evolution of plants with microorganisms, insects, and herbivores along the geological times conducted to the intrinsic production of many different defensive substances some of which can be considered as anti-nutritional factors. These factors being essential for the plant growth and survival, genetic improvement should respect them for better field production rates. Therefore, the removal of these factors, which were developed by the plants to be detrimental for their consumers, can only be achieved at the transformation level. Future food processing methods will have to be imagined to reduce these anti-nutritional factors as economically as possible for both environment and market. Lessons should be taken from the ancient times and from the traditional practices and recipes used as early as the domestication of legumes started. Crossing different data obtained from modern practices or from those developed in the past, it appears that the traditional food processes, being essentially wet, managed empirically or not to eliminate most of the deleterious compounds including phytoestrogens, anti-nutritional substances, and allergens.

References

1. Caracuta V, Barzilai O, Khalaily H, Milevski I, Paz Y, Vardi J, Regev L, Boaretto E (2015) The onset of faba bean farming in the Southern Levant. Sci Rep 5:14370–14379
2. Abbo S (2011) Experimental growing of wild pea in Israel and its bearing on Near Eastern plant domestication. Ann Bot 107:1399–1404
3. Ladizinsky G (1993) Lentil domestication: on the quality of evidence and arguments. Econ Bot 47:60–64 (17, 18, 20)

4. Kerem Z, Lev-Yadun S, Gopher A, Weinberg P, Abbo S (2007) Chickpea domestication in the Neolithic Levant through the nutritional perspective. J Archaeol Sci 34:1289–1293
5. Werker E, Marbach I, Mayer AM (1979) Relation between the anatomy of the testa, water permeability and the presence of phenolics in the genus *Pisum*. Ann Bot 43:765–771
6. Butler A (1989) In: Harris DR, Hillman GC (eds) Foraging and farming. Unwin and Hayman, London, pp 390–407
7. Hillman GC, Wales S, McClaren F, Evans J, Butler A (1993) Identifying problematic remains of ancient plant foods: a comparison of the role of chemical, histological and morphological criteria. World Archaeol 25:94–121
8. Zohary D, Hopf M (1973) Domestication of pulses in the old world. Legumes were companions of wheat and barley when agriculture began in the Near East. Science 182:887–894
9. van Zeist W, Bottema S (1966) Palaeobotanical investigation at Ramad. Ann Archeol Arabes Syr 16:179–180
10. Helbaek H (1964) First impressions of the Çatal Hüyük plant husbandry. Anatol Stud 14:121–123
11. Helbaek H (1969) Plant collecting, dry-farming and irrigation agriculture in prehistoric Deh Luran. In: Hole F, ICV F, Neely JA (eds) Prehistory and human ecology of the Deh Luran Plain. Memoirs of the museum of anthropology, no 1. University of Michigan, Ann Arbor, pp 383–426
12. Helbaek H (1970) In: Mellaart J (ed) Excavations at Hacilar. Edinburgh University Press, Edinburgh, p 189
13. Garrard A (1999) Charting the emergence of cereal and pulse domestication in Southwest Asia. Environ Archaeol 4:67–86
14. Abbo S, Shtienberg D, Lichtenzveig J, Lev-Yadun S, Gopher A (2003) The chickpea, summer cropping, and a new model for pulse domestication in the ancient near east. Q Rev Biol 78(4):37–50
15. Cubero JI (1974) On the evolution of *Vicia faba* L. Theor Appl Genet 45:47–51
16. Gaut BS (2014) The complex domestication history of the common bean. Nat Genet 46(7):663–664
17. Bitocchi E, Bellucci E, Giardini A, Rau D, Rodriguez M, Biagetti E, Santilocchi R, Spagnoletti Zeuli P, Gioia T, Logozzo G, Attene G, Nanni L, Papa R (2013) Molecular analysis of the parallel domestication of the common bean (*Phaseolus vulgaris*) in Mesoamerica and the Andes. New Phytol 197:300–313
18. Beebe S, Skroch PW, Tohme J, Duque MC, Pedraza F, Nienhuis J (2000) Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. Crop Sci 40:264–273
19. Singh SP, Gepts P, Debouck DG (1991) Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ Bot 45:379–396
20. Gepts P, Kmiecik K, Pereira P, Bliss FA (1988) Dissemination pathways of common bean (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. I. The Americas. Econ Bot 42:73–85
21. Papa R, Gepts P (2003) Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. Theor Appl Genet 106:239–250
22. Atchison GW, Nevado B, Eastwood RJ, Contreras-Ortiz N, Reynel C, Madriñán S, Filatov DA, Hughes CE (2016) Lost crops of the Incas: origins of domestication of the Andean pulse crop 'tarwi' *Lupineus mutabilis*. Am J Bot 103(9):1592–1606
23. Bonavia D (1982) Preceramico Peruano. Los Gavilanes. Mar, Desierto y Oasis en La Historia del Hombre. Corporacio'n Financiera de Desarrollo S.A. COFIDE and Instituto Arqueologico Aleman, Lima
24. Krapovickas A, Gregory WC (1994) Taxonomıa del genero Arachis (Leguminosae). Bonplandia 8:1–186

25. Grabiele M, Chalup A, German Robledo G, Seijo G (2015) Genetic and geographic origin of domesticated peanut as evidenced by 5S rDNA and chloroplast DNA sequences. Plant Syst Evol 298:1151–1165
26. Smartt J (1990) Grain legumes: evolution and genetic resources. Cambridge University Press, Cambridge, pp 140–175
27. Kongjaimun A, Kaga A, Tomooka N, Somta P, Vaughan DA, Srinives P (2012) The genetics of domestication of yardlong bean, *Vigna unguiculata* (L.) Walp. ssp. *unguiculata* cv.-gr. *sesquipedalis*. Ann Bot 109:1185–1200
28. D'Andrea AC, Kahlheber S, Logan X, Watson DJ (2007) Early domesticated cowpea (*Vigna unguiculata*) from Central Ghana. Antiquity 81:686–698
29. D'Andrea AC, Logan AL, Watson DJ (2006) Oil palm and prehistoric subsistence in tropical West Africa. J Afr Archaeol 4(2):195–222
30. Coulibaly S, Pasquet RS, Papa R, Gepts P (2002) AFLP analysis of the phenetic organization and genetic diversity of *Vigna unguiculata* L. Walp. Reveals extensive gene flow between wild and domesticated types. Theor Appl Genet 104(2–3):358–366
31. Lambot C (2002) Industrial potential of cowpea. In: Fatokun CA, Tarawali SA, Singh PM, Kormawa PM, Tarmo M (eds) Challenges and opportunities for enhancing sustainable cowpea production. International Institute of Tropical Agriculture, Ibadan, pp 367–375
32. Basu S, Mayes S, Davey M, Roberts JA, Azam-Ali SN, Mithen R, Pasquet RS (2007) Inheritance of 'domestication' traits in bambara groundnut (*Vigna subterranea* (L.) Verdc.). Euphytica 157:59–68
33. Philippson G, Serge Bahuchet S (1994) Cultivated crops and bantu migrations in central and eastern Africa: a linguistic approach. Archaeol Res Afr 29–30(1):103–120
34. Frahm-Leliveld JA (1953) Some chromosome numbers in tropical leguminous plants. Euphytica 2:46–48
35. Deng Z, Qin L, Gao Y, Weisskopf AR, Zhang C, Fuller DCQ (2015) From early domesticated rice of the middle Yangtze Basin to millet, rice and wheat agriculture: archaeobotanical macro-remains from Baligang, Nanyang Basin, Central China (6700–500 BC). PLoS One 10(10): e0139885
36. Hymowitz T (1970) On the domestication of the soybean. Econ Bot 24:408–421
37. Fehr WR (1980) Soybean. In: Ferh W, Hadley HH (eds) Hybridization of crop plants. American Society of Agronomy, Madison, pp 589–599
38. Canadian Food Inspection Agency (1996) The biology of *Glycine max* (L.) Merr. (Soybean) Biology Document BIO1996–10; 11p
39. Willis H (1989) Growing great soybeans. Acres USA 1, 6–8
40. Shurtleff W, Huang HT, Aoyagi A (2014) History of soybeans and soyfoods in China and Taiwan, and in chinese cookbooks, restaurants, and Chinese work with soyfoods Outside china Including Manchuria, Hong Kong and Tibet (1024 BCE to 2014). Soyinfo Center, Lafayette 3015p
41. Sprent JI (2008) 60 Ma of legume nodulation. What's new? What's changing? J Exp Bot 59:1081–1084
42. Doyle JJ (2011) Phylogenetic perspectives on the origins of nodulation. Mol Plant-Microbe Interact 24:1289–1295
43. Sprent JI (2007) Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. New Phytol 174:11–25
44. Ivanov S, Fedorova EE, Limpens E, De Mita S, Genre A, Bonfante P, Bisseling T (2012) Rhizobium-legume symbiosis shares exocytotic pathway required for arbuscule formation. PNAS USA 109:8316–8321
45. Mierziak J, Kostyn K, Kulma A (2014) Flavonoids as important molecules of plant interactions with the environment. Molecules 19:16240–16265
46. Pueppke SG (1996) The genetic and biochemical basis for nodulation of legumes by rhizobia. Crit Rev Biotechnol 16:1–51

47. Desbrosses GJ, Stougaard J (2011) Root nodulation: a paradigm for how plant-microbe symbiosis influences host developmental pathways. Cell Host Microbe 10(4):348–358
48. Peters NK, Frost JW, Long SR (1986) A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. Science 233(4767):977–980
49. Hartwig UA, Maxwell CA, Joseph CM, Phillips DA (1990) Chrysoeriol and luteolin released from alfalfa seeds induce nod genes in *Rhizobium meliloti*. Plant Physiol 92:116–122
50. Kuzma MM, Hunt S, Layzell DB (1993) Role of oxygen in the limitation and inhibition of nitrogenase activity and respiration rate in individual soybean nodules. Plant Physiol 101:161–169
51. Ott T, van Dongen JT, Gunther C, Krusell L, Desbrosses G et al (2005) Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development. Curr Biol 15:531–535
52. Udvardi M, Poole PS (2013) Transport and metabolism in legume-rhizobia symbioses. Annu Rev Plant Biol 64:781–805
53. Vance CP, Gantt JS (1992) Control of nitrogen and carbon metabolism in root-nodules. Physiol Plant 85:266–274
54. Pate JS, Atkins CA, White ST, Rainbird RM, Woo KC (1980) Nitrogen nutrition and xylem transport of nitrogen in ureide producing grain legumes. Plant Physiol 65:961–965
55. Heath KD, McGhee KE (2012) Coevolutionary constraints? The environment alters tripartite interaction traits in a legume. PLoS One 7(7):e41567
56. Martínez-Romero E (2009) Coevolution in rhizobium-legume symbiosis? DNA Cell Biol 28 (8):361–370
57. Andam CP, Parker MA (2008) Origins of *Bradyrhizobium* nodule symbionts from two legume trees in the Philippines. J Biogeogr 35:1030–1039
58. Maillet F, Poinsot V, Andre O, Puech-Pages V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469:58–64
59. Smit P, Limpens E, Geurts R, Fedorova E, Dolgikh E, Gough C, Bisseling T (2007) *Medicago* L YK3, an entry receptor in rhizobial nodulation factor signaling1[W]. Plant Physiol 145:183–191
60. Pietraszewska-Bogiel A, Lefebvre B, Koini MA, Klaus-Heisen D, Takken FLW, Geurts R, Cullimore JV, Gadell TWJ (2013) Interaction of *Medicago truncatula* lysin motif receptor-like kinases, NFP and LYK3, produced in *Nicotiana benthamiana* induces defence-like responses. PLoS One 8:e65055
61. Rubio LA, Pérez A, Ruiz R, Guzmán MÁ, Aranda-Olmedo I, Clemente A (2014) Characterization of pea (*Pisum sativum*) seed protein fractions. J Sci Food Agric 94(2):280–287
62. Yaklich RW (2001) β-Conglycinin and glycinin in high-protein soybean seeds. J Agric Food Chem 49:729–735
63. Cabello-Hurtado F, Keller J, Ley J, Sanchez-Lucas R, Jorrín-Novo JV, Aïnouche A (2016) Proteomics for exploiting diversity of lupine seed storage proteins and their use as nutraceuticals for health and welfare. J Proteome 143:57–68
64. Duranti M, Restani P, Poniatowska M, Cerletti P (1981) The seed globulins of *Lupineus albus*. Phytochemistry 20:2071–2075
65. Barać M, Cabrilo S, Pešić M, Stanojević S, Pavlićević M, Maćej O, Ristić N (2011) Functional properties of pea (*Pisum sativum*, L.) protein isolates modified with chymosin. Int J Mol Sci 12(12):8372–8387
66. De Pace C, Delre V, Scarascia Mugnozza GT, Maggini F, Cremonini R, Frediani M, Cionini PG (1991) Legumin of *Vicia faba* major: accumulation in developing cotyledons, purification, mRNA characterization and chromosomal location of coding genes. Theor Appl Genet 83(1):17–23
67. Derbyshire E, Wright DJ, Boulter D (1976) Legumin and vicilin, storage proteins of legume seeds. Phytochemistry I5:3–24

68. Pusztai A, Stewart JC (1980) Molecular size, subunit structure and microhet§of glycoprotein II from the seeds of kidney bean (*Phaseolus vulgaris* L.). Biochim Biophys Acta 623(2):418–428
69. Carbonaro M, Grant G, Cappelloni M, Pusztai A (2000) Perspectives into factors limiting in vivoèjn4 digestion of legume proteins: antinutritional compounds or storage proteins? J Agric Food Chem 48(3):742–749
70. Bouchenak M, Lamri-Senhadji M (2013) Nutritional quality of legumes, and their role in cardiometabolic risk prevention: a review. J Med Food 16(3):185–198
71. Nosworthy MG, Medina G, Franczyk AJ, Neufeld J, Appah P, Utioh A, Frohlich P, House JD (2018) Effect of processing on the in vitro and in vivo protein quality of red and green lentils (*Lens culinaris*). Food Chem 240:588–593
72. Tömösközi S, Lásztity R, Haraszi R, Baticz O (2001) Isolation and study of the functional properties of pea proteins. Nahrung 45(6):399–401
73. Rachwa-Rosiak D, Nebesny E, Budryn G (2015) Chickpeas – composition, nutritional value, health benefits, application to bread and snacks: a review. Crit Rev Food Sci Nutr 55(8):1137–1145
74. Lizarazo CI, Lampi AM, Liu J, Sontag-Strohm T, Piironen V, Stoddard FL (2017) Nutritive quality and protein production from grain legumes in a boreal climate. J Sci Food Agric 97(6):2053–2064
75. Nosworthy MG, Franczyk A, Zimoch-Korzycka A, Appah P, Utioh A, Neufeld J, House JD (2017) Impact of processing on the protein quality of pinto bean (*Phaseolus vulgaris*) and buckwheat (*Fagopyrum esculentum* Moench) flours and blends, as determined by *in vitro* and *in vivo* methodologies. J Agric Food Chem 65(19):3919–3925
76. Prathiba KM, Reddy MU (1994) Nutrient composition of groundnut cultures (*Arachis hypogaea* L.) in relation to their kernel size. Plant Foods Hum Nutr 45(4):365–369
77. Hussain MA, Basahy AY (1998) Nutrient composition and amino acid pattern of cowpea (*Vigna unguiculata* (L.) Walp, Fabaceae) grown in the Gizan area of Saudi Arabia. Int J Food Sci Nutr 49(2):117–124
78. Yao DN, Kouassi KN, Erba D, Scazzina F, Pellegrini N, Casiraghi MC (2015) Nutritive evaluation of the Bambara groundnut Ci12 landrace [*Vigna subterranea* (L.) Verdc. (Fabaceae)] produced in Côte d'Ivoire. Int J Mol Sci 16(9):21428–21441
79. Mubarak AE (2005) Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Food Chem 89:489–495
80. Kouris-Blazos A, Belski R (2016) Health benefits of legumes and pulses with a focus on Australian sweet lupines. Asia Pac J Clin Nutr 21(1):1–17
81. Staniak M, Księżak J, Bojarszczuk J (2014) Chapter 6: Mixtures of legumes with cereals as a source of feed for animals. In: Organic agriculture towards sustainability. Tech Open Publisher, pp 123–145. https://doi.org/10.5772/58358
82. Maphosa Y, Jideani VA (2017) Chapter 6: The role of legumes in human nutrition. In: Functional food – improve health through adequate food. Intechopen Publisher, pp 103–121. https://doi.org/10.5772/intechopen.69127
83. Gupta YP (1987) Anti-nutritional and toxic factors in food legumes: a review. Plant Foods Hum Nutr 37(3):201–228
84. Bate-Smith EC, Swain T (1962) Flavonoid compounds. In: Mason HS, Florkin AM (eds) Comparative biochemistry. Academic, New York, pp 755–809
85. Jansman AJM, Longstaff M (1993) Nutritional effects of tannins and vicine/covicine in legume seeds. In: van der Poel AFB, Huisman J, Saini HS (eds) Proceedings of the second international workshop on "Antinutritional factors (ANFS) in legume seeds". Pers Wageningen, Wageningen, pp 301–316
86. Shimelis EA, Rakshit SK (2007) Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. Food Chem 103:161–172

87. Zia-ur-Rehman, Shah WH (2001) Tannin contents and protein digestibility of black grams (*Vigna mungo*) after soaking and cooking. Plant Food Hum Nutr 56:265–273
88. Duodu KG, Taylor JRN, Belton PS, Hamakerc BR (2003) Factors affecting sorghum protein digestibility. J Cereal Sci 38:117–131
89. Elkin RG, Freed MB, Hamaker BR, Zhang Y, Parsons CM (1996) Condensed tannins are only partially responsible for variations in nutrient digestibilities of sorghum grain cultivars. J Agric Food Chem 44:848–853
90. Jansman AFJ, Frohlich AA, Marquardt RR (1994) Production of proline-rich proteins by the parotid glands of rats is enhanced by feeding diets containing tannins from faba beans (*Vicia faba* L.). J Nutr 124:249–258
91. Mehansho H, Asquith TN, Butler LG, Rogler JC, Carlson DM (1992) Tannin mediated induction of proline-rich protein synthesis. J Agric Food Chem 40:93–97
92. Gilani GS, Xiao CW, Cockell KA (2012) Impact of Antinutritional Factors in Food Proteins on the Digestibility of Protein and the Bioavailability of Amino Acids and on Protein Quality. Brit J Nutr 108:S315–S332
93. Harland BF, Oberleas D (1987) Phytates in foods. World Rev Nutr Diet 52:235–259
94. Chitra U, Vimala V, Singh U, Geervani P (1995) Variability in phytic acid content and protein digestibility of grain legumes. Plant Foods Hum Nutr 47:163–172
95. Batista KA, Prudencio SH, Fernandes KE (2010) Changes in functional properties and anti-nutritional factors of extruded hard-to-cook common beans (*Phaseolus vulgaris* L.). J Food Sci 75: C286–C290
96. Vaintraub IA, Bulmaga VP (1991) Effect of phytate on the *in vitro* activity of digestive proteinases. J Agric Food Chem 39:859–861
97. Lothia D, Hoch H, Kievernagel Y (1987) Influence of phytate on *in vitro* digestibility of casein under physiological conditions. Plant Foods Hum Nutr 37:229–235
98. Ravindran V, Cabahug S, Ravindran G, Bryden WL (1999) Influence of microbial phytase on apparent ileal amino acid digestibility of food stuffs for broilers. Poult Sci 78:699–706
99. Selle PH, Ravindran V, Caldwell RA, Bryden WL (2000) Phytate and phytase; consequences for protein utilisation. Nutr Res Rev 13:255–278
100. Gulewicz P, Ciesiołka D, Frias J, Vidal-Valverde C, Frejnagel S, Trojanowska K, Gulewicz K (2000) Simple method of isolation and purification of alpha-galactosides from legumes. J Agric Food Chem 48(8):3120–3123
101. Fan P-H, Zang M-T, Xing J (2015) Oligosaccharides composition in eight food legumes species as detected by high-resolution mass spectrometry. J Sci Food Agric 95:2228–2236
102. Gangola MP, Jaiswal S, Khedikar YP, Chibbar RN (2014) A reliable and rapid method for soluble sugars and RFO analysis in chickpea using HPAEC-PAD and its comparison with HPLC-RI. Food Chem 154:127–133
103. Cerning-Béroard J, Filiatre-Verel A (1980) Characterization and distribution of soluble and insoluble carbohydrates in lupine seeds. Z Lebensm Unters Forsch 171(4):281–285
104. Tharanathan RN, Wankhede DB, Rao M, Rao RR (1975) Carbohydrate composition of groundnuts (*Arachis hypogea*). J Sci Food Agric 26(6):749–754
105. Adeleke OR, Adiamo OQ, Fawale OS, Olamiti G (2017) Effect of soaking and boiling on Antinutritional factors, oligosaccharide contents and protein digestibility of newly developed Bambara groundnut cultivars Turk. J Agric Food Sci Technol 5(9):1006–1014
106. Devindra S, Rao SJ, Krishnaswamy P, Bhaskar V (2011) Reduction of α-galactoside content in red gram (*Cajanus cajan* L.) upon germination followed by heat treatment. J Sci Food Agric 91(10):1829–1835
107. Olson AC, Gray GM, Grambmann MR, Wagner IR (1981) Flatus causing factors in legumes. In: Ory RL (ed) Antinutrients and natural toxicants in food. Food and Nutritional Press, Westport, pp 275–294

108. Liener IE (1994) Implications of antinutritional components in soybean food. Crit Rev Food Sci Nutr 34:31–37
109. De Hoff PL, Brill LM, Hirsch AM (2009) Plant lectins: the ties that bind in root symbiosis and plant defense. Mol Gen Genomics 282(1):1–15
110. He S, Simpson BK, Sun H, Ngadi MO, Ma Y, Huang T (2018) Phaseolus vulgaris lectins: a systematic review of characteristics and health implications. Crit Rev Food Sci Nutr 58(1):70–83
111. Loris R, Hamelryck T, Bouckaert J, Wyns L (1998) Legume lectin structure. BBA-Protein Struct M 1383(1):9–36
112. Kumar S, Sharma A, Das M, Jain SK, Dwivedi PD (2014) Leucoagglutinating phytohemagglutinin: purification, characterization, proteolytic digestion and assessment for allergenicity potential in BALB/c mice. Immunopharmacol Immunotoxicol 36(2):138–144
113. Hirabayashi J, Kuno A, Tateno H (2011) Lectin-based structural glycomics: a practical approach to complex glycans. Electrophoresis 32(10):1118–1128
114. Gabius H-J, André S, Jiménez-Barbero J, Romero A, Solís D (2011) From lectin structure to functional glycomics: principles of the sugar code. Trends Biochem Sci 36(6):298–313
115. Brewer CF, Brown Iii RD, Koenig SH (1983) Metal ion binding and conformational transitions in concanavalin A: a structure-function study. J Biomol Struct Dyn 1(4):961–997
116. Menard S, Cerf-Bensussan N, Heyman M (2010) Multiple facets of intestinal permeability and epithelial handling of dietary antigens. Mucosal Immunol 3(3):247–259
117. Bardocz S, Grant G, Ewen S, Duguid T, Brown D, Englyst K, Pusztai A (1995) Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. Gut 37(3):353–360
118. King T, Pusztai A, Clarke E (1980) Kidney bean (Phaseolus vulgaris) lectin-induced lesions in rat small intestine: 1. Light microscope studies. J Comp Pathol 90(4):585–595
119. Miyake K, Tanaka T, Mcneil PL (2007) Lectin-based food poisoning: a new mechanism of protein toxicity. PLoS One 2(8):e687
120. Pusztai A, Palmer R (1977) Nutritional evaluation of kidney beans (*Phaseolus vulgaris*): the toxic principle. J Sci Food Agric 28:620–623
121. Vasconcelos IM, Oliveira JTA (2004) Antinutritional properties of plant lectins. Toxicon 44(4):385–403
122. Rougé P, Culerrier R, Granier C, Rancé F, Barre A (2010) Characterization of IgE-binding epitopes of peanut (*Arachis hypogaea*) PNA lectin allergen cross-reacting with other structurally related legume lectins. Mol Immunol 47(14):2359–2366
123. Brash AR (1999) Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem 274:23679–23682
124. Porta H, Rocha-Sosa M (2002) Plant lipoxygenases, physiological and molecular features. Plant Physiol 130:15–21
125. Zhu-Salzman K, Salzman RA, Ahn JE, Koiwa H (2004) Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. Plant Physiol 134(1):420–431
126. Moran PJ, Thompson GA (2001) Molecular responses to aphid feeding in Arabidopsis in relation to plant defense pathways. Plant Physiol 125(2):1074–1085
127. Mai VC, Drzewiecka K, Jeleń H, Narożna D, Rucińska-Sobkowiak R, Kęsy J, Floryszak-Wieczorek J, Gabryś B, Morkunas I (2014) Differential induction of *Pisum sativum* defense signaling molecules in response to pea aphid infestation. Plant Sci 221-222:1–12
128. Lenis JM, Gillman JD, Lee JD, Shannon JG, Bilyeu KD (2010) Soybean seed lipoxygenase genes: molecular characterization and development of molecular marker assays. Theor Appl Genet 120(6):1139–1149
129. Khokhar S, Owusu-Apenten RK (2003) Antinutritional factors in food legumes and effects of processing. In: Squires VR (ed) The role of food, agriculture, forestry and fisheries in human nutrition – Vol IV. Encyclopedia of Life Support Systems (EOLSS), Oxford, pp 82–116
130. Johnson IT, Gee JM, Price K, Curl C, Fenwick GR (1986) Influence of saponins on gut permeability and active nutrient transport in vitro. J Nutr 116(11):2270–2277

131. Anderson RL, Wolf WJ (1995) Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. J Nutr 125:581S–585S
132. El-Adaway TA (2002) Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Foods Hum Nutr 57 (1):83–97
133. Vadivel V, Janardhanan (2005) Nutritional and antinutritional characteristics of seven south Indian wild legumes. Plant Food Hum Nutr 60:69–75
134. Kansal R, Kumar M, Kuhar K, Gupta IRN, Subrahmanyam B, Koundal KR, Gupta VK (2008) Purification and characterization of trypsin inhibitor from *Cicer arietinum* L. and its efficacy against Helicoverpa armigera Braz. J Plant Physiol 20(4):313–322
135. Balail NG (2014) Effect of De cortication and roasting on trypsin inhibitors and tannin contents of cowpea (*Vigna unguiculata* L. Walp) seeds. Pak J Biol Sci 17:864–867
136. Lin G, Bode W, Huber R, Chi C, Engh RA (1993) The 0.25-nm X-ray structure of the Bowman-Birk-type inhibitor from mung bean in ternary complex with porcine trypsin. Eur J Biochem 212(2):549–555
137. Liener I (1979) Significance for humans of biologically active factors in soybeans and other food legumes. J Am Oil Chem Soc 56(3):121–129
138. Liener IE (1995) Possible adverse effects of soybean anticarcinogens. J Nutr 125(3):744S–750S
139. Friedman M, Brandon DL (2001) Nutritional and health benefits of soy proteins. J Agric Food Chem 49:1069–1086
140. Lajolo FM, Genovese MI (2002) Nutritional significance of lectins and enzyme inhibitors from legumes. J Agric Food Chem 50(22):6592–6598
141. Pusztai A, Grant G, Bardocz S, Baintner K, Gelencser E, Ewen SWB (1997) Both free and complexed trypsin inhibitors stimulate pancreatic secretion and change duodenal enzyme levels. Am J Phys 35:G340–G350
142. Maneepun S (2003) Traditional processing and utilization of legumes in processing and utilization of legumes report of the APO seminar on processing and utilization of legumes, Japan, 9–14 Oct 2000 ©APO 2003, ISBN: 92-833-7012-0, pp 53–62
143. Khokhar S, Chauhan BM (1986) Antinutritional factors in moth bean (Vigna aconitifolia): varietal differences and effects of methods of domestic processing and cooking. J Food Sci 51(3):591–594
144. Prabhakaran MP, Perera CO, Valiyaveettil S (2006) Effect of different coagulants on the isoflavone levels and physical properties of prepared firm tofu. Food Chem 99(3):492–499
145. Noguchi A (2003) Modern processing and utilization of legumes – recent research and industrial achievements in soybean foods in Japan in processing and utilization of legumes report of the APO seminar on processing and utilization of legumes, Japan, 9–14 Oct 2000 ©APO 2003, ISBN: 92-833-7012-0, pp 63–74
146. Une S, Nonaka K, Akiyama J (2016) Effects of hull scratching, soaking, and boiling on Antinutrients in Japanese red sword bean (Canavalia gladiata). J Food Sci 81(10):C2398–C2404
147. Bau HM, Villaume C, Méjean L (2000) Effects of soybean (Glycine max) germination on biologically active components, nutritional values of seeds, and biological characteristics in rats. Nahrung 44(1):2–6
148. Khalil AH, Mansour EH (1995) The effect of cooking, autoclaving and germination on the nutritional quality of faba beans. Food Chem 54:177–182
149. Fernandez-Lopez A, Lamothe V, Delample M, Denayrolles M, Bennetau-Pelissero C (2016) Removing isoflavones from modern soyfood: why and how? Food Chem 210:286–294
150. Liu Z, Li W, Sun J, Zeng Q, Huang J, Yu B, Huo J (2004) Intake of soy foods and soy isoflavones by rural adult women in China. Asia Pac J Clin Nutr 13(2):204–209
151. Cassidy A, Bingham S, Setchell KD (1994) Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr 60(3):333–340
152. Chen KI, Erh MH, Su NW, Liu WH, Chou CC, Cheng KC (2012) Soyfoods and soybean products: from traditional use to modern applications. Appl Microbiol Biotechnol 96(1):9–22

153. Hotz C, Gibson RS (2007) Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. J Nutr 137(4):1097–1100
154. Lönnerdal B, Sandberg A-S, Sandström B, Kunz C (1989) Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. J Nutr 119:211–214
155. Sandberg A-S, Brune M, Carlsson N-G, Hallberg L, Skoglund E, Rossander-Hulthen L (1999) Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. Am J Clin Nutr 70:240–246
156. Hurrell RF (2004) Phytic acid degradation as a means of improving iron absorption. Int J Vitam Nutr Res 74:445–452
157. Teucher B, Olivares M, Cori H (2004) Enhancers of iron absorption: ascorbic acid and other organic acids. Int J Vitam Nutr Res 74:403–419
158. Ibrahim SS, Habiba RA, Shatta AA, Embaby HE (2002) Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. Nahrung 46(2):92–95
159. Siulapwa N, Mwambungu A (2014) Nutritional value of differently processed soybean seeds. Int J Res Agric Food Sci 2(6):8–16
160. Martín-Cabrejas MA, Sanfiz B, Vidal A, Mollá E, Esteban R, López-Andréu FJ (2004) Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (*Phaseolus vulgaris* L.). J Agric Food Chem 52(2):261–266
161. Chen Y, Xu Z, Zhang C, Kong X, Hua Y (2014) Heat-induced inactivation mechanisms of Kunitz trypsin inhibitor and Bowman-Birk inhibitor in soymilk processing. Food Chem 154:108–116
162. Miyagi Y, Shinjo S, Nishida R, Miyagi C, Takamatsu K, Yamamoto T, Yamamoto S (1997) Trypsin inhibitor activity in commercial soybean products in Japan. J Nutr Sci Vitaminol (Tokyo) 43(5):575–580
163. Landete MJ, Hernández T, Robredo S, Dueñas M, de Las RB, Estrella I, Muñoz R (2015) Effect of soaking and fermentation on content of phenolic compounds of soybean (*Glycine max* cv. Merit) and mung beans (*Vigna radiata* [L] Wilczek). Int J Food Sci Nutr 66(2):203–209
164. Mahungu SM, Diaz-Mercado S, Li J, Schwenk M, Singletary K, Faller J (1999) Stability of isoflavones during extrusion processing of corn/soy mixture. J Agric Food Chem 47(1):279–284
165. Bennetau-Pelissero C (2017) Positive or negative effects of isoflavones: toward the end of a controversy. Food Chem 225:293–301
166. Bennetau-Pelissero (2013) Chapter 77: Isoflavonoids and phytoestrogenic activity. In: Ramawat KG, Merillon JM (eds) Natural products edition. Springer, Berlin/Heidelberg, pp 2381–2431
167. Verma AK, Kumar S, Das M, Dwivedi PD (2013) A comprehensive review of legume allergy. Clin Rev Allergy Immunol 45(1):30–46
168. Maria John KM, Khan F, Luthria DL, Garrett W, Natarajan S (2017) Proteomic analysis of antinutritional factors (ANF's) in soybean seeds as affected by environmental and genetic factors. Food Chem 218:321–329
169. Somoza ML, Blanca-Lopez N, Perez Alzate D, Garcimartin MI, Ruano FJ, Anton-Laiseca A, Canto G (2015) Allergy to legumes in adults: descriptive features. J Allergy Clin Immunol 135:AB254
170. Eigenmann PA (2009) Mechanisms of food allergy. Pediatr Allergy Immunol 20:5–11
171. Astwood JD, Leach JN, Fuchs RL (1996) Stability of food allergens to digestion in vitro. Nat Biotechnol 14:1269–1273
172. Egger M, Mutschlechner S, Wopfner N, Gadermaier G, Briza P, Ferreira F (2006) Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. Allergy 61(4):461–476
173. Rance F, Dutau G (1997) Practical strategy for the diagnosis of food allergies. Pediatr Pulmonol Suppl 16:228–229

174. Bouakkadia H, Boutebba A, Haddad I, Vinh J, Guilloux L, Sutra JP, Sénéchal H, Poncet P (2015) Immunoproteomics of non water-soluble allergens from 4 legumes flours: peanut, soybean, sesame and lentil. Ann Biol Clin 73(6):690–704
175. Verma AK, Kumar S, Das M, Dwivedi PD (2012) Impact of thermal processing on legume allergens. Plant Foods Hum Nutr 67(4):430–441
176. Davis PJ, Williams SC (1998) Protein modification by thermal processing. Allergy 53:102–105
177. Chung SY, Butts CL, Maleki SJ, Champagne ET (2003) Linking peanut allergenicity to the processes of maturation, curing, and roasting. J Agric Food Chem 51:4273–4277

Soybean Bioactive Molecules: Current Trend and Future Prospective

11

Brij Pal Singh, Deepika Yadav, and Shilpa Vij

Contents

Abstract

Bioactive food components or functional foods have recently received significant attention because of their widely advertised positive effects on health beyond basic nutrition. Soybean, a leguminous crop native to East Asia, is renowned for

B. P. Singh (✉) · D. Yadav · S. Vij
Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India
e-mail: bpsingh03@gmail.com; dpu408685@gmail.com; shilpavijn@yahoo.co.in

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_4

high protein and often used to replace the animal proteins in an individual's diet, due to fact that is only vegetable food contains all the essential amino acids. Therefore, FDA authorized a health claim for soy protein that 25 g of soy protein per day may reduce the risk of heart disease. Besides, soybean comprises isoflavones, phytosterols, saponins, and other basic nutritive constituents, such as lipids, vitamins, minerals, oligosaccharides, and biological active peptides, that are of strong therapeutic values. The potential health benefits of soybean/soy bioactive components include protect heart health, anticancer, reduce the effects of menopause, promotes bone health, improve metabolism, and decrease the risk of diabetes. Fermentation is considered as one of the best means to eliminate unpleasant beany flavors, which limit the wide consumption of soybean. Soy isoflavones appear to have estrogen-like activity because they structurally resemble to estrogen and bind to estrogen receptors. Daidzein, genistein, and glycitein are three major glycosidic forms of isoflavones found in soybeans responsible for most of health benefits. Soy bioactive peptides are specific fragments of major soy proteins β-conglycinin and glycinin, and they can be released by enzymatic hydrolysis, food processing, and/or fermentation. Apart from that, saponins derived from soybean appear to have strong cancer inhibitory properties. Moreover, several experimental trials revealed the ability of soy phytosterols to lower cholesterol. In spite of remarkable biological functions of soy bioactive compounds, their large-scale production and commercialization are limited. Therefore, there is much required need to emphasize large-scale production, mechanism of action, and bioavailability of these components. Henceforth, this chapter comprises the current scenario and future prospective of major soy bioactive compounds and their associated health benefits.

Keywords
Soybean · Phytoestrogens · Isoflavones · Bioactive Peptides · Lunasin · Saponins · Phytosterols · Angiotensin-converting enzyme

1 Introduction

Soybean (*Glycine max*) is considered one of the widely consumed legume crops in the world. Usually, soybean seeds consist of about 36.5% protein, 19.9% lipids, 30% carbohydrates, and 9.3% dietary fiber. Soybean has the highest Protein Digestibility Corrected Amino Acid Score (PDCAAS) among legumes, indicating that soy protein provides most of the amino acids required for human nutrition [1]. Soybean has been regarded as an important protein source for Eastern people. The recent knowledge of soy nutritional and functional properties has considerably increased the interest and consumption of soy products in the Western world. The consumer is looking for more nutritious and healthy products, concerning with the consequences of his/her food choices and life styles. Several studies have demonstrated the associated advantages of the use of soy products in preventing heart disease, obesity, blood cholesterol, cancer, diabetes, kidney disease, osteoporosis, blood pressure

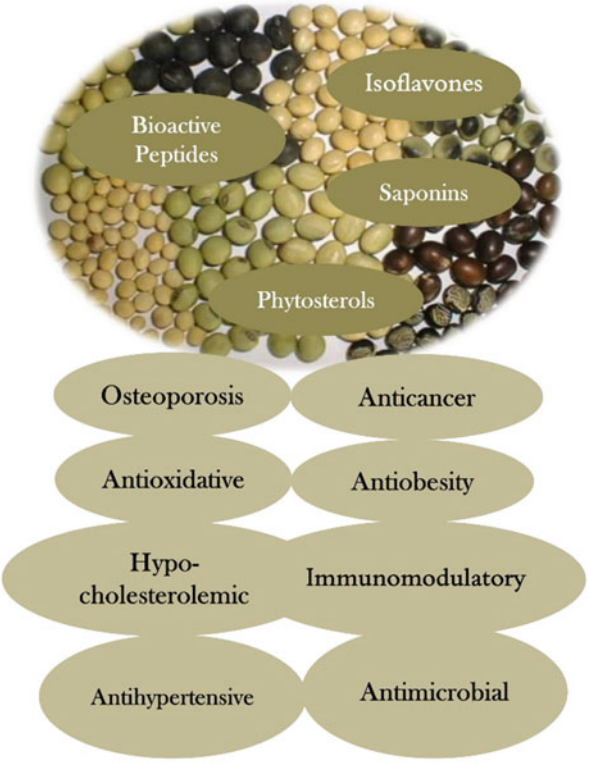


Fig. 1 Soybean bioactive components and associated health benefits

regulation, and menopause symptoms. Soybean and soy-based products contain phytochemicals such as isoflavones, saponins, phytosterols, and bioactive peptides that promote health (Fig. 1) [2]. Food and Drug Administration (FDA) authorized "Soy Protein Health Claim" on October 26, 1999, that 25 g of soy protein a day may reduce the risk of heart disease. Since then market is very much responsive to this health claim and soy foods had penetrated rapidly into Western cultures and diets [3]. Nutritional supplements based on soy (*Glycine max* L.) extract are a rapidly growing segment in the food and health care market [4]. Soy also benefits bone health, which is a concern for aging people [5]. The types of soy products available in market include soy-based cheeses, tofu, soy yoghurt, soy ice cream, soy sauce, and soy vegetable burgers. Also soybean flour, soybean concentrates, and isolated soy proteins are some of ingredients used in the food industry.

Although soy-based foods provide a range of health benefits to consumers, the consumption of soy milk or other soy-based products is hindered due to the presence of unpleasant off-flavors in soybeans. These characteristic flavors are caused by *n*-hexanal and pentanal, which occur in beans as a product of breakdown of unsaturated fatty acids [6]. In addition to these aldehydes, various oligosaccharides including raffinose and stachyose present in soybean may cause a gastrointestinal discomfort to consumers [6, 7]. Raffinose and stachyose are α-galactosides

comprising three and four monomeric units respectively, and are nondigestible in the gut due to the absence of α-galactosidase in the human intestinal mucosa. Consequently, intact oligosaccharides pass directly into the lower intestine where they are metabolized by bacteria that possess this enzyme, resulting in the production of gases [8]. Fermentation is one of the best ways to eliminate such problem and also to improve the acceptability. Fermentation improves the bioavailability of isoflavones, assists in digestion of protein, provides more soluble calcium, enhances intestinal health, and supports immune system. Moreover, nowadays, soy milk has been emerging as an interesting alternative to dairy products for lactose-intolerant population because it is free from lactose. Also, soybeans are less expensive and more abundant than bovine milk, and do not contain cholesterol [9].

Soybeans comprise the highest concentration of isoflavones among all plant foods. The associated health benefits of isoflavones include cancer prevention, reduced risk of osteoporosis, valuable role in chronic renal disease, and protection against cardiovascular disorders. Besides isoflavones, phytocompounds such as phytosterols and saponins have anticancer, hypocholesterolemic, and immunostimulatory activities in human beings. Soy bioactive peptides are specific fractions of soy proteins which have beneficial effect on living organisms. Usually, biological activities of soy peptides are encrypted in proteins, once they released from their parental proteins through hydrolysis, they may influence the major body systems such as cardiovascular, digestive, nervous, and immune. Henceforth, this chapter comprises the current scenario of major bioactive compounds of soybean and their potential health benefits. Future prospective in the field of soy bioactive molecules as technological and functional properties has also discussed.

2 Soy Isoflavones

Isoflavones are a group of naturally occurring heterocyclic polyphenols mostly found in soybean. They are present in soy and soy-derived products such as soy milk, meal, sauce, and tofu, both as free aglycones and as conjugates. Isoflavones are also known as phytoestrogens because they are found in plants and appear to have estrogen-like activity. They are structurally similar to estrogen and bind to estrogen receptors. Interest in soy isoflavones has increased in recent decades, due to their potential preventive activity against human chronic diseases, such as cardiovascular disease, cancer, osteoporosis, menopausal symptoms, and cognitive function [10–12]. Twelve isoflavones have been reported to be present in soybean, consisting of four chemical forms with each form having three compounds, such as malonyl-β-glucoside, acetyl-β-glucoside, and aglycones [13]. Three major groups of isoflavones found in soybeans are genistein, daidzein, and glycitein [14]. Isoflavones generally exist in soybeans and soy foods as aglycones (daidzein, genistein, and glycitein), β-glycosides (daidzin, genistin, and glycitin), acetylglycosides (6″-O-acetyldaidzin, 6″-O-acetylgenistin, and 6″-O-acetylglycitin), and malonylglycosides (6″-O-malonyldaidzin, 6″-O-malonylgenistin, and 6″-O-malonylglycitin) (Fig. 2) [15].

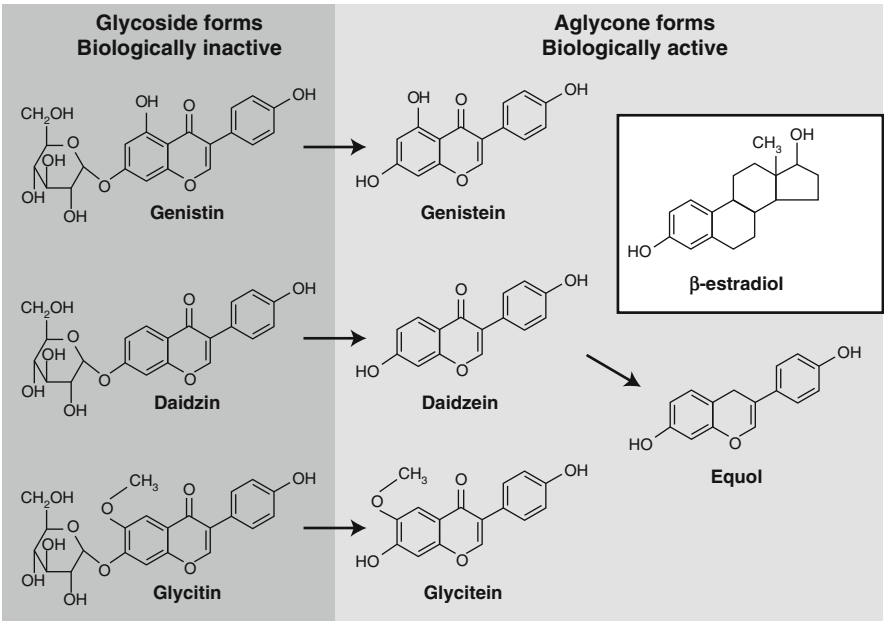


Fig. 2 Molecular structure of isoflavones. Isoflavones presents in a biologically inactive glycoside form (genistin, daidzin and glycitin) in soybean. β-Glucosidases cleave the glucosyl residue and generate biologically active aglycones form (genistein, daidzein, and glycitein) of isoflavones during digestion. Daidzein can be further metabolized into equol

The bioavailability of isoflavones can be influenced by their chemical form in foods and the susceptibility to degradation during heating [16]. Genistein and daidzein, in soybean, are mainly present in the form of their glycosides. Thereafter the consumption of soybeans or soy-based food products, intestinal microorganism's hydrolyzed isoflavones to its unconjugated form i.e., daidzein, genistein and glycitein which are more estrogenic and have better bioavailability [17]. Moreover, equol is a byproduct of bacterial metabolism of daidzein. Equol is reported to have more potent estrogenic activity, greater affinity for estrogen receptors, unique antiandrogenic, and antioxidant activity [18]. Earlier studies have documented that malonyl genistin is the most abundant form of isoflavones in soybean, followed by malonyl glycitin, daidzin, daidzein, genistein, and glycitin. It is generally believed that isoflavones hydrolyzed glycosides to their corresponding aglycones prior to gastrointestinal absorption. Isoflavones when ingested are metabolized extensively in the intestinal tract, absorbed, transported to the liver, and undergo enterohepatic recycling. Intestinal bacterial glucosidases cleave sugar moieties and release the biologically active isoflavones, daidzein, and genistein. These active isoflavones can be further biotransformed by bacteria to the specific metabolites, equol [19], desmethylangolensin, and p-ethylphenol [20]. All these phytoestrogens are then eliminated, mainly by the kidney, and therefore share the physiological features and behavior of endogenous estrogens [21].

2.1 Soy Isoflavones for Women Health

Ingestion of isoflavones containing soybean foods has reported as an alternative for hormone replacement therapy for post-menopausal women. Several epidemiological evidence suggest that isoflavones are beneficial for breast cancer and osteoporosis. Osteoporosis is an age-related disorder that affects most of the aged population in the world [22]. Osteoporosis most often occurs after menopause in women, when the ovaries stop producing estrogen. Soy isoflavones have the potential to overcome bone-specific effects, and evidence from epidemiologic studies supports that dietary isoflavones reduced osteoporotic bone loss induced by menopause through decreasing the bone resorption and stimulating the bone formation. Isoflavones can act as an antiresorptive and bone-sparing agent in preventing osteoporosis. The phenolic rings in their structures are critical structural elements to bind estrogen receptors (ERs) and exert estrogen-like effects [23]. Animal studies, as well as double-blind placebo-controlled trials in humans, suggest that genistein can help restore bone protection [24]. In contrary, the ingestion of high concentrations of isoflavones has reported to adversely affect the reproduction in several animal species. Daily ingestion of soy products has been reported to lengthen the menstrual cycle and suppress the usual midcycle surge in pituitary gonadotropins in premenopausal women [25].

2.2 Soy Isoflavones as Anticancer

The bioactive forms of isoflavones, especially the aglycones, have benefits against cardiovascular diseases and cancers and function by acting as antiestrogens [26]. Breast cancer is the one of most frequently diagnosed cancer in female worldwide and occurs as an interaction of genes and diet. It has been reported that the consumption of phytoestrogens enriched soy food/soy products is traditionally high in Japan, where the annual breast cancer incidence rate is only 0.033%, whereas this figure reached to 0.08% in Western European countries and in the USA, where people consumed less soybean [27]. Genistein and daidzein, two principal components in soy products, have been focused nowadays for their role in cancer prevention. Genistein is a well-known tyrosine kinase inhibitor [28] and inhibits topoisomerase and angiogenesis. Additionally, isoflavones have been thought to act as anticancer agents in part by their ability to scavenge oxidants involved in carcinogenesis. Genistein, which possesses weak estrogenic activity, has been shown to act in animal models as an antiestrogen and, therefore, may play a protective role in hormonally influenced cancers, such as breast cancer. Studies have shown that injection of the isoflavone daidzein reduced *N*-methyl-*N* nitrosourea-induced mammary carcinogenesis by approximately 20% [29]. These findings highlight the importance of daidzein as an anticancer agent and may offer therapeutic potential against colon cancer. Moreover, there are also many reports about the protective effect of soy against oxidative stress, which can also lead to numerous disorders, e.g., neurodegenerative conditions, chronic inflammation, and cancer [30]. An in vitro study on human intestinal Caco-2 cells revealed that

genistein, daidzein, and equol are able to reduce LPS-induced inflammatory responses from intestinal cells, interfering with NF-kB dependent molecular mechanisms [31].

2.3 Soy Isoflavones for Cardiovascular Disease (CVD)

In vitro investigations have demonstrated the hydrogen-donating abilities of isoflavones and metabolites, inhibition of lipid peroxidation, and the ability to interact with the oxidants such as hypochlorous acid and peroxynitrite [32]. Cell culture studies suggest that isoflavones may enhance the cellular antioxidant network by increasing metallothione in mRNA levels, inhibiting peroxynitrite-mediated LDL oxidation by delaying tyrosine nitration [33], and activating glutathione peroxidase [34] (thereby increasing levels of cellular reduced glutathione) or inhibiting superoxide production, and thus cell-mediated LDL modification [35]. A number of studies have shown that the consumption of soy is antiatherogenic, and the bioactive components in this regard are the isoflavones [36–38]. These polyphenols have the potential to scavenge lipid-based peroxyl radicals, and it is possible that the prevention of lipid peroxidation is an important mechanism underlying the protective effects of soy consumption. This contention is supported by the inhibition of copper-dependent LDL oxidation by addition of purified forms of the isoflavones, genistein, daidzein, and biochanin [39]. It has also been reported that genistein can inhibit LDL oxidation, which plays a potential role in atherosclerosis [40], by scavenging reactive oxygen species (ROS) or blocking generation of ROS involved in numerous pathological events. The shift of LDL particle size to a larger, less atherogenic pattern as a result of soy protein consumption and improvement in endothelial function independent of lipoprotein changes represent two other potential soy-mediated protective mechanisms [41, 42].

2.4 Soy Isoflavones for Diabetes Mellitus

Isoflavones content in soy foods possess antioxidant activity and α-glucosidase inhibitory activity, which have proved effective in the treatment of type 2 diabetes mellitus through lowering of the blood glucose level [43]. Similarly, Lee [44] reported that genistein and soy protein isolate contribute to alleviating the adverse effect of diabetes mellitus by enhancing the lipid metabolism as well as the hepatic antioxidant defense system. Genistein and soy protein isolate supplements may be beneficial for correcting the hyperglycemia and preventing diabetic complications. However, their mechanism of action is yet to be elucidated. A well-controlled study on 32 postmenopausal women with type 2 diabetes documented beneficial effect with high isoflavones soy protein supplement. The high isoflavones soy protein supplement has significantly reduced fasting insulin and insulin-resistance values in this population [45].

2.5 Soy Isoflavones as Antimicrobial Agent

The antibacterial activity of flavonoids against *S. aureus*, including antibiotic-resistant strains, and *S. epidermidis* has been reported by Harborne and Williams (2000), and there is an increasing interest in the use of plant flavonoids for treating human diseases [46]. The prokaryotic type II topoisomerases (DNA gyrase and topoisomerase IV) are targets for broad-spectrum antibiotics [47]. Genistein is a topoisomerase II inhibitor and has been shown to stimulate topoisomerase IV-mediated DNA cleavage in *E. coli* [48]. In addition, genistein inhibits the release of inflammatogenic substances by activated macrophages [49] and neutrophils [50], thereby contributing to the downregulation of inflammatory responses. According to Verdrengh and coworkers, genistein may represent a new type of antistaphylococcal agent, whose activity appears to involve the stabilization of the covalent topoisomerase II-DNA cleavage complex [51].

3 Soy Bioactive Peptides

Bioactive peptides can be defined as a specific fragments of proteins that have positive impact on body functions or conditions and may ultimately influence health [52, 53]. Mellander [54] was the first to suggest that casein-derived phosphorylated peptides enhanced vitamin D–independent bone calcification in rachitic infants [55]. Numerous bioactive peptides from different sources having various biological functions have been identified since the first observation of bioactive peptides. Soybean is economically the most important source of vegetable protein for millions of people and a potential source of bioactive peptides. Besides, bioactive peptides have been isolated and characterized from other food protein sources, including milk, egg, fish, oyster, cereal, and radish seeds [56–58]. Soybeans, an excellent source of dietary peptides, have antihypertensive, anticholesterolemic, and antioxidant activities, and appear to prevent cancer [59, 60]. Soybean contains around 40% of protein, and the major soy proteins are known as β-conglycinin and glycinin, which account for 65–80% of total proteins [61]. β-Conglycinin and glycinin are the precursor of most of the peptides isolated from soy bean [62]. Several peptides have been isolated, purified, and characterized from soybean having ACE-inhibitory, antioxidant, anticancer, immunomodulatory, hypocholesteromic, antibacterial, and insulin-modulating activities (Table 1) [67, 78–81].

It has well established now that bioactive peptides can be produced from different food proteins by enzymatic hydrolysis during gastrointestinal digestion and by fermentation of food [52]. Enzymatic hydrolysis has been recognized as one of the most common method for bioactive peptide production. Single and/or multiple specific or nonspecific proteases, i.e., pepsin, bromelain, trypsin, chymotrypsin, and papain, can be used efficiently for bioactive peptide production [82, 83]. Many bioactive peptides have been experimentally generated using various commercial proteases [84]. These enzymes are also used in combination to release more effective and stable bioactive peptides [85]. In addition, several enzymes of plant

Table 1 List of some potential bioactive peptides derived from soybean/ soy based products

Peptides sequences	Biological effects
Val-Pro-Pro	ACE inhibitory [63, 64]
Ile-Pro-Pro	ACE inhibitory [63, 64]
Thr-Pro	ACE inhibitory [63]
Ala-Phe-His	ACE inhibitory [55]
Ile-Phe-Leu	ACE inhibitory [65]
Trp-Leu	ACE inhibitory [65]
Ile-Ile	ACE inhibitory [66]
Ile-Phe-Tyr	ACE inhibitory [66]
Leu-Phe-Tyr	ACE inhibitory [66]
Phe-Phe-Tyr-Tyr	ACE inhibitory [66]
Tyr-Val-Val-Phe-Lys	ACE inhibitory [67]
lle-Pro-Pro-Gly-Val-Pro-Try-Trp-Thr	ACE inhibitory [67]
His-His-Leu	Antihypertensive [7]
Pro-Gly-Thr-Ala-Val-Phe-Lys	Antihypertensive [68]
Ala-Asp-Phe-Val-Leu-Asp-Asn-Glu-Gly-Asn-Phe-Leu-Glu-Asn-Gly-Gly-Thr-Tyr-Tyr-Ile	Antioxidant and ACE inhibitory [53]
Pro-Gly-Thr-Ala-Val-Phe-Lys	Antimicrobial [69]
Ile-Lys-Ala-Phe-Lys-Glu-Ala-Thr-Lys-Val-Asp-Lys-Val-Val-Val-Leu-Trp-Thr-Ala	Antimicrobial [69]
His-Thr-Ser-Lys-Ala-Leu-Leu-Asp-Met-Leu-Lys-Arg-Leu-Gly-Lys	Antimicrobial [70]
His-Cys-Gln-Arg-Pro-Arg and Gln-Arg-Pro-Arg	Immunomodulatory [71]
Gln-Arg-Pro-Arg	Immunomodulatory [71]
His-Cys-Gln-Arg-Pro-Arg	Immunomodulatory [72]
Gln-Arg-Pro-Arg	Immunomodulatory [72]
Leu-Pro-Tyr-Pro	Hypocholesterolemic [73, 74]
Leu-Pro-Tyr-Pro-Arg	Hypocholesterolemic [71]
Ile-Ala-Val-Pro-Gly-Glu-Val-Ala	Hypocholesterolemic [74]
Leu-Pro-Tyr-Pro-Arg	Antiobesity [75]
Pro-Gly-Pro	Antiobesity [75]
X-Met-Leu-Pro-Ser-Try-Ser-Pro-Try	Anticancer [76]
Ser-Lys-Trp-Gln-His-Gln-Gln-Asp-Ser-Cys-Arg-Lys-Gln-Lys-Gln-Gly-Val-Asn-Leu-The-Pro-Cys-Glu-Lys-His-Ile-Met-Glu-Lys-Ile-Glm-Gly-Arg-Gly-Asp-Asp-Asp-Asp-Asp-Asp-Asp-Asp-Asp	Anticancer (Lunasin) [77]

origin, papain and pronase, can be used for protein hydrolysis of soy flour and wheat flour [86]; soy protein hydrolysates have been enzymatically prepared by several commercially available proteases, alcalase, flavourzyme, trypsin, papain, protease, and peptidase [87]. Enzymatic digestion of β-conglycinin and glycinin hydrolyzed more active amino acid R groups that lead to increased antioxidant activity. It has been reported that additional advantage of hydrolysis can be the development of

hydrophobicity since proteolysis unfolds the protein chains. Moreover, hydrolysis leads to production of small bioactive peptides, and the bitterness of peptides of below 1000 Da is much less than fractions with a higher molecular mass [88].

Further, structural and chemical changes that occur during processing of food proteins may also lead to release of bioactive peptides [89]. Apart from that, fermentation is also an efficient way to produce bioactive peptides and food grade hydrolyzed proteins. Lactic acid bacteria (LAB), a large group of beneficial bacteria widespread in nature and also found in our digestive systems, are generally used for bioactive peptide production. They are best known for their role in the preparation of fermented products, not only because of their physiological significance but also because of their technological importance in texture and flavor development [90]. The proteolytic system of lactic acid bacteria, e.g., *Lactococcus (L.) lactis*, *Lactobacillus (Lb.) helveticus*, and *Lb. delbrueckii* ssp. *bulgaricus*, has proteinases that are capable of releasing a large number of different oligopeptides (4–8 amino acid). The oligopeptide transport system, main route for nitrogen entry into the cell and peptidases, located intracellularly is required for complete degradation of accumulated peptides [91, 92]. Lactic acid bacteria, possessing α-galactosidase enzyme, can hydrolyze the soy oligosaccharides (sucrose, raffinose, and stachyose) during fermentation and reduce its beany flavor and flatulence [6, 7, 93]. During fermentation of soy milk, proteins are degraded into simpler forms like oligopeptides, dipeptides, and tripeptides and serve as a good source of bioactive peptides. Comparatively microbial fermentation is the cheapest process instead of enzymatic hydrolysis for bioactive peptide production because microorganisms are a cheap source of proteases and are recognized as safe. Upon administration bioactive peptides may affect the major body systems, i.e., cardiovascular, digestive, immune, and nervous. The beneficial effects of bioactive peptides include antimicrobial, antioxidative, antithrombotic, antihypertensive, antidiabetic, anticancer, antiobesity, and immunomodulatory [94–97].

3.1 Effect of Soy Bioactive Peptides on Blood Pressure Regulation

Antihypertensive peptides are the greatly studied bioactive peptides in soy foods. Antihypertensive or angiotensin-converting enzyme (ACE) inhibitory peptides block the first step in the rennin-angiotensin system and interrupt the negative feedback effects of angiotensin II. The rennin-angiotensin system regulates blood pressure and fluid balance in body. ACE is a nonspecific dipeptidyl carboxy peptidase and converts the inactive decapeptide angiotensin I by cleaving dipeptide from the C-terminus into the potent vasoconstricting octapeptide angiotensin II in the rennin-angiotensin system (RAS), which has a tendency to increase blood pressure [98]. Besides, ACE also catalyzes the degradation of bradykinin, a blood pressure–lowering nonapeptide in the kallikrein-kinin system (Fig. 3) [99–101]. Inhibition of ACE is considered to be a useful therapeutic approach in the treatment of hypertension. Hypertension is also considered as a main cause of several risk factors, such as heart failure, stroke, coronary heart disease, and myocardial infarction.

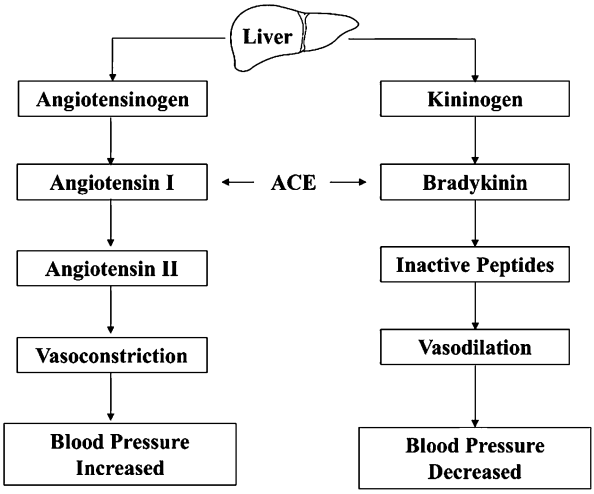


Fig. 3 Role of angiotensin-converting enzyme (ACE) in blood pressure regulation

Soy bioactive peptides, i.e., VPP (valyl-prolyl-proline), IPP (isoleucyl-prolyl-proline), and TP (Tyrosin-Proline), are known to have ACE-inhibitory activity and blood pressure lowering effect [63]. Similarly, bioactive peptides from fermented soy product (miso and doenjang) have been shown ACE-inhibitory activity in spontaneously hypertensive rats, mainly associated with tripeptides (VPP and IPP) [64]. Moreover, an ACE-inhibitory tripeptide Ala-Phe-His have been isolated from soybeans fermented by *Bacillus natto* and *Bacillus subtilis* [55]. It has also observed that peptides prepared from alcalase hydrolysis of soy protein significant decrease systolic blood pressure (SBP) in spontaneously hypertensive rats [102]. Fractionated whey from fermented soy milk with probiotics, *L. casei, L. acidophilus, L. bulgaricus, S. thermophilus*, and *B. longum*, has reported to exhibit antihypertensive activity [103]. Two ACE-inhibitory peptides have also been isolated having Ile-Phe-Leu and Trp-Leu amino acid sequence [65]. Fermented soybean extract were also used for the production of ACE-inhibitory peptide [104]. Shimakage and coworkers identified novel ACE-inhibitory peptides, such as Ile-Ile, Ile-Asp, Ile-Phe-Tyr, Leu-Phe-Tyr, Leu-Tyr-Tyr, Phe-Phe-Tyr-Tyr, and Trp-His-Pro, from protease-treated natto and soy milk [66]. Alauddin et al. [105] has recently identified the ACE-inhibitory peptide and studied its hypotensive effect from processed soy milk (PSM); the ingestion of PSM may lower high blood pressure and ameliorate cardiovascular diseases related to hypertension without causing adverse side effects in spontaneously hypertensive rat. Nakahara et al. [106] identified two antihypertensive peptide Seryl–tyrosine (Ser-Tyr) and glycyl–tyrosine (Gly-Tyr) from fermented soy sauce. Angiotensin-I converting enzyme (ACE-I) inhibitory activity of prepared soy whey proteins were fractionated into different fractions using ultrafiltration and found that unfractionated whey protein had the highest ACE-I inhibition activity. The study also indicated that soy–whey protein fraction (>50 kDa) had good solubility, emulsion activity, and stability, while the

unfractionated whey protein exhibited the strongest ACE-I inhibition percentage (19%) [107]. Recently, around 33 peptides were identified from soy milk fermented by a *Lactobacillus plantarum* strain, having ACE-inhibitory activity [53].

3.2 Effect of Soy Bioactive Peptides on Oxidative Stress

Antioxidants are recognized as a substances that can significantly decrease the unfavorable effects of reactive species. Reactive species can be formed during cellular metabolism of human system and plays important roles in cell signaling, apoptosis, gene expression, and ion transportation. However, when the production of these molecules is uncontrolled, poor cellular defenses lead to damaging of protein, lipid, and nucleic acids by the process called oxidative stress. However, human beings possess defense and repair systems for oxidative stress, but these innate antioxidative systems are usually not enough to prevent them from the oxidative stress [108]. Apart from several natural antioxidative compounds and vitamins, food-derived bioactive peptides have now been explored as an effective antioxidant agent. Antioxidant food supplements or bioactive peptides may be used to help the human body and animals to reduce the oxidative damage [109]. It has been noticed that several amino acid residues, i.e., histidine, tyrosine, tryptophan, phenylalanine, proline, and leucine, contribute for antioxidant capacity of peptides [110].

Antioxidative peptides can inhibit oxidation through multiple pathways, including inactivation of reactive oxygen species, scavenging free radicals, and chelation of pro-oxidative transition metals [111]. Several amino acids, such as Tyr, Met, His, Lys, and Trp, are generally accepted as antioxidants. On the same line, antioxidative peptide from β-conglycinin fraction of soy protein has been isolated by Chen et al. [112] using protease of microbial origin. Similarly, Coscueta et al. [113] reported that hydrolysis of soybean meal protein isolate has potentially enhanced ABTS and ORAC antioxidant capacity by treatment with corolase PP. Antioxidative peptides have been produced from soy protein isolate through hydrolysis with a food grade pancreatic trypsin/chymotrypsin with an antioxidative capacity of 113 mg TEAC/g [114].

In a recent study, *Lactobacillus plantarum* strain has been used as a starter to ferment soy whey in order to study antioxidative potential. The results demonstrated that fermented soy whey possessed more radical scavenging capacity as compared to unfermented, which is directly linked to generation of bioactive peptides during fermentation [115]. Similarly, Sanjukta et al. [116] reported 3.1–24 folds increment in total antioxidant activity of two soybean varieties of Sikkim Himalayan region of India by fermentation with *Bacillus subtilis.* Recently, we have also identified 35 antioxidative peptides from soy milk fermented by a *Lactobacillus plantarum* strain [53]. Bioactive peptides has also identified in soybean-based infant formulas having around 90% soy protein isolate. Around 120 antioxidant peptides were characterized by using ultrafiltration followed by HPLC-ESI-Q-ToF-MS/MS [117]. Antioxidant activity of lunasin (a soy peptide) has also been reported as a potent scavenger of peroxyl and superoxide radicals. The protective role of lunasin on cell viability and

antioxidant defenses of human Caco-2 cells has also been studied and found that direct antioxidant action of lunasin on enterocytes exposed to oxidizing species makes this peptide a promising agent to preserve the integrity of intestinal mucosa against oxidative damage–related diseases [118].

3.3 Antimicrobial Soy Bioactive Peptides

The growing problem of resistance to conventional antibiotics and the necessity for new antibiotics has stimulated an interest in the development of antimicrobial peptides (AMPs) as human therapeutics [119]. AMPs are peptides that can kill microorganisms, and they often exhibit a broad spectrum of activity against Gram Positive and Gram Negative bacteria [120]. Antimicrobial peptides are widely distributed in nature and are essential to the immune system. They are the organism's first line of defense against colonization by exogenous microorganisms, and they play a fundamental role in regulating bacterial populations on the mucosa and other epithelial surfaces. Therefore, using natural sources of antimicrobial compounds has enormous potential because they have characteristics such as low toxicity and high specificity. The mechanisms of these natural antimicrobial compounds can be better understood if we compare their modes of action against bacterial (unicellular) and animal (multicellular) cells. Bacterial cells have a layer rich in negatively charged phospholipids pointing toward the external environment, facilitating their interactions with peptides, most of which are positively charged. In contrast, animal cells are mainly composed of uncharged lipids in the outermost layer, and the negatively charged regions are pointed toward the cell interior (cytoplasm) [121, 122]. Soy proteins are excellent source of bioactive compounds. The biological activity of the glycinin and β-conglycinin hydrolysates was confirmed, and glycinin peptides were found to produce stronger antimicrobial effects than β-conglycinin peptides [123]. Antimicrobial peptides ranging from 15 to 40 amino acids in length, most of which are hydrophobic and cationic, are generally involved in innate immunity. These peptides include two or more positively charged residues provided by arginine, lysine, or, in acidic environments, histidine, and a large proportion of hydrophobic residues. They can exhibit an efficient role in the host defense against the most frequent pathogenic bacteria that interact directly with them and therefore scavenging them. Such peptides provide protection against bacteria, fungi, and viruses by acting on the cell membranes of the pathogens [124]. Recently, we have studied antimicrobial activity of bioactive peptide fractions derived from fermented soy milk. Five kilo dalton fraction has showed highest activity against most of the tested pathogens, among them highest activity has reported against *E.coli* (12 ± 0.57) followed by *S. dysenteriae* (11 ± 0.57), *L. monocytogenous* (10 ± 0.57), and *B. cereus* (10 ± 0.57 mm) [125]. Two soy peptides, i.e., PGTAVFK and IKAFKEATKVDKVVVLWTA, have tested against *Pseudomonas aeruginosa* and *Listeria monocytogenes* biofilms; both the peptides showed strong inhibitory activity against *Listeria monocytogenes* [69]. In a recent study, a soy meal fermented by a

Bacillus subtilis strain has found to produce antimicrobial peptides with great antimicrobial activity against *Vibrio alginolyticus* and *V. parahaemolyticus* [70].

3.4 Soy Bioactive Peptides as Immune System Modulator

Soy bioactive peptides have also been reported to enhance immune function. Two peptides from soy protein hydrolysate, i.e., His-Cys-Gln-Arg-Pro-Arg and Gln-Arg-Pro-Arg, have been found to exhibit phagocytosis stimulatory activity [72]. Usually, immunomodulatory peptides can enhance immune cell functions, such as lymphocyte proliferation, natural killer (NK) cell activity, antibody synthesis, and cytokine regulation. A soy protein hydrolysates prepared from insoluble soy protein has displayed highest immunomodulatory activity on proliferation of murine splenic lymphocytes and phagocytic effect of peritoneal macrophages [87]. Similarly, a phagocytic enhancing peptide has been isolated from trypsin digests of α-subunit of β-conglycinin soy proteins [61]. Lunasin, a soy peptide, exerts synergistic effects with cytokine Il-12 or Il-2 on modulating the expression of a number of genes in NK cells, resulting in strong NK activation with enhanced cytotoxicity, which is associated with higher levels of IFNγ and granzyme B expressed by both CD56 bright and dim populations [126]. Lunasin and other lunasin-like peptides, purified from defatted soybean flour, inhibited inflammation in LPS-induced macrophage by suppressing NFkB pathway. Out of three purified peptides (5, 8, and 14 kDa), the 5 kDa peptide inhibited most potently the proinflammatory markers including interleukin-6 production, nuclear factor-kappa B, cyclooxygenase-2 expression, nitric oxide production, inducible nitric oxide synthase, nuclear translocation, and p50 nuclear translocation [127]. Recently, Tung et al. [128] studied stimulatory effects of lunasin on innate immune cells by regulating expression of a number of genes that are important for immune responses. Lunasin-treated conventional DCs (cDCs) not only expressed elevated levels of costimulatory molecules (CD86, CD40) but also exhibited upregulation of cytokines (IL1B, IL6) and chemokines (CCL3, CCL4). Lunasin-treated cDCs induced higher proliferation of allogeneic CD4+ T cells when comparing with medium control treatment in the mixed leukocyte reaction (MLR).

3.5 Effect of Soy Bioactive Peptides on High Cholesterol

Hypercholesterolemia, also called high cholesterol, is the presence of high levels of cholesterol in the blood. It is a significant risk factor in the development of heart diseases and one of the major causes of deaths worldwide. Hypercholesterolemia is a state of high blood lipids and elevated levels of lipoproteins in the blood. Dietary proteins can modulate the effect of high serum cholesterol concentrations. The hypocholesterolemic activity of food protein and peptides involves stimulation of bile acids secretion, changes in cholesterol metabolism in the liver, hormonal effects, and regulation of cholesterol receptors [129, 130]. The effects of soy-based product

on cardiovascular diseases (CHD) have considered through its impact on blood cholesterol. It has been reported that the released bioactive peptides lower the cholesterol levels when soy protein was subjected to protease digestion in the digestive tract [131]. It has now been well established that the dietary intakes of 20 g of soy protein per day for 5 weeks would be effective in reducing the CHD risk among high-risk, middle-aged men [132]. Similarly, the effect of fermented soy milk on rats fed a high cholesterol diet was investigated to clarify the cholesterol-lowering function [133]. Wang et al. (2008) reported that soy protein reduced the level of circulating triglycerides and cholesterol in hypocholesterolemic subjects [134]. Similarly Sugano et al. [135] documented that soy protein hydrolysate of peptic digest has a stronger serum cholesterol lowering effect in comparison to intact soy protein in rats. Soy peptides may also bind to phospholipids and exert serum cholesterol lowering activity in humans [136]. Keeping the cholesterol lowering effect of soy protein in mind, Food and Drug Administration (FDA) approved a health claim linking foods that are naturally rich in soy protein reduce coronary heart disease [137].

LPYP, a tetrapeptide, has been isolated from soy protein glycinin with hypocholesterolemic effect [73]. Zhong and coworkers studied the in vivo hypocholesterolemic effect of soy peptides [138], and they found that cholesterol micellar solubility inhibitory rate of soy peptides was more than 45%. Similarly, Ferreira and coworkers (2010) reported cholesterol-lowering effect of β-conglycinin and glycinin soy proteins in rats fed a high-cholesterol diet [139]. A recent study showed effect of a symbiotic fermented soy product supplemented with okara (a by-product from soybean) on cardiovascular disease risk markers in healthy men. In a randomized two groups study, subjects consumed daily 100 g of soy-based product fermented with *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* subsp. *lactis* Bb-12, and *Streptococcus thermophilus* (starter culture), and LDL-C mean decreased significantly resulting in a significant improvement of the LDL-C/HDL-C ratio was observed [140]. Three peptides from soy glycinin, IAVPGEVA, IAVPTGVA, and LPYP, have been studied by Lammi et al. [74] for their hypocholesterolemic activity. HepG2 cells treated with these peptides interfere with the catalytic activity of 3-hydroxy-3-methylglutaryl CoA reductase (HMGCoAR) and modulate the cholesterol metabolism through the activation of the LDLR-SREBP2 pathway, increasing the ability of HepG2 cells to uptake the LDL.

3.6 Soy Bioactive Peptides for Weight Management

A condition when excess fat has accumulated in body to the extent that may have an adverse effect on health called obesity. Obesity has now been considered as a major health problem in most of the countries, which is associated with higher incidence of CVD, type 2 diabetes, obstructive sleep apnea, cancer, osteoarthritis, and depression [141]. Consumption of dietary proteins, i.e., soy, casein, and whey protein, has reported antiobesity effect. An in vitro study revealed that soy protein has greater antiobesity effect in comparison to casein and whey protein in obese rats/or mice

[142]. Soy protein may also lead to lower hepatic deposits of triglycerides [143]. Two peptides Leu-Pro-Tyr-Pro-Arg and Pro-Gly-Pro from soybean glycinin protein have strong antiobesity potential [75]. Several animal studies showed that soy protein ingestion lead to lipid-lowering effect by reducing hepatic cholesterol content and enhancing removal of LDL [144]. Soy proteins/peptides have directly affected the hepatic cholesterol metabolism and LDL receptor activity. Evidence from animal models suggested that soy protein has strongly influenced lipogenesis in the liver. Similarly, dietary soybean proteins have reported to reduce the concentrations of plasma triglycerides in rat liver. These effects have mainly associated with the reduction of hepatic lipogenic enzymes, such as glucose-6-phosphate dehydrogenase, malic enzyme, fatty acid synthetase, and acetyl-CoA carboxylase (ACC), which concluded that soy protein reduces liver triglycerides by inhibition of hepatic fatty acid synthesis in liver [145]. Soy protein can improve insulin resistance and lipid levels by activation of PPAR (peroxisome-proliferator activated receptors), which is the main transcription factor responsible for the regulation of expression of genes involved in glucose homeostasis, lipid metabolism, and fatty acid oxidation. On the same line, Jang and coworkers (2008) studied in vitro antiobesity effect of soy peptides derived from black soybean. They observed that the diet-induced obese mice fed a high-fat diet along with soy peptides gained less body weight in comparison to control [146].

3.7 Anticancer Soy Bioactive Peptides

Lunasin, a peptide isolated from soybean, is well known for their anticancer effect [77]. According to reports, lunasin can prevent cellular transformation induced by chemical carcinogens and the viral oncogenes RAS and E1A [147, 148]. Hsieh and coworkers (2010) reported that lunasin has been strongly inhibited cell proliferation and cancerous foci formation in cells treated with 7,12-dimethylbenz (a) anthracene (DMBA) and 3-methylcholanthrene-treated (MCA) [149]. Similarly, an in vivo study also documented anticancer effect of lunasin induced by DMBA [150]. As safety point of view reports claimed that lunasin does not affect the growth rate of normal and established cancer cell lines in spite of cancer-preventive properties. Soy proteins has several mechanisms for cancer prevention including increased mammary gland differentiation, decreased activation of procarcinogens to carcinogens, and regulation of genes in signal transduction pathways underlying tumor initiation, promotion, and/or progression [151]. Rayaprolu and coworkers [152] reported anticancer properties (more than 65%) of soy protein isolates against human colon (HCT-116, Caco-2), liver (HepG-2), and lung (NCL-H1299) cancer cell lines. Similarly, oral administration of lunasin has reported to reduce liver metastasis by 56–94% [153]. Downregulation of inflammation related genes was reported by Hwang et al. [154] when breast cancer MCF7 cells were treated with fermented soybean extracts. Similarly, Yang et al. [155] reported that lunasin can effectively suppress allergic airway inflammation.

4 Soy Saponins

Saponins are compounds containing a steroid or triterpenoid aglycone linked to one or more oligosaccharide moieties [156]. These high-molecular weight glycosides are found in many plant-derived drugs. Saponins, which derive their name from their ability to form stable, soap-like foams in aqueous solutions, constitute a complex and chemically diverse group of compounds [157]. Soy saponins are amphiphilic compounds and categorized as triterpenoic saponins [158]. Soybeans and soy-based products contain approximately 1–5% saponins. It has been estimated that there are at least 40 different saponins in soybeans. Most of the soy saponins identified till date have a soyasapogenol conjugated at the 3-position with a 2′-glycosylated glucuronic acid residue [159].

4.1 Health Effects of Soy Saponins

Soy saponins are well known for the inhibition of cancer cells [160]. Clinical studies have suggested that saponins affect the immune system in ways that help protect the human body against cancers [157]. At high concentrations, saponins cause cell damage by disrupting the cell membrane or inducing apoptosis [161]. The membranolytic activity of soybean saponins as indicated by their interaction with human colon carcinoma cells explains their role as anticarcinogens. Previous reports indicated that soybean saponins suppress the growth of colon tumor cells in vitro, and a 2% crude soybean saponins diet inhibited a carcinogen-induced colonic aberrant formation in rats. Soybean saponins have been shown to contain 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), and DDMP (1 mg/mL) possesses a strong radical scavenging activity equivalent to 17.1 units of superoxide dismutase [162]. Soybean saponins significantly inhibit the release of prostaglandin E2 (PGE2), nitric oxide (NO), tumor necrosis factor α (TNFα), and monocyte chemotactic protein- 1 (MCP-1) in a dose-dependent manner [163]. They also downregulate the expression of cyclooxygenase-2 (COX-2) or inducible nitric oxide synthase (iNOS) at mRNA/protein levels. The anti-inflammatory properties of soybean saponins are mediated by the necrosis factor-κB (NF-κB) signaling pathway, blocking the degradation of inhibitory proteins known as IκB-α. Kang et al. [163] concluded that the anti-inflammatory action of soybean saponins may be useful for developing preventive agents against inflammatory diseases as well as suppressing tumor progression. On the same line, Tsai and coworkers (2010) reported that soy saponins decreased the number of viable human colon cancer cells in a dose-dependent manner and suppressed 12-otetradecanol-phorbol-13-acetate-stimulated PKC activity. Cells treated with saponins developed cytoplasmic vesicles and the cell membrane became rougher and more irregular and eventually disassembled [158].

Apart from those other beneficial properties of soy saponins includes hypocholesterolemic, hemolytic, and immunostimulatory activities [11]. Moreover, soy saponins were reported to have antiviral and antioxidative activities. The blood

cholesterol-lowering properties of soybean saponins are also of particular interest in human nutrition. Saponins cause a depletion of body cholesterol by preventing its reabsorption, thus increasing its excretion. Studies on rats have shown soybean saponins to have an anabolic effect on bone components, suggesting its role as a nutritional factor in the prevention of osteoporosis [135]. Nishida and coworkers (1993) reported that the inhibition of radical-initiated lipid peroxidation by soy saponins was responsible for its anticarcinogenic properties [164].

5 Soy Phytosterols

Phytosterols are naturally occurring compounds which are found in all foods of plant origin. Phytosterols have the similar basic function in plants as cholesterol in animals, and they play a key role in cell membrane function. Therefore, they are best known for reducing blood cholesterol levels. Phytosterols comprise a wide variety of molecules that are structurally similar to cholesterol. They are naturally found in pulses, vegetable products, oils, and dried fruits [165]. Soybean phytosterols consist mainly of β-sitosterol, campesterol, and stigmasterol; β-sitosterol content being highest among them.

5.1 Health Effects of Soy Phytosterols

Several experimental trials revealed the ability of phytosterols to reduce LDL-cholesterol, without significantly altering HDL-cholesterol or triglycerides in general [165]. It was reported that phytosterols such as β-sitosterol offer anticancer effects [166], prostatic hyperplasia-lowering effects [167], and stimulation of a plasminogen activating factor [168]. In particular, there are many reviews on cholesterol-lowering activity of phytosterols. It has been demonstrated that soybean phytosterols are able to reduce serum cholesterol by inhibiting the absorption of cholesterol. Therefore, phytosterols are often supplemented into functional foods to improve human health. The FDA has also authorized a food claim for phytosterols: "Food containing at least 0.65 g per serving of plant sterol esters, eaten twice a day with meals for a daily total intake of at least 1.3 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease" [169–171]. Lerman and coworkers (2010) concluded that individuals at high CVD risk benefit from a soy phytosterol containing medical food; thereafter, conduction of a 12-week randomized trial for subjects received a phytochemical-enhanced diet [172]. Similarly, a diet program containing 30 g of soy protein and 4 g of phytosterols was subjected to 27 postmenopausal women per day for 12 week, and observed that total cholesterol, low-density lipoprotein cholesterol, and triacylglycerol have decreased significantly [173]. Moreover, mild-to-moderate hyperlipidemic patients were selected in a study and treated with placebo soy milk powder enriched with phytosterols. After 3 months of trail, the serum total cholesterol, low-density lipoprotein cholesterol, and non-high-density lipoprotein cholesterol levels decreased by 9.3%, 11.4%, and 12.6%, respectively [174].

6 Future Prospective

Over the last two decades, scientific research on soybean-derived bioactive compounds, such as isoflavones, peptides, and protein hydrolysates, has been detonated, which displays a broad scope of functions. However, these bioactive molecules are less potent in comparison to pharmaceutical drugs; conversely, they have minimum or no side effects because nature has provided the mechanism for their metabolism and utilization or excretion. Usually, bioactivity of compounds in vitro cannot be directly related to its in vivo effect because they may likely encounter degradation and modification in the intestine, vascular system, and liver. Therefore, they need to remain active during digestion and be transported through the intestinal wall into the blood. However, several differences are observed between in vitro models and in vivo studies [175, 176]. Therefore, bioavailability of bioactive compounds is one of the major areas of research which requires focus. It was evident that only a small portion of bioactive molecules are sufficient to exert the specific function at tissue level once they pass through the intestine barrier [177]. For instance, a numbers of mechanisms for absorption of peptides are available, such as paracellular route, passive diffusion, transport via carrier, and endocytosis. The lymphatic system is also a possible route of peptides absorption; here absorption of peptides is mainly affected by their permeability via the capillary of the portal circulation and lipid solubility [88]. The small peptides absorbed more readily instead of large peptides. They are able to cross the intestinal barrier and exert their biofunctionality at the specific target organs [178–180].

In spite of remarkable array of biological functions by soy bioactive molecules, it may be surprising that only a few have commercialized and reached to the market. Therefore, new strategies are needed to establish economical and efficient industrial scale production of bioactive compounds with different action. However, the high cost and multistep process limited the large-scale production of bioactive compounds. Therefore, advance and precise technologies are needed for hydrolysis, separation, and purification of bioactive molecules with high potency and yield. Also, downstream processing such as drying, cellular disruption, extraction, and production cost–related studies of bioactive molecules need specific attention. Moreover, bioinformatics approach should be explored for prediction and analysis of specific bioactivity of molecules. Molecular studies are also needed to examine the mechanisms by which these compounds exert their actions.

7 Conclusions

Soybean has a great potential of supplying bioactive compounds that are functionally significant. However, the raw beans itself are not accepted throughout because it contains some antinutrients that can disrupt digestion activities in the stomach, leading to cramping and associated discomfort. Therefore, these bioactive components can be incorporated in functional and fresh foods, dietary supplements, and even pharmaceuticals with the aim of delivering specific health benefits. Microbial

fermentation is also an effective way to increased bioactivity and acceptability of soybean. The effect of soybean/soy-derived bioactive components on emerging lifestyle complications, such as metabolic syndrome and cardiovascular disorders, has increased demands worldwide. Apart from that, soybean products can be good substitutes for animal products because, unlike some other beans, soybean offers a complete protein profile. Soybeans contain all the essential amino acids except methionine, which must be supplied in the diet because they cannot be synthesized by the human body. Besides the potential health effect of soybean-derived bioactive molecules, the bioavailability and safety of these products should be ensured as most priority. More definite clinical studies are needed to ensure any side effect. Advanced molecular and bioinformatics approaches should also be explored for target-specific studies of soy bioactive molecules.

References

1. Vagadia BH, Vanga SK, Raghavan V (2017) Inactivation methods of soybean trypsin inhibitor–a review. Trends Food Sci Technol 64:115–125
2. Lee CC, Dudonné S, Dubé P, Desjardins Y, Kim JH, Kim JS, Kim JE, Park JH, Lee KW, Lee CY (2017) Comprehensive phenolic composition analysis and evaluation of Yak-Kong soybean (Glycine max) for the prevention of atherosclerosis. Food Chem 234:486–493
3. Fukushima D (2001) Recent progress in research and technology on soybeans. Food Sci Technol Res 7:8–16
4. Usui T (2006) Pharmaceutical prospects of phytoestrogens. Endocr J 53:7–20
5. Messina M, Gugger E, Alekel D (2001) Soy protein, soybean isoflavones, and bone health: a review of the animal and human data. In: Handbook of Nutraceuticals and Functional Foods, vol 20. CRC Press, Florida, pp 77–98
6. Scalabrini P, Rossi M, Spettoli P, Matteuzzi D (1998) Characterization of Bifidobacterium strains for use in soymilk fermentation. Int J Food Microbiol 39:213–219
7. Shin H, Lee J, Pestka J, Ustunol Z (2000) Growth and viability of commercial Bifidobacterium spp in skim milk containing oligosaccharides and inulin. J Food Sci 65:884–887
8. Tsangalis D, Shah N (2004) Metabolism of oligosaccharides and aldehydes and production of organic acids in soymilk by probiotic bifidobacteria. Int J Food Sci Technol 39:541–554
9. Hati S, Vij S, Mandal S, Malik RK, Kumari V, Khetra Y (2014) α-Galactosidase activity and oligosaccharides utilization by lactobacilli during fermentation of soy milk. J Food Process Preserv 38:1065–1071
10. Sirtori C (2001) Risks and benefits of soy phytoestrogens in cardiovascular diseases, cancer, climacteric symptoms and osteoporosis. Drug Saf 24:665–682
11. Li YR, Yun TT, Liu S, Qi WT, Zhao LQ, Liu JR, Li AK (2016) Analysis of water-soluble bioactive compounds in commonly consumed soymilk in China. J Food Compost Anal 46:29 35
12. Kašparovská J, Dadáková K, Lochman J, Hadrová S, Křížová L, Kašparovský T (2017) Changes in equol and major soybean isoflavone contents during processing and storage of yogurts made from control or isoflavone-enriched bovine milk determined using LC–MS (TOF) analysis. Food Chem 222:67–73
13. Kao T, Chen B (2002) An improved method for determination of isoflavones in soybean powder by liquid chromatography. Chromatographia 56:423–430
14. Cassidy A, Bingham S, Setchell K (1994) Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr 60:333–340
15. Wang H, Murphy P (1994) Isoflavone content in commercial soybean foods. J Agric Food Chem 42:1666–1673

16. Birt D, Hendrich S, Wang W (2001) Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol Ther 90:157–177
17. Setchell KDR, Clerici C (2010) Equol: history, chemistry, and formation. J Nutr 140:1355–1362
18. Rossi M, Amaretti A, Roncaglia L, Leonardi A, Raimondi S (2010) Dietary isoflavones and intestinal microbiota: metabolism and transformation into bioactive compounds. In: Thompson MJ (ed) Isoflavones: biosynthesis, occurrence and health effects. Nova Science Publisher, New York, pp 137–161
19. Axelson M, Kirk D, Farrant R, Cooley G, Lawson A, Setchell K (1982) The identification of the weak oestrogen equol [7-hydroxy-3-(4′-hydroxyphenyl) chroman] in human urine. Biochem J 201:353–357
20. Joannou G, Kelly G, Reeder A, Waring M, Nelson C (1995) A urinary profile study of dietary phytoestrogens. The identification and mode of metabolism of new isoflavonoids. J Steroid Biochem Mol Biol 54:167–184
21. Setchell KD, Brown NM, Zimmer-Nechemias L, Brashear WT, Wolfe BE, Kirschner AS, Heubi JE (2002) Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am J Clin Nutr 76:447–453
22. Arjmandi B, Lucas E, Khalil D, Devareddy L, Smith B, McDonald J, Arquitt AB, Payton ME, Mason C (2005) One year soy protein supplementation has positive effects on bone formation markers but not bone density in postmenopausal women. Nutr J 4:8
23. Zheng X, Lee SK, Chun OK (2016) Soy isoflavones and osteoporotic bone loss: a review with an emphasis on modulation of bone remodeling. J Med Food 19:1–4
24. Morabito N, Crisafulli A, Vergara C, Gaudio A, Lasco A, Frisina N, D'Anna R, Corrado F, Pizzoleo MA, Cincotta M, Altavilla D (2002) Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. J Bone Miner Res 17:1904–1912
25. Setchell KD, Gosselin SJ, Welsh MB, Johnston JO, Balistreri WF, Kramer LW, Dresser BL, Tarr MJ (1987) Dietary estrogens-a probable cause of infertility and liver disease in captive cheetahs. Gastroenterology 93:225–233
26. Lin PY, Lai HM (2006) Bioactive compounds in legumes and their germinated products. J Agri Food chem 54:3807–3814
27. Mourouti N, Panagiotakos DB (2013) Soy food consumption and breast cancer. Maturitas 76:118–122
28. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe SI, Itoh N, Shibuya M, Fukami Y (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Che 262:5592–5595
29. Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R, Schweigerer L (1993) Genistein, a dietary-derived inhibitor of in vitro angiogenesis. Proc Natl Acad Sci 90:2690–2694
30. Szymczak G, Wójciak-Kosior M, Sowa I, Zapała K, Strzemski M, Kocjan R (2017) Evaluation of isoflavone content and antioxidant activity of selected soy taxa. J Food Comp Anal 57:40–48
31. Calvello R, Aresta A, Trapani A, Zambonin C, Cianciulli A, Salvatore R, Clodoveo ML, Corbo F, Franchini C, Panaro MA (2016) Bovine and soybean milk bioactive compounds: effects on inflammatory response of human intestinal Caco-2 cells. Food Chem 210:276–285
32. Patel RP, Boersma BJ, Crawford JH, Hogg N, Kirk M, Kalyanaraman B, Parks DA, Barnes S, Darley-Usmar V (2001) Antioxidant mechanisms of isoflavones in lipid systems: paradoxical effects of peroxyl radical scavenging. Free Radic Biol Med 31:1570–1581
33. Lai H, Yen G (2002) Inhibitory effect of isoflavones on peroxynitrite-mediated low-density lipoprotein oxidation. Biosci Biotechnol Biochem 66:22–28
34. Suzuki K, Koike H, Matsui H, Ono Y, Hasumi M, Nakazato H, Okugi H, Sekine Y, Oki K, Ito K, Yamamoto T (2002) Genistein, a soy isoflavone, induces glutathione peroxidase in the human prostate cancer cell lines LNCaP and PC-3. Int J Cancer 99:846–852

35. Hwang J, Wang J, Morazzoni P, Hodis H, Sevanian A (2003) The phytoestrogen equol increases nitric oxide availability by inhibiting superoxide production: an antioxidant mechanism for cell-mediated LDL modification. Free Radic Biol Med 34:1271–1282
36. Anthony M, Clarkson T, Williams J (1998) Effects of soy isoflavones on atherosclerosis: potential mechanisms. Am J Clin Nutr 68:1390–1393
37. Tikkanen M, Wahala K, Ojala S, Vihma V, Adlercreutz H (1998) Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci 95:3106–3110
38. Wiseman H, D O'Reilly J, Adlercreutz H, Mallet AI, Bowey EA, Rowland IR, Sanders TA (2000) Isoflavone phytoestrogens consumed in soy decrease F2-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am J Clin Nutr 72:395–400
39. Hwang J, Sevanian A, Hodis H, Ursini F (2000) Synergistic inhibition of LDL oxidation by phytoestrogens and ascorbic acid. Free Radic Biol Med 29:79–89
40. Kapiotis S, Hermann M, Held I, Seelos C, Ehringer H, Gmeiner B (1997) Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage by atherogenic LDL. Arterioscler Thromb Vasc Biol 17:2868–2874
41. Cuevas A, Irribarra V, Castillo O, Yanez M, Germain A (2003) Isolated soy protein improves endothelial function in postmenopausal hypercholesterolemic women. Eur J Clin Nutr 57:889–894
42. Steinberg F, Guthrie N, Villablanca A, Kumar K, Murray M (2003) Soy protein with isoflavones has favorable effects on endothelial function that are independent of lipid and antioxidant effects in healthy postmenopausal women. Am J Clin Nutr 78(1):123–130
43. Niamnuy C, Nachaisin M, Laohavanich J, Devahastin S (2011) Evaluation of bioactive compounds and bioactivities of soybean dried by different methods and conditions. Food Chem 129:899–906
44. Lee J (2006) Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats. Life Sci 79:1578–1584
45. Jayagopal V, Albertazzi P, Kilpatrick ES, Howarth EM, Jennings PE, Hepburn DA, Atkin SL (2002) Beneficial effects of soy phytoestrogen intake in postmenopausal women with type 2 diabetes. Diabetes Care 25:1709–1714
46. Harborne J, Williams C (2000) Advances in flavonoid research since 1992. Phytochemistry 55:481–504
47. Heisig P (2001) Inhibitors of bacterial topoisomerases: mechanisms of action and resistance and clinical aspects. Planta Med 67:3–12
48. Bernard FX, Sable S, Cameron B, Provost J, Desnottes JF, Crouzet J, Blanche F (1997) Glycosylated flavones as selective inhibitors of topoisomerase IV. Antimicrob Agents Chemother 41:992–998
49. Kim Y, Jang Y, Kim D, Ko H, Han E, Lee C (2001) Differential regulation of protein tyrosine kinase on free radical production, granule enzyme release, and cytokine synthesis by activated murine peritoneal macrophages. Biochem Pharmacol 61:87–96
50. Naucler C, Grinstein S, Sundler R, Tapper H (2002) Signaling to localized degranulation in neutrophils adherent to immune complexes. J Leukoc Biol 71:701–710
51. Verdrengh M, Collins L, Bergin P, Tarkowski A (2004) Phytoestrogen genistein as an anti-staphylococcal agent. Microb Infect 6:86–92
52. Singh BP, Vij S, Hati S (2014) Functional significance of bioactive peptides derived from soybean. Peptides 54:171–179
53. Singh BP, Vij S (2017) Growth and bioactive peptides production potential of Lactobacillus plantarum strain C2 in soy milk: a LC-MS/MS based revelation for peptides biofunctionality. LWT-Food Sci Technol 86:293–301
54. Mellander O (1950) The physiological importance of the casein phosphopeptide calcium salts. II. Peroral calcium dosage of infants. Some aspects of the pathogenesis of rickets. Acta Soc Bot Pol 55:247–255
55. Korhonen H, Pihlanto A (2003) Food-derived bioactive peptides-opportunities for designing future foods. Curr Pharm Des 9:1297–1308
56. Matsui T, Matsufuji H, Seki E, Osajima K, Nakashima M, Osajima Y (1993) Inhibition of angiotensin I-converting enzyme by *Bacillus licheniformis* alkaline protease hydrolyzates derived from sardine muscle. Biosci Biotechnol Biochem 57:922–925

57. Li CH, Matsui T, Matsumoto K, Yamasaki R, Kawasaki T (2002) Latent production of angiotensin I-converting enzyme inhibitors from buckwheat protein. J Pept Sci 8:267–274
58. Yoshikawa M, Takahashi M, Yang S (2003) Delta opioid peptides derived from plant proteins. Curr Pharm Des 9:1325–1330
59. de Mejia E, Ben O (2006) Soybean bioactive peptides: a new horizon in preventing chronic diseases. Sex Reprod Menopause 4:91–95
60. Vij S, Hati S, Yadav D (2011) Biofunctionality of probiotic soy yoghurt. Food Nutr Sci 2:502
61. Maruyama N, Maruyama Y, Tsuruki T, Okuda E, Yoshikawa M, Utsumi S (2003) Creation of soybean β-conglycinin β with strong phagocytosis-stimulating activity. Biochim Biophys Acta, Proteins Proteomics 1648:99–104
62. Gibbs BF, Zougman A, Masse R, Mulligan C (2004) Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. Food Res Int 37:123–131
63. Seppo L, Jauhiainen T, Poussa T, Korpela R (2003) A fermented milk high in bioactive peptides has a blood pressure–lowering effect in hypertensive subjects. Am J Clin Nutr 77:326–330
64. Tsai JS, Lin YS, Pan BS, Chen TJ (2006) Antihypertensive peptides and γ-aminobutyric acid from prozyme 6 facilitated lactic acid bacteria fermentation of soymilk. Process Biochem 41:1282–1288
65. Kuba M, Tanaka K, Tawata S, Takeda Y, Yasuda M (2003) Angiotensin I-converting enzyme inhibitory peptides isolated from tofuyo fermented soybean food. Biosci Biotechnol Biochem 67:1278–1283
66. Shimakage A, Shinbo M, Yamada S (2012) ACE inhibitory substances derived from soy foods. Int J Biol Macromol 12:72–80
67. Kodera T, Nio N (2002) Angiotensin converting enzyme inhibitors. PCT Int Appl, WO 2002055546 A1 18 43 p. Kokai Tokkyo Koho
68. Kitts DD, Weiler K (2003) Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. Curr Pharm Des 9:1309–1323
69. Dhayakaran R, Neethirajan S, Weng X (2016) Investigation of the antimicrobial activity of soy peptides by developing a high throughput drug screening assay. Biochem Biophys Rep 6:149–157
70. Cheng AC, Lin HL, Shiu YL, Tyan YC, Liu CH (2017) Isolation and characterization of antimicrobial peptides derived from Bacillus Subtilis E20-fermented soybean meal and its use for preventing Vibrio infection in shrimp aquaculture. Fish Shellfish Immunol 67:270–279
71. Yoshikawa M, Fujita H, Matoba N, Takenaka Y, Yamamoto T, Yamauchi R, Tsuruki H, Takahata K (2000) Bioactive peptides derived from food proteins preventing lifestyle-related diseases. Biofactors 12:143–146
72. Mercier A, Gauthier SF, Fliss I (2004) Immunomodulating effects of whey proteins and their enzymatic digests. Int Dairy J 14:175–183
73. Kwon DY, SW O, Lee JS, Yang HJ, Lee SH, Lee JH, Lee YB, Sohn HS (2002) Amino acid substitution of hypocholesterolemic peptide originated from glycinin hydrolyzate. Food Sci Biotechnol 11:55–61
74. Lammi C, Zanoni C, Arnoldi A (2015) IAVPGEVA, IAVPTGVA, and LPYP, three peptides from soy glycinin, modulate cholesterol metabolism in HepG2 cells through the activation of the LDLR-SREBP2 pathway. J Funct Foods 14:469–478
75. Takenaka Y, Utsumi S, Yoshikawa M (2000) Introduction of enterostatin (VPDPR) and a related sequence into soybean proglycinin A1aB1b subunit by site-directed mutagenesis. Biosci Biotechnol Biochem 64:2731–2733
76. Kim SE, Kim HH, Kim JY, Kang YI, Woo HJ, Lee HJ (2000) Anticancer activity of hydrophobic peptides from soy proteins. Biofactors 12:151–155
77. Galvez AF, Revilleza MJR, De Lumen BO (1997) A novel methionine-rich protein from soybean cotyledon: cloning and characterization of cDNA (accession no. AF005030). Plant gene register# PGR97-103. Plant Physiol 114:1567–1569
78. Yokomizo A, Takenaka Y, Takenaka T (2002) Antioxidative activity of peptides prepared from Okara protein. Food Sci Technol Res 8:357–359

79. Chen HM, Muramoto K, Yamauchi F, Nokihara K (1996) Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. J Agric Food Chem 44:2619–2623
80. Chen HM, Muramoto K, Yamauchi F, Fujimoto K, Nokihara K (1998) Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. J Agric Food Chem 46:49–53
81. Nishi T, Hara H, Tomita F (2003) Soybean β-conglycinin peptone suppresses food intake and gastric emptying by increasing plasma cholecystokinin levels in rats. J Nutr 133:352–357
82. Clemente A (2000) Enzymatic protein hydrolysates in human nutrition. Trends Food Sci Technol 11:254–262
83. Agyei D, Danquah MK (2011) Industrial-scale manufacturing of pharmaceutical-grade bioactive peptides. Biotechnol Adv 29:272–277
84. Najafian L, Babji AS (2012) A review of fish-derived antioxidant and antimicrobial peptides: their production, assessment, and applications. Peptides 33:178–185
85. Byun T, Kofod L, Blinkovsky A (2001) Synergistic action of an X-prolyl dipeptidyl aminopeptidase and a non-specific aminopeptidase in protein hydrolysis. J Agric Food Chem 49:2061–2063
86. Franek F, Hohenwarter O, Katinger H (2000) Plant protein hydrolysates: preparation of defined peptide fractions promoting growth and production in animal cells cultures. Biotechnol Prog 16:688–692
87. Kong X, Guo M, Hua Y, Cao D, Zhang C (2008) Enzymatic preparation of immunomodulating hydrolysates from soy proteins. Bioresour Technol 99:8873–8879
88. Sarmadia BH, Ismail A (2010) Antioxidative peptides from food proteins: a review. Peptides 31:1949–1956
89. Korhonen H, Pihlanto-Leppäla A, Rantamäki P, Tupasela T (1998) Impact of processing on bioactive proteins and peptides. Trends Food Sci Technol 9:307–319
90. Savijoki K, Ingmer H, Varmanen P (2006) Proteolytic systems of lactic acid bacteria. Appl Microbiol Biotechnol 71:394–406
91. Kunji ER, Mierau I, Hagting A, Poolman B, Konings WN (1996) The proteotytic systems of lactic acid bacteria. Antonie Van Leeuwenhoek 70:187–221
92. Christensen JE, Dudley EG, Pedersin JA, Steele JL (1999) Peptidases and amino acid catabolism in lactic acid bacteria. Antonie Leeuwenhoek 76:217–246
93. Dhananjay S, Kulkarni S, Kapanoor KG, Naganagouda VK, Veerappa HM (2006) Reduction of flatus-inducing factors in soymilk by immobilized α-galactosidase. Biotechnol Appl Biochem 45:51–57
94. Shimizu M (2004) Food-derived peptides and intestinal functions. Biofactors 21:43–48
95. Fekete AA, Givens DI, Lovegrove JA (2013) The impact of milk proteins and peptides on blood pressure and vascular function: a review of evidence from human intervention studies. Nutr Res Rev 26:177–190
96. Li-Chan EC (2015) Bioactive peptides and protein hydrolysates: research trends and challenges for application as nutraceuticals and functional food ingredients. Curr Opin Food Sci 1:28–37
97. Nongonierma AB, FitzGerald RJ (2015) The scientific evidence for the role of milk protein-derived bioactive peptides in humans: a review. J Funct Foods 17:640–656
98. Natesh R, Schwager SL, Sturrock ED, Acharya KR (2003) Crystal structure of the human angiotensin-converting enzyme–lisinopril complex. Nature 421:551–554
99. Johnston CI (1992) Renin-angiotensin system: a dual tissue and hormonal system for cardiovascular control. J Hypertens 10:S27
100. Cat AND, Touyz RM (2011) A new look at the renin–angiotensin system focusing on the vascular system. Peptides 32:2141–2150
101. Jao CL, Huang SL, Hsu KC (2012) Angiotensin I-converting enzyme inhibitory peptides: inhibition mode, bioavailability, and antihypertensive effects. Biomedicine 2:130–136
102. Wu J, Ding X (2001) Hypotensive and physiological effect of angiotensin converting enzyme inhibitory peptides derived from soy protein on spontaneously hypertensive rats. J Agric Food Chem 49:501–506

103. Chiang WD, Tsou MJ, Tsai ZY, Tsai TC (2006) Angiotensin I-converting enzyme inhibitor derived from soy protein hydrolysate and produced by using membrane reactor. Food Chem 98:725–732
104. Rho SJ, Lee JS, Chung YI, Kim YW, Lee HG (2009) Purification and identification of an angiotensin I-converting enzyme inhibitory peptide from fermented soybean extract. Process Biochem 44:490–493
105. Alauddin M, Shirakawa H, Hiwatashi K, Shimakage A, Takahashi S, Shinbo M, Komai M (2015) Processed soymilk effectively ameliorates blood pressure elevation in spontaneously hypertensive rats. J Funct Foods 14:126–132
106. Nakahara T, Yamaguchi H, Uchida R (2012) Effect of temperature on the stability of various peptidases during peptide-enriched soy sauce fermentation. J Biosci Bioeng 113:355–359
107. Lassissi TA, Hettiarachchy NS, Rayaprolu SJ, Kannan A, Davis M (2014) Functional properties and angiotensin-I converting enzyme inhibitory activity of soy–whey proteins and fractions. Food Res Int 64:598–602
108. Li S, Zhao Y, Zhang L, Zhang X, Huang H, Li D, Niu C, Yang Z, Wang Q (2012) Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. Food Chem 135:1914–1919
109. Yang JH, Mau JL, Ko PT, Huang LC (2000) Antioxidant properties of fermented soybean broth. Food Chem 71:249–254
110. Ranamukhaarachchi S, Meissner L, Moresoli C (2013) Production of antioxidant soy protein hydrolysates by sequential ultrafiltration and nanofiltration. J Membr Sci 429:81–87
111. Elias RJ, Kellerby SS, Decker EA (2008) Antioxidant activity of proteins and peptides. Crit Rev Food Sci Nutr 48:430–441
112. Chen HM, Muramoto K, Yamauchi F (1995) Structural analysis of antioxidative peptides from soybean. Beta-Conglycinin. J Agric Food Chem 43:574–578
113. Coscueta ER, Amorim MM, Voss GB, Nerli BB, Picó GA, Pintado ME (2016) Bioactive properties of peptides obtained from Argentinian defatted soy flour protein by Corolase PP hydrolysis. Food Chem 198:36–44
114. Beermann C, Euler M, Herzberg J, Stahl B (2009) Anti-oxidative capacity of enzymatically released peptides from soybean protein isolate. Eur Food Res Technol 229:637–644
115. Xiao Y, Wang L, Rui X, Li W, Chen X, Jiang M, Dong M (2015) Enhancement of the antioxidant capacity of soy whey by fermentation with Lactobacillus plantarum B1–6. J Funct Foods 12:33–44
116. Sanjukta S, Rai AK, Muhammed A, Jeyaram K, Talukdar NC (2015) Enhancement of antioxidant properties of two soybean varieties of Sikkim Himalayan region by proteolytic *Bacillus subtilis* fermentation. J Funct Foods 14:650–658
117. Puchalska P, Marina ML, García MC (2014) Isolation and identification of antioxidant peptides from commercial soybean-based infant formulas. Food Chem 148:147–154
118. García-Nebot MJ, Recio I, Hernández-Ledesma B (2014) Antioxidant activity and protective effects of peptide lunasin against oxidative stress in intestinal Caco-2 cells. Food Chem Toxicol 65:155–161
119. Maroti G, Kereszt A, Kondorosi E, Mergaert P (2011) Natural roles of antimicrobial peptides in microbes, plants and animals. Res Microbiol 162:363–374
120. Kamatou GPP, Viljoen AM, Gono-Bwalya AB, Van Zyl RL, Van Vuuren SF, Lourens ACU, Baser KHC, Demirchi B, Lindsey KL, Staden JV, Steenkamp P (2005) The in vitro pharmacological activities and a chemical investigation of three south African salvia species. Ethnopharmacol 102:382–390
121. Matsuzaki K (1999) Why and how are peptide–lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Bio Biophy Acta 1462:1–10
122. de Castro RJS, Sato HH (2015) Biologically active peptides: processes for their generation, purification and identification and applications as natural additives in the food and pharmaceutical industries. Food Res Int 74:185–198

123. Vasconcellos FCS, Woiciechowski AL, Soccol VT (2014) Antimicrobial and antioxidant properties of β-conglycinin and glycinin from soy protein isolate. Int J Curr Microbiol. Appl Sci 3:144–157
124. Vattem DA, Lin YT, Ghaedian R, Shetty K (2005) Cranberry synergies for dietary management of *Helicobacter pylori* infections. Process Biochem 40:1583–1592
125. Singh BP, Vij S, Hati S, Singh D, Kumari P, Minj J (2015) Antimicrobial activity of bioactive peptides derived from fermentation of soy milk by *Lactobacillus plantarum* C2 against common foodborne pathogens. Int J Fermented Foods 4:99–99
126. Chang HC, Lewis D, Tung CY, Han L, Henriquez SM, Voiles L, Robertson MJ (2014) Soy peptide lunasin in cytokine immunotherapy for lymphoma. Cancer Immunol Immunother 63:283–295
127. de Mejia EG, Dia VP (2009) Lunasin and lunasin-like peptides inhibit inflammation through suppression of NF-κB pathway in the macrophage. Peptides 30:2388–2398
128. Tung CY, Flores S, Han L, Yao S, Zhou B, Sun J, Chang HC (2014) Activation of dendritic cells by soypeptide lunasin as a novel vaccine adjuvant (VAC6P. 942). J Immunol 192:140–143
129. Anthony MS, Clarkson TB, Bullock BC, Wagner JD (1997) Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. Arterioscler Thromb Vasc Biol 17:2524–2531
130. Udenigwe CC, Rouvinen-Watt K (2015) The role of food peptides in lipid metabolism during Dyslipidemia and associated health conditions. Int J Mol Sci 16:9303–9313
131. Potter SM (1995) Overview of proposed mechanisms for the hypocholesterolemic. J Nutr 125:606S
132. Sagara M, Kanda T, NJelekera M, Teramoto T, Armitage L, Birt N, Brit C, Yamori Y (2004) Effects of dietary intake of soy protein and isoflavones on cardiovascular disease risk factors in high risk, middle-aged men in Scotland. J Am Coll Nutr 23:85–91
133. Kobayashi M, Hirahata R, Egusa S, Fukuda M (2012) Hypocholesterolemic effects of lactic acid-fermented soymilk on rats fed a high cholesterol diet. Forum Nutr 4:1304–1316
134. Wang SH, Zhang C, Yang YL, Diao M, Bai MF (2008) Screening of a high fibrinolytic enzyme producing strain and characterization of the fibrinolytic enzyme produced from *Bacillus subtilis* LD-8547. World J Microbiol Biotechnol 24:475–482
135. Sugano M, Goto S, Yamada Y, Yoshida K, Hashimoto Y, Matsuo T, Kimoto M (1990) Cholesterol-lowering activity of various undigested fractions of soybean protein in rats. J Nutr 120:977–985
136. Hori G, Wang MF, Chan YC, Komtatsu T, Wong Y, Chen TH, Yamamoto K, Nagaoka S, Yamamoto S (2001) Soy protein hydrolyzate with bound phospholipids reduces serum cholesterol levels in hypercholesterolemic adult male volunteers. Biosci Biotechnol Biochem 65:72–78
137. Donkor ON, Henriksson A, Singh TK, Vasiljevic T, Shah NP (2007) ACE-inhibitory activity of probiotic yoghurt. Int Dairy J 17:1321–1331
138. Zhong F, Liu J, Ma J, Shoemaker CF (2007) Preparation of hypocholesterol peptides from soy protein and their hypocholesterolemic effect in mice. Food Res Int 40:661–667
139. Ferreira ES, Silva MA, Demonte A, Neves VA (2010) β -Conglycinin (7S) and glycinin (11S) exert a hypocholesterolemic effect comparable to that of fenofibrate in rats fed a high-cholesterol diet. J Funct Foods 2:175–283
140. Bedani R, Rossi EA, Cavallini DCU, Pinto RA, Vendramini RC, Augusto EM, Abdalla DSP, Saad SMI (2015) Influence of daily consumption of synbiotic soy-based product supplemented with okara soybean by-product on risk factors for cardiovascular diseases. Food Res Int 73:142–148
141. Hubert HB, Feinleib M, McNamara PM, Castelli WP (1983) Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham heart study. Circulation 67:968–977
142. Aoyama T, Fukui K, Nakamori T, Hashimoto Y, Yamamoto T, Takamatsu K, Sugano M (2000) Effect of soy and milk whey protein isolates and their hydrolysates on weight reduction in genetically obese mice. Biosci Biotechnol Biochem 64:2594–2600

143. Ascencio C, Torres N, Isoard-Acosta F, Gómez-Pérez FJ, Hernández-Pando R, Tovar AR (2004) Soy protein affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats. J Nutr 134:522–529
144. Greaves KA, Wilson MD, Rudel LL, Williams JK, Wagner JD (2000) Consumption of soy protein reduces cholesterol absorption compared to casein protein alone or supplemented with an isoflavone extract or conjugated equine estrogen in ovariectomized cynomolgus monkeys. J Nutr 130:820–826
145. Iritani N, Nagashima K, Fukuda H, Katsurada A, Tanaka T (1986) Effects of dietary proteins on lipogenic enzymes in rat liver. J Nutr 116:190–197
146. Jang EH, Moon JS, Ko JH, Ahn CW, Lee HH, Shin JK, Park CS, Kang JH (2008) Novel black soy peptides with antiobesity effects: activation of leptin-like signaling and AMP-activated protein kinase. Int J Obes 32:1161–1170
147. Jeong HJ, Jeong JB, Kim DS, Park JH, Lee JB, Kweon DH, Ben O (2002) The cancer preventive peptide lunasin from wheat inhibits core histone acetylation. Cancer Lett 255:42–48
148. Lam Y, Galvez A, de Lumen BO (2003) Lunasin suppresses E1A-mediated transformation of mammalian cells but does not inhibit growth of immortalized and established cancer cell lines. Nutr Cancer 47:88–94
149. Hsieh CC, Hernández-Ledesma B, De Lumen BO (2010) Soybean peptide lunasin suppresses in vitro and in vivo 7, 12-Dimethylbenz anthracene induced tumorigenesis. J Food Sci 75:311–316
150. Ben O (2005) Lunasin: a cancer-preventive soy peptide. Nutr Rev 63:16–21
151. Badger TM, Ronis MJ, Simmen RC, Simmen FA (2005) Soy protein isolate and protection against cancer. J Am Coll Nutr 24:146–149
152. Rayaprolu SJ, Hettiarachchy NS, Chen P, Kannan A, Mauromostakos A (2013) Peptides derived from high oleic acid soybean meals inhibit colon, liver and lung cancer cell growth. Food Res Int 50:282–288
153. Dia VP, de Mejia EG (2013) Potential of lunasin orally-administered in comparison to intraperitoneal injection to inhibit colon cancer metastasis in vivo. J Cancer Ther 4:34–43
154. Hwang JS, Yoo HJ, Song HJ, Kim KK, Chun YJ, Matsui T, Kim HB (2011) Inflammation-related signaling pathways implicating TGFβ are revealed in the expression profiling of MCF7 cell treated with fermented soybean, Chungkookjang. Nutr Cancer 63:645–652
155. Yang X, Zhu J, Tung CY, Gardiner G, Wang Q, Chang HC, Zhou B (2015) Lunasin alleviates allergic airway inflammation while increases antigen-specific tregs. PLoS One 10:e0115330
156. Liener I (1994) Implications of antinutritional components in soybean foods. Crit Rev Food Sci Nutr 34:31–67
157. Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y (2004) Saponins from edible legumes: chemistry, processing, and health benefits. J Med Food 7:67–78
158. Tsai CY, Chen YH, Chien YW, Huang WH, Lin SH (2010) Effect of soy saponin on the growth of human colon cancer cells. World J Gastroenterol 16:3371
159. Kerwin SM (2004) Soy saponins and the anticancer effects of soybeans and soy-based foods. Curr Med Chem Anticancer Agents 4:263–272
160. Konoshima T, Kokumai M, Kozuka M, Tokuda H, Nishino H, Iwahima A (1992) Anti-tumor-promoting activities of afromosin and soyasaponin I isolated from Wistaria brachybotrys. J Nat Prod 55:1776–1778
161. Kim SY, Son KH, Chang HW, Kang SS, Kim HP (1999) Inhibition of mouse ear edema by steroidal and triterpenoid saponins. Arch Pharm Res 22:313–316
162. Yoshiki Y, Okubo K (1995) Active oxygen scavenging activity of DDMP (2, 3-dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-one) saponin in soybean seed. Biosci Biotechnol Biochem 59:1556–1557
163. Kang JH, Sung MK, Kawada T, Yoo H, Kim YK, Kim JS, Yu R (2005) Soybean saponins suppress the release of proinflammatory mediators by LPS-stimulated peritoneal macrophages. Cancer Lett 230:219–227

164. Nishida K, Ohta Y, Araki Y, Ito M, Nagamura Y (1993) Inhibitory effects of group A saponin and group B saponin fractions from soybean seed hypocotyls on radical-initiated lipid peroxidation in mouse liver microcosms. J Clin Biochem Nutr 15:175–184
165. Quilez J, Garcia-Lorda P, Salas-Salvado J (2003) Potential uses and benefits of phytosterols in diet: present situation and future directions. Clin Nutr 22:343–351
166. Awad A, Fink C (2000) Phytosterols as anticancer dietary components: evidence and mechanism of action. J Nutr 130:2127–2130
167. Klippel K, Hiltl D, Schipp B (1997) A multicentric, placebo-controlled, double-blind clinical trial of β-sitosterol (phytosterol) for the treatment of benign prostatic hyperplasia. Br J Urol 80:427–432
168. Kojima S, Soga W, Hagiwara H, Shimonaka M, Saito Y, Inada Y (1986) Visible fibrinolysis by endothelial cells: effect of vitamins and sterols. Biosci Rep 6:1029–1033
169. Jones P, MacDougall D, Ntanios F, Vanstone C (1997) Dietary phytosterols as cholesterol-lowering agents in humans. Can J Physiol Pharmacol 75:217–227
170. Wong N (2001) The beneficial effects of plant sterols on serum cholesterol. Can J Cardiol 17:715–721
171. Ostlund RE Jr (2004) Phytosterols and cholesterol metabolism. Curr Opin Lipidol 15:37–41
172. Lerman RH, Minich DM, Darland G, Lamb JJ, Chang JL, Hsi A, Bland JS, Tripp ML (2010) Subjects with elevated LDL cholesterol and metabolic syndrome benefit from supplementation with soy protein, phytosterols, hops rho iso-alpha acids, and Acacia Nilotica proanthocyanidins. J Clin Lipidol 4:59–68
173. Lukaczer D, DeAnn JL, Lerman RH, Darland G, Schiltz B, Tripp M, Bland JS (2006) Effect of a low glycemic index diet with soy protein and phytosterols on CVD risk factors in postmenopausal women. Nutrition 22:104–113
174. Dong S, Zhang R, Ji YC, Hao JY, Ma WW, Chen XD, Xiao R, HL Y (2016) Soy milk powder supplemented with phytosterol esters reduced serum cholesterol level in hypercholesterolemia independently of lipoprotein E genotype: a random clinical placebo-controlled trial. Nutr Res 36:879–884
175. Shargel L, AB Y (1999) Applied Biophamaceutics & Pharmacokinetics, 4th edn. McGraw-Hill, New York
176. Hur SJ, Lim BO, Decker EA, McClements DJ (2011) In vitro human digestion models for food applications. Food Chem 125(1):1–12
177. Gardner MLG (1988) Gastrointestinal absorption of intact proteins. Annu Rev Nutr 8:329–350
178. Grimble GK (1994) The significant of peptides in clinical nutrition. Annu Rev Nutr 14:419–447
179. Roberts PR, Burney JD, Black KW, Zaloga GP (1999) Effect of chain length on absorption of biologically active peptides from the gastrointestinal tract. Digestion 60:332–337
180. Hausch F, Shan L, Santiago NA, Gray GM, Khosla C (2002) Intestinal digestive resistance of immunodominant gliadin peptides. Am J Physiol Gastrointest Liver Physiol 283:996–1003

Wheat Bran Proteins

12

René R. Balandrán-Quintana and Ana María Mendoza-Wilson

Contents

Abstract

Wheat pericarp, known as bran after milling, is the protective envelope of the grain. It is constituted by seven layers, grouped into three major sections: inner (aleurone), intermediate, and outer bran, respectively. Thousands of proteins are expressed during the wheat grain development, many of them in pericarp, so wheat bran protein content is 15%. Aleurone proteins are mostly storage and metabolic enzymes, while those of intermediate and outer bran layers are mainly involved in stress/defense. Bioactivity of wheat bran proteins has been scarcely explored. Some evidences suggest a role in regulating fat metabolism, which could be preventive of obesity and nonalcoholic fatty liver disease.

R. R. Balandrán-Quintana (✉) · A. M. Mendoza-Wilson
Centro de Investigación en Alimentación y Desarrollo, A.C., Coordinación de Tecnología de Alimentos de Origen Vegetal, Hermosillo, Son., México
e-mail: rbalandran@ciad.mx; mwilson@ciad.mx

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_57

Antihypertensive peptides have been isolated from wheat bran, as well as soluble proteins of low molecular weight with antibacterial properties. Other uses for wheat bran protein extracts, such as enzyme inhibitors in food processing or as reservoirs of proteins for nanoparticle self-assembly, are discussed.

Keywords

Cereals · Bioactive peptides · Wheat bran proteomics · Wheat pericarp · Pericarp proteins · Wheat aleurone · Agro industrial by-products

Abbreviations

2-DE	Two-dimensional electrophoresis
ACE	Angiotensin I converting enzyme
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
Arg	Arginine
ATP	Adenosine triphosphate
CD	Degrees day after anthesis
DAA	Days after anthesis
DiFA (AT)	Dehydroferulic acid (aryltetralin form)
DiFA (BF)	Dehydrodiferulic acid (benzofuran form)
Gln	Glutamine
Ile	Isoleucine
Leu	Leucine
NASH	Nonalcoholic steatohepatitis
Phe	Phenylalanine
PPO	Polyphenol oxidase
Pro	Proline
ROS	Reactive oxygen species
Thr	Threonine
Tyr	Tyrosine
Val	Valine

1 Introduction

In this chapter, proteins from wheat bran are reviewed. Wheat is the third most important cereal world-wide, aside from rice and maize, in terms of production [1]. However, it represents the first supply of proteins and energy for human beings, either directly or indirectly, since it is processed to fabricate a variety of foods and also is used for livestock feed [2, 3].

The most common processing method for the wheat grain is milling, from which flour is obtained as the main product while bran and germ are major by-products [4]. Traditionally, most of the produced bran has been intended to animal consumption, whereas the remaining fraction has found applications in a few food groups as a source of dietary fiber [5]. In recent decades, an increasing interest for wheat bran

has arisen since many potential benefits to human health, derived from its consumption, have been discovered [5–7]. In this context, the wheat bran proteins, despite being of better quality than those of the flour, are underutilized because most are enclosed in cells, surrounded by a cell wall made up of cellulose and hemicelluloses, besides being complexed with polysaccharides, so their bioaccessibility is poor. This entrapment and/or complexation also make the extraction and isolation of proteins a difficult task [8].

Proteins from wheat endosperm have been well studied because of their technological importance [9], but not those of bran. The state of the art in this respect is that wheat bran proteins are distributed among the different layers, aleurone having the most part of them. The classes of proteins range from storage proteins to enzymes involved in cellular metabolism and stress/defense. Most of the proteins in aleurone are globulins, whereas those in middle and outer layers are mainly albumins with metabolic and defense activity [10]. Until now, with the assistance of mass spectrometry and bioinformatics, a great diversity of proteins have been identified from the thousands of spots revealed in two-dimensional electrophoresis (2-DE) gels from bran of mature and developing grains [11–16]. Distribution of proteins among the different layers that compose the bran has been possible to elucidate through proteomic studies assisted by electronic, fluorescence, and optical microscopies [10]. It is also known that pericarp proteins are under strict genetic control because no significant differences in their expression have been found between species or between growing conditions.

Although at the present time not all the proteins which are contained in the 2-DE Coomassie-stained spots have been identified, the available information has permitted to explain in some detail how the wheat bran proteome is interrelated to provide the grain with an arsenal of chemical compounds and enzymes. New evidences showing the advantages that could be taken from the properties of wheat bran proteins, highlighting those of importance to human health, are also discussed.

2 Overview on Wheat Grain

Common wheat (*Triticum aestivum*) is classified within the family of grasses *Poaceae*, subfamily *Pooideae*, and represents only one of the nine species of *Triticeae* tribe that conform to this subfamily, among which also are barley (*Hordeum vulgaris*), rye (*Secale cereale*), and triticale (*Triticosecale* sp.), as well as other wheat species known by their common name as Emmer or farro (*T. dicoccum*); hard Macaroni (*T. durum*); Einkorn the small spelt (*T. monococcum*); Spelt (*T. spelta*), and Rivet or cone (*T. turgidum*). The name *T. aestivum* comprises several forms of hexaploid wheat, although not necessarily all are common or have the necessary characteristics for the bread making. Within each species, there are also subspecies or varieties. The terms variety and cultivar are sometimes used interchangeably; however in a botanical sense, a variety (or subspecies) is the result of natural selection, whereas a cultivar is a plant that has been domesticated, i.e., is the product of a selection made by man, so it does not represent an evolutionary

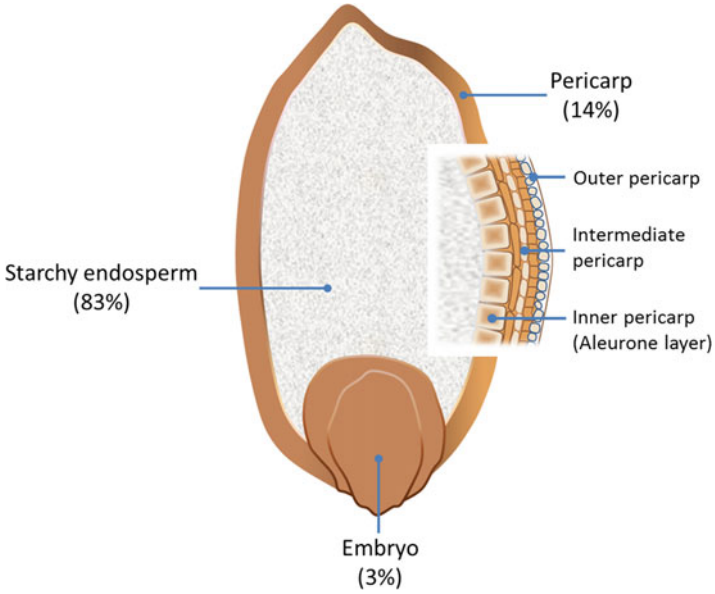


Fig. 1 Schematic representation of the major morphological components of the wheat caryopsis and their distribution by grain weight

status [17]. In this chapter, when reference is made to a cultivar, the taxonomic name will be written, followed by the name of the cultivar in quotation marks.

Wheat grain is a one-seeded fruit or caryopsis, which is constituted by three major morphological components [18] (Fig. 1):

1. Embryo
2. Starchy endosperm
3. Pericarp

The embryo is the reproductive organ from which a new plant is born after fertilization. Embryo represents the smallest percentage of the grain weight, only 3%, and its chemical composition is based on lipids and proteins. The starchy endosperm is the most voluminous part, representing approximately 83% of the grain weight, and is composed basically of starch and proteins. The proteins of the endosperm possess chemical characteristics that grant them the functionality required for the elaboration of bread. This quality makes wheat a unique cereal and is one of the main sources of energy and protein in human food [19]. Pericarp constitutes approximately 14% by weight of the caryopsis. It is an important source of dietary fiber and bioactive compounds, among which phenolic compounds stand out because of their antioxidant capacity [20, 21]. It also contains important amounts of vitamins, minerals, and proteins (Table 1).

Table 1 Chemical composition of wheat pericarp or bran

Component	Amount (per 100 g, unless otherwise indicated)	References
Water	9.9 g	[22]
Protein	15.6 g	[22]
Total lipid (fat)	4.25 g	[22]
n-Fatty acids	0.66 g	[23]
Monoglycerides	0.26 g	[23]
Diglycerides	0.22 g	[23]
Triglycerides	0.20 g	[23]
n-Alkylresorcinols	0.23 g	[23]
Sterols	0.05 g	[23]
Fiber, total dietary	42.8 g	[22]
Sugars	0.41 g	[22]
Xylose	47 mol%	[21]
Arabinose	24 mol%	[21]
Glucose	15 mol%	[21]
Minerals		
Potassium, K	1,182 mg	[22]
Phosphorus, P	1,013 mg	[22]
Magnesium, Mg	611 mg	[22]
Carbohydrates, by difference	64.51 g	[22]
Vitamins		
Thiamin	0.52 mg	[22]
Riboflavin	0.58 mg	[22]
Niacin	13.6 mg	[22]
Vitamin B-6	1.3 mg	[22]
Folate, DFE	79 μg	[22]
Vitamin A	9 IU	[22]
Vitamin E (alpha-tocopherol)	1.49 mg	[22]
Vitamin K	1.9 μg	[22]
Phenolics	(μg/g cell wall)	
Trans-ferulic acid	≈3,600	[21]
5-8′DiFA (BF)	≈1,000	[21]
Cis-ferulic acid	≈1,000	[21]
8-0-4′DiFA	≈1,000	[21]
8-8′DiFA (AT)	≈1,000	[21]

2.1 Protein Synthesis at Different Stages of Grain Development

As in any other biological system, cereal proteins are synthesized in response to an intricate molecular signaling system. In general terms, the ultimate aim of such a system is the formation of a new seed, which is equipped with the necessary to generate another plant and to defense itself against a hostile environment. Embryo, aleurone, and endosperm act in coordination and in a differentiated way after fertilization. From the

embryo, the new plant is born, while the endosperm and aleurone provide all the reserve substances and enzymes necessary for its development [12].

There are variations in literature regarding the number and duration of the stages through which the wheat grain develops. In order to give an overview of the proteins that are expressed along the wheat grain formation, here will be adopted the three-stage development [3, 16]. Duration of each stage is given in days after anthesis (DAA), i.e., days after the flowering period, and sometimes is also expressed as degrees day after anthesis (°CD), i.e., the accumulated daily average temperature, in Celsius degrees. These three stages are:

1. Early development (lag phase) [≈0–16 DAA; ≈ 0–250 °CD]
2. Accumulation of storage compounds (filling phase) [≈14–28 DAA]
3. Maturation-desiccation [≈28 DAA to maturity]

The events occurring in the early development stage and lasting of each one are:

A. Fertilization and development of coenocytic endosperm (0–1 DAA).
B. Cellularization (3–6 DAA; ≈50–100 °CD).
C. Differentiation (7–8 DAA; ≈115–135 °CD). In this event, the formation of main cell types occurs, such as transfer cells, aleurone, starchy endosperm, and cells surrounding the embryo.
D. Cell wall initiation and intense mitotic activity (8–10 DAA; ≈135–165 °CD).
E. End of cell division (at 16 DAA; ≈250 °CD).

Protein expression starts from the beginning of wheat grain formation, as evidenced by the 492 Coomassie-stained spots that have been observed in 2-DE gels from wheat grains in the early development stage (first 2 weeks, from ovary fertilization to 280 °CD) [16]. From these spots, 249 proteins were identified, 52.6% corresponding to enzymes involved in cellular and carbohydrate metabolism; 17.4% to environmental and information processing; 14.6% to genetic information processing; 13.4% to stress and defense responses, and 0.4% to cellular processes, whereas 1.6% were unknown. The most important changes in the proteome, which is defined as "the assortment of proteins produced at a specific time in a particular cell or tissue type" (https://www.nature.com/scitable/definition/proteome-297) at the early stage were observed at 125–195 °CD, when proteins with molecular mass 10–110 kDa were expressed and identified as storage proteins (glutenins and gliadins) [16].

Gliadins (prolamins) are not only the major storage proteins in the wheat grain but also determine the viscoelasticity of dough, so their study has been fundamental for targeting the quality of wheat [24]. Thus, proteome differences between wheat cultivars having different gluten quality properties have been studied. The analysis and identification of proteins in developing grains of *T. aestivum* "Jimai 20" and *T. aestivum* "Zhoumai 16" by 2-DE, assisted by MALDI-TOF/TOF-MS, resulted in variable patterns of protein expression. From 174 Coomassie-stained spots, 84 unique proteins were identified. Fourteen protein spots accumulated in higher abundance in "Jimai 20" than in "Zhoumai 16," the former having superior gluten

quality. Among proteins, the NAD-dependent isocitrate dehydrogenase, triticin precursor, LMW-s glutenin subunit, and replication factor C-like protein were identified, which are likely to be associated with superior gluten quality [24].

In general, proteins identified in the early stage are involved in the cellular metabolism, such as the enzymes of amino acid synthesis, energy production, cell wall initiation, etc., or in DNA repair and replication, cytoskeleton, and structure. At the beginning of the grain formation, also starch granules-associated proteins are expressed. Stable expression of many proteins involved in signal transduction, sugar metabolism, and stress-defense is carried out between 150–280 °CD. By the 195 °CD period, proteins responsible for folding and degradation are expressed, whereas the starch granule-associated proteins continue increasing constantly their expression up to 280 °CD. Heat shock proteins (HSP) are expressed in the early events [16]. In other study, 138 unique proteins were identified by 2-DE and tandem MALDI-TOF/TOF-MS in the 6–20 DAA development period of "Chinese spring" and "NIL-31" wheat cultivars. Proteins involved in carbohydrate metabolism were the most abundant (32.02%), followed by those of synthesis/assembly/degradation of proteins (13.16%), stress/defense (10.53%), and energy production and transport (9.65%) [25].

Nevertheless the resolution attained by 2-DE, it has limitations, as for example the difficulty for separating hydrophobic proteins. In this regard, other study has permitted the identification of up to 1762 proteins in the early stage, comprising from 4 to 12 DAA, by using isobaric tag for relative and absolute quantitation (iTRAQ) and LC–MS/MS methods [26]. Most of the proteins were involved in metabolism, including cell division, cytoskeleton, carbohydrate metabolism, lipid metabolism, nitrogen metabolism, protein synthesis, signal transduction, translation, and transport [26].

Thus, irrespective of the number of identified proteins, results obtained between distinct methodologies are consistent in that the early stage is characterized by an intense activity of enzymes involved in cellular metabolism, and this pattern is similar among wheat species [25–28].

In the middle and late stages of grain development (21–42 DAA), by means of 2-DE assisted by tandem MALDI-TOF/TOF-MS, 130 proteins have been identified in the Coomassie-stained spots. According to differential functions, proteins were classified and distributed as stress/defense (35.4%), carbohydrate metabolism (21.5%), protein synthesis/assembly/degradation (3.1%), storage proteins (6.9%), energy production and transportation (7.7%), photosynthesis (6.2%), transcription/translation (3.9%), signal transduction (4.6%), and unknown function groups (10.8%) [29].

Proteome characterization of the elite wheat *T. aestivum* L. "Shaan 253" in the filling stage (15–30 DAA) by using an iTRAQ approach, resulted in the identification of 859 differentially expressed proteins [30]. Results showed a down regulation over time of proteins involved in energy and starch metabolism. On the other hand, storage proteins (high and low molecular weight glutenins) were upregulated, especially at 25–30 DAA. Most of the stress/defense proteins showed upregulation during grain filling, with high expression levels at 30 DAA, i.e., alpha-amylase inhibitors, enzymes involved in the response to drought and oxidative stress, serpins, and xylanase inhibitors, whereas others, like some peroxidases and some HSP, were downregulated with age [30].

3 Wheat Bran

Bran is a slang word used for referring to cereal pericarp, the botanical tissue enveloping the grain and that gives mechanical support and protection to the endosperm [13] (Fig. 1). Wheat pericarp consists of seven layers, which together account for approximately 14% in weight of the grain. For simplicity, authors frequently group these layers to divide the pericarp into three great sections [31] (Fig. 1):

1. Inner pericarp. Consists of the aleurone, a monolayer of vegetative cells that surrounds the endosperm and the germ. Although aleurone botanically belongs to the endosperm, it is considered the innermost part of pericarp because it remains adhered to it after grain milling [32].
2. Intermediate pericarp. Comprises three layers, composed by the cross cells, tube cells, and testa, all of them made up from dead cells in the mature grain.
3. Outer pericarp. Also is composed of dead cells and comprise the hyaline layer, beeswing and the outer layer.

Pericarp layers are deposited at different developmental stages of the wheat grain, originally they were made up of living cells, each layer having a different function [33]. In the last stage of development, when grain is desiccated to reach the maturity, aleurone remains as a layer of vegetative cells. Aleurone constitutes an essential source of minerals, chemical compounds, and proteins, necessary for the growing of a new plant after fertilization [34]. On the other hand, the cells of the intermediate and outer pericarp layers undergo cell death, serving as mechanical support and protection for the mature grain [31]. However, they not only act as a physical shield but also are dynamic structures since contain a variety of enzymes intended to defend the grain against environmental stresses and pathogenic attacks [10]. Thus, through the development of the wheat grain, a great variety of proteins are expressed, many of them in the pericarp layers [13], resulting in that wheat bran has a total protein content of approximately 15% in weight [22].

After milling, the wheat endosperm is recovered as the main product (the flour) with an approximate yield of 72%, whereas pericarp and germ are obtained as by-products, with average yields of 15% and 2%, respectively [32]. As said before, once separated from endosperm and germ, in the miller's argot, the pericarp is called bran. Bran is obtained in the mill as flakes of 67 μm thick [35]. Mainly two classes of bran are obtained, which are graded as human or animal consumption. Animal consumption grade bran is mixed with impurities, such as straw and other classes of offal. Human consumption grade bran is the purest class of bran and is almost completely composed of bran flakes to which remainders of endosperm and some germ particles are adhered [32].

Traditionally, approximately 90% of the wheat bran is used for animal consumption, whereas the remaining 10% has found applications as ingredient in the elaboration of processed foods, mainly bread and cereal snacks [36, 37], or is used for its inclusion in healthy diets [5]. Wheat bran has been largely appreciated by its high content of dietary fiber and more recently by the benefits to human health which are

attributed to a diversity of bioactive compounds (Table 1). Among the chemical composition of wheat bran, proteins are highlighted, as these nutriments are present in a relatively high proportion (more than 14% DW) [22, 37]. Also is known that their essential amino acid composition is better than that of the flour [38]. However, wheat bran proteins have been largely ignored in terms of a rational use, probably because of the drawbacks arising during their extraction [8].

4 Wheat Bran Proteins

There is a copious literature on proteins from wheat endosperm. In fact, wheat endosperm is by far one of the most studied protein systems because of their technological and economical importance [9]. Wheat bran proteins have not been the subject of such an extensive study but, fortunately, plant biochemistry has been in charge of providing most of the information about it. Accumulated knowledge about classes, structures, properties, and distribution of proteins in the wheat bran is important as allows understanding their role in the grain. Finding explanations to phenomena related to their application, as well as predicting new uses, are also scope of interest in this subject.

It is worth mentioning the great impetus given by 2-DE to the large-scale study of the functions of proteins. In 2-DE, the protein separation is performed in two steps, by isoelectric focusing in the first one and then as a function of the molecular mass. After staining, usually with the Coomasie blue reagent, a number of spots are visualized on the gels, each one corresponding to a unique protein [39].

Proteins from the wheat pericarp or bran will be analyzed in this review in the context of grain development, as well as in terms of potential benefits and applications, at the light of the newest available information. Due to the space constraint, the analysis is not intended to be exhaustive but is expected to serve as a guide to awaken the interest to innovate in relation to the uses of these proteins.

4.1 Distribution of Proteins Among the Various Layers of the Wheat Bran

The methods used to prepare wheat bran for chemical characterization depends on the purpose of such characterization and this issue is very important when it comes to characterize proteins. Since bran has endosperm residues, if the purpose is to characterize the bran proteins in bulk, or fractioning them according to solubility, endosperm residues must be removed from samples by brushing [40] or by rapid washing while immersed in water [41]. Further defatting with solvent of the washed bran is also desirable. On the other hand, if bran will be used for technological purposes, where the presence of endosperm is irrelevant, preparation (if any) will probably consist in only homogenizing the particle size through sieving. In some other cases, stabilization to inhibit enzymatic activities, mainly lipases and proteases, is mandatory [42].

In proteomic studies, whole pericarp or its layers are invariably dissected by hand after imbibing the wheat grains in water. The latter has the objective of facilitating the manual dissection of individual layers. Manual separation of the bran into layers has permitted to analyze the histology and chemical composition of each one. This technique is advantageous because it permits to extract proteins from individual layers, so cross-contamination between layers is avoided; however, this is a laborious task.

The study of proteins from wheat bran dates from the 1920s and the details known until now have been gradually revealed. At that time, wheat bran was subjected to successive extraction steps with different solvents in order to obtain the Osborne fractions [43]. Wheat bran proteins were first classified as albumins, globulins, and prolamins, according to their solubility in water, dilute salt solution, and 70% alcohol, respectively [44]. In further studies, their amino acid content was reported [45], as well as their biological value, assayed in murine model [46]. Since proteins were obtained as a bulk, little or nothing was known about properties or functions of individual proteins. Gel electrophoresis and gel chromatography have helped to separate the proteins and elucidate their molecular masses [47]. In turn, separation has allowed testing some more specific properties, for example, those related to enzymatic activity [48].

Dissecting the bran in its layers and extracting proteins from each layer has been a good approach to study their distribution in the grain pericarp. Microscopic techniques have advanced to such an extent that it is now possible to observe the precise sites in which the different chemical components of cells and tissues are located. The high resolution of the current microscopes allows capturing in detail the cellular compartments. It is also taken advantage of the specific interaction of some cellular components with monoclonal antibodies and fluorescent molecules or with molecules which develop well-defined colors [49]. This makes possible the labeling of components of interest and, therefore, to identify their histological location by fluorescence microscopy or conventional optical microscopy, respectively [50].Intrinsic fluorescence of cellular components is exploited too, as is the case of phenolic compounds and proteins. It is also common to couple one or more microscopic techniques with the chemical labeling or the intrinsic fluorescence, to observe even more details. For example, in the particular case of wheat bran, the resolution of the scanning electron microscope coupled to the sensitivity of the confocal fluorescence microscopy, have allowed the fluorescence immunolocalization of defense proteins in bran cross-sections [10].

Identification and elucidation of protein functions has greatly advanced in recent decades thanks to proteomics, which relies on 2-DE, mass spectrometry, and nucleotide sequencing databases [51–53]. Proteomics is the study of all the proteins of a cell or tissue, rather than individual proteins, in order to integrate the information and thus respond to biological questions [53]. It is a tool which has permitted deepening on the knowledge on distribution and functions of the wheat bran proteins [11].

Proteins in wheat bran are distributed among their different layers [10]. Those of the outer layers are the most easily extracted because they are water soluble; these proteins are basically defense-stress enzymes. Those in the middle layer are a little more difficult to extract, most are oxidative-stress and defense-related proteins. On

the other hand, proteins from the aleurone layer are enclosed in the cells, so their extraction is invariably assisted by cell wall-disrupting methods, such as shear force and ultrasound, among others, or the use of hydrolytic enzymes [8]. Most of the aleurone proteins act as storage proteins, although some also have hydrolytic functions in protein and starch metabolism during germination and grain filling.

4.1.1 Proteins from the Outer Layers

The pericarp of the wheat grain represents the first line of defense against biotic and abiotic stresses, providing a series of protective barriers:

1. Gives mechanical resistance against invasive attempts by insects and pathogenic microorganisms;
2. Has a probable role in regulating the movement of water into dry grains, which is important to germination, preharvest sprouting, expression of dormancy, and conditioning for optimum milling performance [54];
3. Is sheltered with an arsenal of chemical compounds and enzymes, aimed at repelling biotic attacks if mechanical damage cannot be avoided.

The strength of the wheat grain pericarp is provided by the combination of individual mechanical properties of its layers [31]. In the caryopsis development, pericarp plays a key role because it performs photosynthesis and supplies oxygen to the endosperm, besides dissipating excessive energy during the grain-filling [55]. On the other hand, some of the chemical compounds acting against biotic stresses are structural constituents of the mature grain coat, such as the phenolics and some proteins [21]. Others, for example, many enzymes, are produced de novo or activated during one or more stages of the grain development. The components of this protective machinery act not necessarily in isolation, but sometimes they do it together or synchronously. Details on the complicated defense mechanisms of cereal grains are out of the scope of this chapter. Here, the functions of those proteins which are constitutive of layers of wheat bran will be reviewed.

Production of proteins in the course of caryopsis development obeys to gene expression. Such expression is space differentiated and responds to programmed events, which are necessary for the grain to reach a characteristic phenotype and to be equipped for repelling biotic and abiotic stresses [56]. Aleurone and peripheral layers are formed in the early stages of development of the wheat caryopsis [57]. Aleurone provides protection to the endosperm and acts as a reserve of plant hormones, minerals, vitamins, and other nutrients, including proteins. Eventually, these nutriments will be available in the mature grain to participate in the beginning of a new life cycle. Peripheral layers are those comprising the outer and middle pericarp, which, as said before, are the protective tissues of the grain. Some authors include aleurone within the peripheral layers. In this review, this detail if is considered appropriate will be noted.

Proteomic studies on the peripheral layers (including the internal pericarp, hyaline layer, testa, and aleurone) of hexaploid wheat grains, from anthesis to physiological maturity (0–700 °CD), have resulted in the identification of 207 proteins.

Classified in functional categories, the dominating proteins in peripheral layers are those involved in metabolism (32%), storage (25%), stress/defense (17%), and genetic information and processing (12%), whereas the remaining are unknown or hypothetical (6%), involved in environmental information and processing (3%), ATP interconversion proteins (3%), and proteins involved in biosynthesis of secondary metabolites (2%) [15].

The enzyme profiles are in good correlation to the cellular events associated to each development stage in cereal grains, with metabolic proteins having their maximum expression at the early stages (up to 397 °CD). Dominating proteins are those involved in energy metabolism, including photosynthesis and ROS (reactive oxygen species). At 204 °CD, a significant decrease of protein folding, PS, and signal transduction occurs, which is associated to the end of cell differentiation. The maximum functional diversity has been found at 295 °CD, in whose profile the largest category corresponds to the carbohydrate metabolism (enzymes of the citric acid cycle, glycolysis, and sucrose synthesis) as well as ribonucleoproteins, the latter involved in posttranscriptional changes. At the 397–455 °CD stage, when the grain color changes from green to pale yellow, a drastic decrease in the profile of proteins belonging to energy metabolism is observed, indicating that maturity is in progress [15].

In the late stages, proteins which indicate response to hypoxia, such as alcohol dehydrogenase and pyruvate decarboxylase, are identified, as well as enzymes involved in detoxification and oxidative stress. The two latter groups, which include ascorbate-peroxidase, glyoxalase, and thioredoxin peroxidase, are also identified in all stages of development. In the early stages, their presence is due to the production of ROS by photosynthesis in the green pericarp, whereas in the late stages probably help in maintaining the aleurone alive as this is the only tissue that remains alive in the grain after maturity. Finally, in the latter stages (700 °CD), the protein profile is dominated by oxidative stress proteins and by storage and defense-related proteins. Globulins 2, 3, 3B, and 3C have been identified, which are probably specific to aleurone. Xylanase inhibiting proteins, as well as chitinases and endo-chitinases are also dominating in the latter stages [15].

4.2 Proteins from Aleurone

The cereal grain aleurone is the main stock of phytic acid and minerals, besides to be a reservoir of hydrolytic enzymes that act on starch and endosperm proteins during the germination process [58]. Many of the nutrients and bioactive compounds present in the wheat grain are concentrated in the bran [59], especially in the aleurone layer. This layer is rich in B complex vitamins since 82% of all niacin, 61% of pyridoxine, and 37% of riboflavin are contained in it. Between 43% and 61% of the total minerals are also contained in wheat aleurone, being particularly abundant phosphorus, potassium and magnesium. Likewise, aleurone has 43.8% of hemicelluloses (pentosans). Its protein content of 30% represents 15.3% of the total protein content of the wheat grain, at the time that provides 30% of the total Lys, the limiting essential amino acid in wheat [60].

Proteome from wheat aleurone is strongly genetically controlled as significant differences between species have not been found in proteomic studies. Aleurone is the layer of wheat bran with the most abundant variety of proteins. The number of Coomassie-stained spots observed in 2-DE gels of wheat aleurone ranges from approximately 700 to 1300. Proteins from aleurone and peripheral layers of *T. aestivum* "Chinese Spring" and "Recital" did not show quantitative differences. 2-DE gels of the protein extracts revealed 518 to 754 Coomassie-stained spots in the range of 12.6–76 kDa, irrespective to the cultivar [9, 10].

The proteomic study of aleurone cells, isolated from *T. aestivum* "Babber," resulted in 672 Coomassie-stained spots in the 2-D electrophoresis gels. Among these spots, 387 (58% of the total) were identified as globulin-like storage proteins, whereas the remainder 285 corresponded to proteins involved in carbohydrate metabolism, protein synthesis, stress, and defense. The major proteins were 7S globulin storage protein; xylanase inhibitors (XIP-I, XIP-III, TAXI-I); chitinases (26 kDa endochitinase 1 precursor, chitinase class II); pathogenesis related-4 protein; secretory protein; alpha-amylase/subtisilin inhibitor; enolase; dehydrogenases (glucose-3-phosphate-, glucose ribulose- and formate-); cytosolic NADP malic enzyme; aldose reductase, and heat-shock protein 70 (HSP70) [10].

Variation in the number of expressed proteins in aleurone between wheat varieties is higher than that found between species. There have been observed 1258 Coomassie-stained spots in *T. aestivum* aleurone versus 1109 in *T. durum*, with a total of 339 spots differing between genotypes. Among these 339 spots, 30.8% differed within *T. aestivum* varieties and 56.5% within those of *T.durum*, whereas only 12.7% differed between *T.aestivum* and *T. durum* genotypes [14]. On the other hand, 1320 Coomassie-stained spots in 2-DE gels of aleurone from *T. monococcus* have been observed. Eighty eight (91.5% of total) spots were common between *T.aestivum* and *T. monococcus*, which indicates a highly conservated genome A of the hexaploid wheat. Among the 88 spots that differed significantly between species, 53 proteins were identified, 83% of which were storage proteins. Other proteins were lipoprotein, glyceraldehyde-3-phosphate dehydrogenase, and 1-*cis*-peroxiredoxin [61]. Puroindolines are other class of proteins found in aleurone and endosperm, which are products of the major locus that controls the wheat grain hardness. It has been suggested that the action mechanism of puroindolines in affecting the grain texture in soft wheat is by stabilizing the amyloplast membrane during desiccation and maturation. On the other hand, such stabilization does not occur in hard wheat or only to a smaller extent, resulting in a more direct contact between starch and the gluten protein matrix and so in a harder texture [62].

5 Bioactive Properties of Protein and Peptides Isolated from Wheat Bran

Wheat bran proteins are underutilized when wheat bran is consumed by either human or animals. This is because most of proteins are enclosed in the aleurone layer, whose polysaccharide-constituted cell walls (which are resistant to digestion

in the human small intestine) reduce their bioaccessibility [63]. In the commercial pig production, where the main diet consists in plant carbohydrates, a similar case occurs since the nondigestible fiber reduces nutrient and energy digestibility [64]. Since extraction methods for wheat bran proteins are not efficient and non-profitable [8], as far as is known at the present time there are no rationale use of them to take advantage of their functional and nutritive properties. However, scientific evidences regarding potential benefits of proteins or peptides from wheat bran to human health, as well as proposals for their use in fields other than food, could revert this scenario. Interesting discoveries on wheat bran proteins could impact or enhance the perception and interest of people in the consumption of wheat bran, or developing more efficient methods for protein extraction.

It is known that the regular consumption of wheat bran results in reduction of body weight, so it has been suggested as preventive of obesity. However, at the present time, the compounds of bran which are responsible for diminishing body weight have not been yet identified [65]. Fiber is a candidate, but other components could also have a role. It is known the relation of obesity with the lipid metabolism. Fat in excess, which is accumulated in adipose tissues, is further released as glycerol and fatty acids by action of the pancreatic lipase, so the latter is the target of inhibition for developing anti-obesity drugs [66]. In this regard, attention has been directed to search for possible lipase inhibitors as responsible for weight reduction associated to the uptake of wheat bran. The presence of a lipase-inhibitor component solubilized in the aqueous phase of wheat bran was deduced when a loss of 77–94% in the activity of porcine pancreatic lipase on triglyceride hydrolysis was observed when assaying wheat bran at 1% concentration [67]. Such inhibition was reported to occur only with the wheat bran and not when other fibers were assayed, like cellulose or hemicelluloses. Also it was demonstrated that inhibition was independent of adsorption effects on the fiber [67]. Later, it was reported that the lipolysis-inhibitor compound could be a soluble protein [68], which was further corroborated [69]. In fact, two proteins with molecular weights of 24.4 and 27.5 kDa, which inhibited the pancreatic lipase in vitro, were isolated from wheat bran and germ, [70]. The inhibition mechanism was attributed to the interaction between the proteins and the triglyceride substrate, hindering the adsorption of the enzyme on the interface.

In other study, the ability of wheat bran to inhibit the hydrolysis of tributyrin, a model lipid, by the calf pre-gastric lipase, has also been demonstrated in vitro [71]. Reductions in lipase activity up to 30.5% were observed with suspensions of wheat bran, which was soaked by 24 h. Components responsible for the inhibitory effects were not identified, but it was suggested that some low molecular weight compounds, such as polysaccharides or proteins, could be involved [71]. The physiological relevance of lipase inhibition by wheat bran in humans remains to be elucidated, but data suggest that wheat bran proteins likely play a role during fat digestion.

Wheat bran proteins also could be effective protectors against bacterial infections. Proteins with molecular weight $<$90 kDa, isolated from a soluble extract of wheat bran by size exclusion chromatography, may interfere in the attachment of

enterotoxigenic *E. coli* to intestinal porcine epithelial cells [72], the latter used as model of study. The identification of proteins by mass spectrometry resulted in several low molecular weight protease inhibitors, such as Serpin-Z2B, Class II chitinase, endogenous alpha-amylase/subtilisin inhibitor, and alpha-amylase/trypsin inhibitor CM3. Also, evidence suggesting that Globulin 3 (one of the 7S storage globulins of wheat) of 66 kDa could be one of the most firmly attached wheat bran proteins to *E. coli* cells was demonstrated. Although no details on the molecular events leading to the interfering process were studied, the information was placed in the context of developing innovative anti-adhesion therapeutic agents to prevent bacterial pathogenesis [72].

Other interesting fact about the wheat bran proteins is what has to do with bioactive peptides. Interest for bioactive peptides is recent because many sources of them have been identified, including cereals [73], in such a way that there are now in the marketplace several products with a variety of declared bioactivities, such as antihypertensive, antistress, immunomodulatory, among others [74]. By taking advantage of the presence of endogenous proteases, bioactive peptides have been generated by autolysis of wheat bran extracts (i.e., by stimulation of endogenous proteolytic activity through promoting suitable conditions). Studies performed both in vitro and with murine models have demonstrated several effects of these peptides, which could be beneficial to human health.

The autolysis of wheat bran and shorts, both by-products of milling, the shorts being constituted by a mixture of fine particles of bran, aleurone, and germ [32], has resulted in the production of peptides with potential antihypertensive effect [75].These peptides strongly inhibit the Angiotensin I converting enzyme (ACE), which is a key enzyme in the regulation of blood pressure so that is the target of inhibition for the development of antihypertensive drugs [76]. Shorts and bran hydrolysates have shown ACE inhibitory activity (IC_{50}) of 0.08 and 0.14 mg protein/mL, respectively. The highest effect obtained is by autolysis of a mixture of bran and shorts for 12 h, pH 3.2, 40 °C, from which six peptides with ACE inhibitory activity have been isolated: Leu-Arg-Pro, Leu-Gln-Pro, Ile-Gln-Pro, Val-Tyr, Ile-Tyr, and Thr-Phe. The first two peptides are of additional interest as they contain the branched amino acid Leu, which activates the pathway of AMP-activated protein kinase (AMPK) [77, 78]. AMPK is thought to be important for regulating fatty acid metabolism [79]. In this context, there is a pathological condition known as the metabolic syndrome, whose liver manifestation is the fatty liver disease of nonalcoholic origin [80, 81]. A progressive form of fatty liver disease is the nonalcoholic steatohepatitis (NASH), which has as secondary pathogenic factors the oxidative stress and the downregulation of AMPK [82, 83]. For these reasons, the effects of the Leu-Arg-Pro and Leu-Gln-Pro tri-peptides obtained by autolysis of wheat bran have been investigated in murine model on oxidative stress and the AMPK pathway [84].In general terms, the administration of both peptides for 10 days to mice in which NASH was induced by a high-fat diet resulted in a modulation of oxidative stress and overregulation of AMPK. In addition, a significant decrease in the severity of NASH disease was observed [84].

6 Potential of Wheat Bran Proteins for Other Uses

If using in bulk, wheat bran proteins could have some applications in food technology [85]. Since long time, wheat bran proteins have been proposed as enrichers for bread-making flour, because of their high digestibility, a well-balanced amino acid profile, and a protein efficiency ratio comparable to that casein [86–88]. Results have shown the feasibility of adding wheat bran protein-rich flour at levels up to 10% with no negative effects on bread volume. On the other hand, sensory evaluation of bread which was added with the protein-rich flour at levels between 15% and 25%, resulted in lower scores for texture, color crust, and flavor, but these were not objectionable [87].Wheat bran protein concentrates have also shown good water- and oil-holding capacities as well as high dispersibility, the latter related with good emulsification and foaming properties [40].

Inhibition of polyphenol oxidases (PPO) by proteins and peptides from several sources has been reported [89–93]. PPO enzymes are responsible for the oxidation of phenolic compounds, which eventually results in browning of fruits and vegetables [94], so are also target of inhibition for preventing enzymatic browning [95]. Under this scenario, hydrolysates obtained after proteolysis of aqueous extracts of wheat bran proteins (i.e., the albumin fraction) have been assayed on buffered extracts of apple. Kinetic data showed PPO inhibition up to 40% at hydrolysate concentration of 1% (w/v). On the other hand, Lineweaver-Burk plots showed a mixed-type inhibition, indicating that inhibitor or inhibitors interacted with both, the enzyme and the enzyme-substrate complex [41]. Inhibitor molecules were not identified, but authors reasoned about a possible role of proteins or peptides [41]. The globulin fraction of wheat bran proteins has also been tested as inhibitor of PPO, using the amino acid l-Tyr as substrate [96]. Tyrosinase is a PPO broadly distributed in organisms, including mushrooms [97], and is usually used as a model in PPO inhibition studies [98]. Competitive inhibition of the activity of mushroom tyrosinase by globulins from wheat bran, with an inhibition degree of 24% at 2 mM of l-Tyr, has been reported [96].

The great variety of proteins found in wheat bran has motivated some researches for finding alternative uses, beyond nutrition or the traditional food science applications. This is an interesting avenue in the context of an integral scheme for using wheat bran, even more when emerging technologies are involved. Such is the case of bionanotechnology, where proteins have shown to have a great potential for the development of nanostructures with a diversity of applications [99–101]. At this respect, the nanoencapsulation in order to protect or making drugs and bioactive compounds more bioavailable is a promising approach in both the pharmaceutics and food industries [102, 103]. Proteins from animal origin, like albumins, gelatin, elastin, and those from milk and whey, have been the most studied as raw material to fabricate protein nanoparticles by different methods [99]. Plant proteins have also been studied, mainly soy proteins, zein, and wheat gliadins [101].

Recently, there have been some proposals for utilizing aqueous extracts of wheat bran as source of water soluble proteins for nanoparticle formation. Nanotubes with 100 nm of internal diameter, outer diameter 200 nm, and more than 30 μm in length

have been obtained through the apparent self-assembly of peptides released after proteolysis of wheat bran albumins in presence of calcium ions [104]. Since aqueous extracts, once dialyzed and lyophilized, had a complex chemical composition (protein accounted 45% DW), the structure of nanotubes was difficult to be attributed only to proteins. Instead, with the assistance of infrared spectroscopy analysis, it was proposed a model in which polysaccharide-protein complexes, upon protease action, behaved as sheets which were then gradually curved themselves with the subsequent formation of a tubular nanostructure stabilized by calcium bridges [104]. On the other hand, it was reported that undialyzed wheat bran aqueous extracts subjected to cold gelation/desolvation results in formation of spherical structures [105]. Cold-set gelation/desolvation is a method for fabricating nanoparticles [106] which is based in the alkali cold gelation of proteins. Cold gelation consists in a first heating step, further cooling to room temperature and, finally, adding divalent ions [107, 108]. In another more detailed study, it was shown the protein nature of the nanoparticles and that these were formed during the heat treatment, long before the addition of calcium, which is advantageous because one step would be omitted during the process [109].

Protein-rich aqueous extracts of wheat bran are also investigated because of their potential feasibility for acting as scaffolds for biomineralization in vitro [105]. Preliminary results show that a variety of minerals can be formed by just adding calcium ions to aqueous extracts of wheat bran. Among the minerals, some which are biologically induced, like calcium phosphates, are highlighted. The latter indicates that phosphorus present in the extracts plays a role as precursor ion and that other components of the extracts could act as the scaffold matrix. Experiments are in progress to investigate the nature of the matrix and mechanisms of biomineralization.

In summary, in wheat bran there is a great diversity of proteins, which is derived from a differential genetic expression of them during the development of the grain. Most of these proteins are concentrated in the aleurone layer, where storage proteins are predominating. In the layers of the intermediate and external bran, there are proteins mainly involved in stress and defense. Although bioaccessibility of the proteins of the aleurone layer is poor, those of the outer and intermediate bran layers could be more accessible and present bioactivity related to the metabolism of lipids and against enteropathogenic bacteria. Wheat bran proteins are also a source of antihypertensive peptides, as well as peptides that activate the signaling pathway regulated by AMPK. The latter is involved in the prevention of steatohepatitis of nonalcoholic origin because of its involvement in the regulation of fatty acid metabolism. No less important are the applications that wheat bran proteins could have in food technology, as inhibitors of enzymatic darkening or for their functional and nutritional properties. Study of wheat bran proteins could also be the scope of disciplines which are related to emerging technologies, such as the bionanotechnology and biomimetics. In Fig. 2 a summary of all this potential is represented.

As final reflection, the great amount and diversity of proteins that have been identified in wheat bran suggest a variety of properties which are not exploited yet. The newest evidences are pointing in direction to innovation possibilities which are worth to be studied.

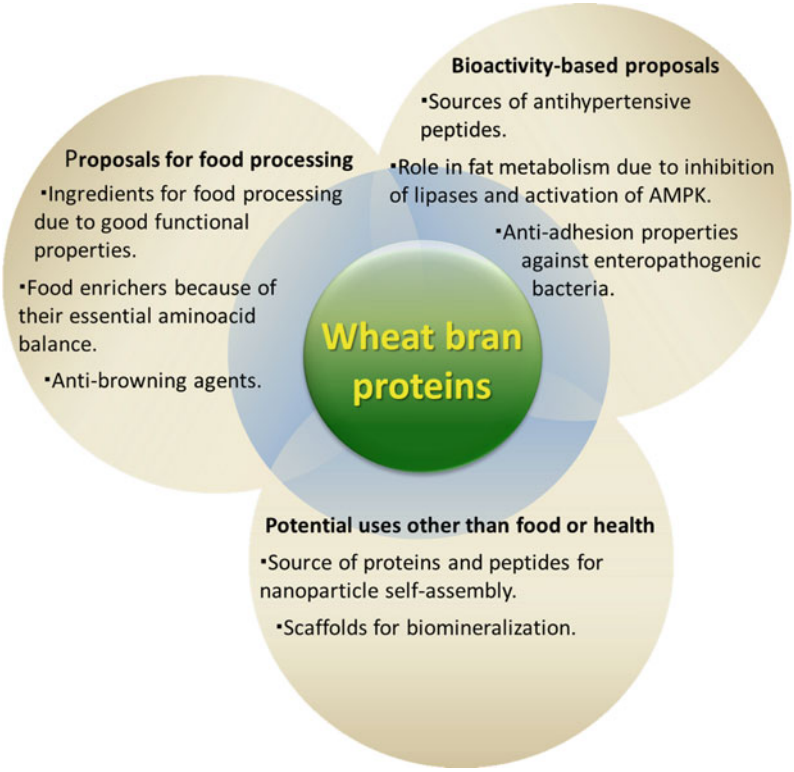


Fig. 2 Summary of the proposals for the use of wheat bran proteins

7 Conclusions

Underutilization of wheat bran proteins and the lack of proposals for their use make the search for innovative alternatives a good opportunity area. Studies showing the potential of wheat bran proteins or peptides in the prevention of hypertension and fat metabolism-related diseases could give some guideline. Also, technological uses of these proteins other than food, for example, in the fabrication of nanoparticles or biomineralization, could be explored in depth in order to add value to wheat bran.

References

1. FAOSTAT (2014) Food and Agriculture Organization of the United Nations – Statistics Division. http://www.fao.org/faostat/en/#data/qc. Accessed 20 Oct 2017
2. Kim K, Anderson J (2015) Forage yield and nutritive value of winter wheat varieties in the southern great plains. Euphytica 202:445–457. https://doi.org/10.1007/s10681-014-1325-8

3. Shewry PR, Mitchell RAC, Tosi P, Wan Y, Underwood C, Lovegrove A, Freeman J, Toole GA, Mills ENC, Ward JL (2012) An integrated study of grain development of wheat (cv. Hereward). J Cereal Sci 56(1):21–30. https://doi.org/10.1016/j.jcs.2011.11.007
4. Posner E (2009) Wheat flour milling. In: Khan K, Shewry R (eds) Wheat chemistry and technology, 4th edn. AACC International, St. Paul, pp 119–152
5. Prückler M, Siebenhandl-Ehn S, Apprich S, Höltinger S, Haas C, Schmid E, Kneifel W (2014) Wheat bran-based biorefinery 1: Composition of wheat bran and strategies of functionalization. LWT – Food Sci Technol 56(2):211–221. https://doi.org/10.1016/j.lwt.2013.12.004
6. Ferguson LR, Harris PJ (1999) Protection against cancer by wheat bran: role of dietary fibre and phytochemicals. Eur J Cancer Prev 8(1):17–25
7. Qu H, Madl RL, Takemoto DJ, Baybutt RC, Wang W (2005) Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. J Nutr 135(3):598–602
8. Balandrán-Quintana R (2018) Recovery of proteins from cereal processing by-products. In: Galanakis C (ed) Sustainable recovery and reutilization of cereal processing by-products. Elsevier Ltd. Kidlington, UK
9. Laubin B, Lullien-Pellerin V, Nadaud I, Gaillard-Martinie B, Chambon C, Branlard G (2008) Isolation of the wheat aleurone layer for 2d electrophoresis and proteomics analysis. J Cereal Sci 48(3):709–714. https://doi.org/10.1016/j.jcs.2008.03.004
10. Jerkovic A, Kriegel AM, Bradner JR, Atwell BJ, Roberts TH, Willows RD (2010) Strategic distribution of protective proteins within bran layers of wheat protects the nutrient-rich endosperm. Plant Physiol 152(3):1459
11. Hu J, Rampitsch C, Bykova NV (2015) Advances in plant proteomics toward improvement of crop productivity and stress resistance. Front Plant Sci 6:209. https://doi.org/10.3389/fpls.2015.00209
12. He M, Zhu C, Dong K, Zhang T, Cheng Z, Li J, Yan Y (2015) Comparative proteome analysis of embryo and endosperm reveals central differential expression proteins involved in wheat seed germination. BMC Plant Biol 15:97. https://doi.org/10.1186/s12870-015-0471-z
13. Chateigner-Boutin A-L, Suliman M, Bouchet B, Alvarado C, Lollier V, Rogniaux H, Guillon F, Larré C (2015) Endomembrane proteomics reveals putative enzymes involved in cell wall metabolism in wheat grain outer layers. J Exp Bot 66(9):2649–2658. https://doi.org/10.1093/jxb/erv075
14. Meziani S, Nadaud I, Gaillard-Martinie B, Chambon C, Benali M, Branlard G (2012) Proteomic analysis of the mature kernel aleurone layer in common and durum wheat. J Cereal Sci 55(3):323–330. https://doi.org/10.1016/j.jcs.2012.01.010
15. Tasleem-Tahir A, Nadaud I, Girousse C, Martre P, Marion D, Branlard G (2011) Proteomic analysis of peripheral layers during wheat (*Triticum aestivum* L.) grain development. Proteomics 11(3):371–379. https://doi.org/10.1002/pmic.201000333
16. Nadaud I, Girousse C, Debiton C, Chambon C, Bouzidi MF, Martre P, Branlard G (2010) Proteomic and morphological analysis of early stages of wheat grain development. Proteomics 10(16):2901–2910. https://doi.org/10.1002/pmic.200900792
17. Morrison LA, Wrigley C (2004) Taxonomic classification of grain species. In: Encyclopedia of grain science, vol 3. Elsevier, New York
18. Grundas S, Wrigley C (2004) Wheat/ultrastructure of the grain, flour, and dough. In: Encyclopedia of grain science, vol 3. Elsevier, New York
19. Barneix AJ (2007) Physiology and biochemistry of source-regulated protein accumulation in the wheat grain. J Plant Physiol 164(5):581–590. https://doi.org/10.1016/j.jplph.2006.03.009
20. Barron C, Surget A, Rouau X (2007) Relative amounts of tissues in mature wheat (*Triticum aestivum* L.) grain and their carbohydrate and phenolic acid composition. J Cereal Sci 45(1):88–96. https://doi.org/10.1016/j.jcs.2006.07.004
21. Parker ML, Ng A, Waldron KW (2005) The phenolic acid and polysaccharide composition of cell walls of bran layers of mature wheat (*Triticum aestivum* L. cv. Avalon) grains. J Sci Food Agric 85(15):2539–2547. https://doi.org/10.1002/jsfa.2304
22. USDA (2016) USDA National Nutrient Database for standard reference. Release 28, slightly revised May 2016. The National Agricultural Library. Accessed 19 Oct 2017

23. Prinsen P, Gutiérrez A, Faulds CB, del Río JC (2014) Comprehensive study of valuable lipophilic phytochemicals in wheat bran. J Agric Food Chem 62(7):1664–1673. https://doi.org/10.1021/jf404772b
24. Guo G, Lv D,Yan X, Subburaj S, Ge P, Li X, Hu Y, Yan Y (2012) Proteome characterization of developing grains in bread wheat cultivars (*Triticum aestivum* L.). BMC Plant Biol 12 (147). http://www.biomedcentral.com/1471-2229/12/147
25. Du D, Gao X, Geng J, Li Q, Li L, Lv Q, Li X (2016) Identification of key proteins and networks related to grain development in wheat (*Triticum aestivum* L.) by comparative transcription and proteomic analysis of allelic variants in *TaGW2-6A*. Front Plant Sci 7:922. https://doi.org/10.3389/fpls.2016.00922
26. Yang M, Dong J, Zhao W, Gao X (2016) Characterization of proteins involved in early stage of wheat grain development by iTRAQ. J Proteome 136:157–166. https://doi.org/10.1016/j.jprot.2016.01.002
27. Arena S, D'Ambrosio C, Vitale M, Mazzeo F, Mamone G, Di Stasio L, Maccaferri M, Curci PL, Sonnante G, Zambrano N, Scaloni A (2017) Differential representation of albumins and globulins during grain development in durum wheat and its possible functional consequences. J Proteome 162(Suppl C):86–98. https://doi.org/10.1016/j.jprot.2017.05.004
28. Mazzeo MF, Di Stasio L, D'Ambrosio C, Arena S, Scaloni A, Corneti S, Ceriotti A, Tuberosa R, Siciliano RA, Picariello G, Mamone G (2017) Identification of early represented gluten proteins during durum wheat grain development. J Agric Food Chem 65(15):3242–3250. https://doi.org/10.1021/acs.jafc.7b00571
29. Zhang N, Chen F, Huo W, Cui D (2015) Proteomic analysis of middle and late stages of bread wheat (*Triticum aestivum* L.) grain development. Front Plant Sci 6:735. https://doi.org/10.3389/fpls.2015.00735
30. Yong C, Yang MM, Dong J, Zhao WC, Gao X (2017) iTRAQ-based quantitative proteome characterization of wheat grains during filling stages. J Integr Agric 16(10):2156–2167. https://doi.org/10.1016/S2095-3119(16)61583-6
31. Antoine C, Peyron S, Mabille F, Lapierre C, Bouchet B, Abecassis J, Rouau X (2003) Individual contribution of grain outer layers and their cell wall structure to the mechanical properties of wheat bran. J Agric Food Chem 51(7):2026–2033. https://doi.org/10.1021/jf0261598
32. Dexter J, Sarkar A (2004) Wheat/dry milling. In: Encyclopedia of grain science, vol 3. Elsevier/Academic, Amsterdam/New York
33. Xiong F, Yu XR, Zhou L, Wang F, Xiong AS (2013) Structural and physiological characterization during wheat pericarp development. Plant Cell Rep 32(8):1309–1320. https://doi.org/10.1007/s00299-013-1445-y
34. Xiong F, Yu XR, Zhou L, Wang Z, Wang F, Xiong AS (2013) Structural development of aleurone and its function in common wheat. Mol Biol Rep 40(12):6785–6792. https://doi.org/10.1007/s11033-013-2795-9
35. Wheat: the raw material (2005) In: Wheat flour milling. References series. AACC International, St. Paul. https://doi.org/10.1094/1891127403.00110.1094/1891127403.001
36. Hemdane S, Leys S, Jacobs PJ, Dornez E, Delcour JA, Courtin CM (2015) Wheat milling by-products and their impact on bread making. Food Chem 187:280–289. https://doi.org/10.1016/j.foodchem.2015.04.048
37. Onipe OO, Jideani AIO, Beswa D (2015) Composition and functionality of wheat bran and its application in some cereal food products. Int J Food Sci Technol 50(12):2509–2518. https://doi.org/10.1111/ijfs.12935
38. Jensen SA, Martens H (1983) The botanical constituents of wheat and wheat milling fractions. II. Quantification by amino acids. Cereal Chem 60(2):172–177
39. Wilkins MR, Pasquali C, Appel RD, Ou K, Golaz O, Sanchez JC, Yan JX, Gooley AA, Hughes G, Humphery-Smith I, Williams KL, Hochstrasser DF (1996) From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. Biotechnology (N Y) 14(1):61–65

40. Idris WH, Babiker EE, El Tinay AH (2003) Fractionation, solubility and functional properties of wheat bran proteins as influenced by pH and/or salt concentration. Nahrung 47(6):425–429. https://doi.org/10.1002/food.200390094
41. Campas-Ríos MDJ, Mercado-Ruiz JN, Valdéz-Covarrubias MA, Islas-Rubio AR, Mendoza-Wilson AM, Balandrán-Quintana RR (2012) Hydrolysates from wheat bran albumin as color-adding agents and inhibitors of apple polyphenol oxidase. J Food Biochem 36(4):470–478. https://doi.org/10.1111/j.1745-4514.2011.00553.x
42. Sudha ML, Ramasarma PR, Venkateswara Rao G (2011) Wheat bran stabilization and its use in the preparation of high-fiber pasta. Food Sci Technol Int 17(1):47–53. https://doi.org/10.1177/1082013210368463
43. Osborne T (1907) The proteins of the wheat kernel. Carnegie Institute, Washington, DC
44. Jones DB, Gersdorff CEF (1923) Proteins of wheat bran: I. Isolation and elementary analyses of a globulin, albumin; and prolamine. J Biol Chem 58(1):117–131
45. Jones DB, Gersdorff CEF (1925) Proteins of wheat bran: II. Distribution of nitrogen, percentages of amino acids and of free amino nitrogen: a comparison of the bran proteins with the corresponding proteins of wheat endosperm and embryo. J Biol Chem 64(2):241–251
46. Murphy JC, Jones DB (1926) Proteins of wheat bran: III. The nutritive properties of the proteins of wheat bran. J Biol Chem 69(1):85–99
47. De Brier N, Gomand SV, Celus I, Courtin CM, Brijs K, Delcour JA (2015) Extractability and chromatographic characterization of wheat (*Triticum aestivum* L.) bran protein. J Food Sci 80(5):C967–C974. https://doi.org/10.1111/1750-3841.12856
48. Yamasaki Y, Konno H, Noda K (2008) Polyphenol oxidase from wheat bran is a serpin. Acta Biochim Pol 55:325–328
49. Dornez E, Holopainen U, Cuyvers S, Poutanen K, Delcour JA, Courtin CM, Nordlund E (2011) Study of grain cell wall structures by microscopic analysis with four different staining techniques. J Cereal Sci 54(3):363–373. https://doi.org/10.1016/j.jcs.2011.07.003
50. Panato A, Antonini E, Bortolotti F, Ninfali P (2017) The histology of grain caryopses for nutrient location: a comparative study of six cereals. Int J Food Sci Technol 52(5):1238–1245. https://doi.org/10.1111/ijfs.13390
51. Shevchenko A, Jensen ON, Podtelejnikov AV, Sagliocco F, Wilm M, Vorm O, Mortensen P, Shevchenko A, Boucherie H, Mann M (1996) Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels. Proc Natl Acad Sci USA 93(25):14440–14445
52. Pandey A, Lewitter F (1999) Nucleotide sequence databases: a gold mine for biologists. Trends Biochem Sci 24(7):276–280
53. Graves PR, Haystead TAJ (2002) Molecular biologist's guide to proteomics. Microbiol Mol Biol Rev 66(1):39–63. https://doi.org/10.1128/MMBR.66.1.39-63.2002
54. Rathjen JR, Strounina EV, Mares DJ (2009) Water movement into dormant and non-dormant wheat (*Triticum aestivum* L.) grains. J Exp Bot 60(6):1619–1631. https://doi.org/10.1093/jxb/erp037
55. Kong LA, Xie Y, Sun MZ, Si JS, Hu L (2016) Comparison of the photosynthetic characteristics in the pericarp and flag leaves during wheat (*Triticum aestivum* L.) caryopsis development. Photosynthetica 54(1):40–46. https://doi.org/10.1007/s11099-015-0153-y
56. Drea S, Leader DJ, Arnold BC, Shaw P, Dolan L, Doonan JH (2005) Systematic spatial analysis of gene expression during wheat caryopsis development. Plant Cell 17(8):2172–2185. https://doi.org/10.1105/tpc.105.034058
57. Becraft PW, Yi G (2011) Regulation of aleurone development in cereal grains. J Exp Bot 62(5):1669–1675. https://doi.org/10.1093/jxb/erq372
58. Becraft P (2007) Aleurone cell development. In: Olsen O (ed) Endosperm, Plant cell monographs, vol 8. Springer, Berlin/Heidelberg. https://doi.org/10.1007/7089_2007_108
59. Mateo Anson N, Hemery YM, Basta A, Haenen GRMM (2012) Optimizing the bioactive potential of wheat bran by processing. Food Funct 3:362–375. https://doi.org/10.1039/c2fo10241b

60. Brouns F, Hemery Y, Price R, Mateo Anson N (2012) Wheat aleurone: separation, composition, health aspects, and potential food use. Crit Rev Food Sci Nutr 52(6):553–568. https://doi.org/10.1080/10408398.2011.589540
61. Meziani S, Nadaud I, Gaillard-Martinie B, Chambon C, Benali M, Branlard G (2014) Proteomic comparison of the aleurone layer in *Triticum aestivum* and *Triticum monococcum* wheat varieties. Curr Proteomics 11(1):71–77
62. Pauly A, Pareyt B, Fierens E, Delcour JA (2013) Wheat (*Triticum aestivum* L. and *T. turgidum* L. Ssp. Durum) kernel hardness: I. Current view on the role of puroindolines and polar lipids. Compr Rev Food Sci Food Saf 12(4):413–426. https://doi.org/10.1111/1541-4337.12019
63. Lattimer JM, Haub MD (2010) Effects of dietary fiber and its components on metabolic health. Nutrients 2(12):1266–1289. https://doi.org/10.3390/nu2121266
64. Jha R, Berrocoso JD (2015) Review: dietary fiber utilization and its effects on physiological functions and gut health of swine. Animal 9(9):1441–1452. https://doi.org/10.1017/S1751731115000919
65. Numan Ahmad M, Rabah Takruri H (2015) The effect of dietary wheat bran on sucrose-induced changes of serum glucose and lipids in rats. Nutr Hosp 32(4):1636–1644. https://doi.org/10.3305/nh.2015.32.4.9457
66. Jeong JY, Jo YH, Kim SB, Liu Q, Lee JW, Mo EJ, Lee KY, Hwang BY, Lee MK (2015) Pancreatic lipase inhibitory constituents from *Morus alba* leaves and optimization for extraction conditions. Bioorg Med Chem Lett 25(11):2269–2274. https://doi.org/10.1016/j.bmcl.2015.04.045
67. Lairon D, Lafont H, Vigne JL, Nalbone G, Leonardi J, Hauton JC (1985) Effects of dietary fibers and cholestyramine on the activity of pancreatic lipase in vitro. Am J Clin Nutr 42(4):629–638
68. Lairon D, Borel P, Termine E, Grataroli R, Chabert C, JC H (1985) Evidence for a proteinic inhibitor of pancreatic lipase in cereals, wheat bran and wheat germ. Nutr Rep Int 32:1107–1113
69. Borel P, Lairon D, Senft M, Chautan M, Lafont H (1989) Wheat bran and wheat germ: effect on digestion and intestinal absorption of dietary lipids in the rat. Am J Clin Nutr 49(6):1192–1202
70. Borel P, Lairon D, Termine E, Grataroli R, Lafont H (1989) Isolation and properties of lipolysis inhibitory proteins from wheat germ and wheat bran. Plant Foods Hum Nutr 39(4):339–348
71. O'Connor CJ, Sun D, Smith BG, Melton LD (2003) The inhibitory effects of brans and their aqueous extracts on the lipolysis of tributyrin catalyzed by calf pregastric lipase. J Food Sci 68(5):1818–1825. https://doi.org/10.1111/j.1365-2621.2003.tb12336.x
72. González-Ortiz G, Bronsoms S, Quarles Van Ufford HC, Halkes SBA, Virkola R, Liskamp RMJ, Beukelman CJ, Pieters RJ, Pérez JF, Martín-Orúe SM (2014) A proteinaceous fraction of wheat bran may interfere in the attachment of enterotoxigenic *E. Coli* K88 (F4+) to porcine epithelial cells. PLoS One 9(8):e104258. https://doi.org/10.1371/journal.pone.0104258
73. Shamloo M, Eck P, Beta T (2015) Angiotensin converting enzyme inhibitory peptides derived from cereals. J Hum Nutr Food Sci 3(1):1057
74. Hayes M, Tiwari BK (2015) Bioactive carbohydrates and peptides in foods: an overview of sources, downstream processing steps and associated bioactivities. Int J Mol Sci 16(9):22485–22508. https://doi.org/10.3390/ijms160922485
75. Nogata Y, Nagamine T, Yanaka M, Ohta H (2009) Angiotensin I converting enzyme inhibitory peptides produced by autolysis reactions from wheat bran. J Agric Food Chem 57(15):6618–6622. https://doi.org/10.1021/jf900857w
76. Anthony CS, Masuyer G, Sturrock ED, Acharya KR (2012) Structure based drug design of angiotensin-I converting enzyme inhibitors. Curr Med Chem 19(6):845–855
77. Li H, Xu M, Lee J, He C, Xie Z (2012) Leucine supplementation increases sirt1 expression and prevents mitochondrial dysfunction and metabolic disorders in high-fat diet-induced obese

mice. Am J Physiol Endocrinol Metab 303(10):E1234–E1244. https://doi.org/10.1152/ajpendo.00198.2012
78. Banerjee J, Bruckbauer A, Zemel MB (2016) Activation of the AMPK/sirt1 pathway by a leucine-metformin combination increases insulin sensitivity in skeletal muscle, and stimulates glucose and lipid metabolism and increases life span in *Caenorhabditis elegans*. Metabolism 65(11):1679–1691. https://doi.org/10.1016/j.metabol.2016.06.011
79. O'Neill HM, Holloway GP, Steinberg GR (2013) AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: implications for obesity. Mol Cell Endocrinol 366(2):135–151. https://doi.org/10.1016/j.mce.2012.06.019
80. Dietrich P, Hellerbrand C (2014) Non-alcoholic fatty liver disease, obesity and the metabolic syndrome. Best Pract Res Clin Gastroenterol 28(4):637–653. https://doi.org/10.1016/j.bpg.2014.07.008
81. Benedict M, Zhang X (2017) Non-alcoholic fatty liver disease: an expanded review. World J Hepatol 9(16):715–732. https://doi.org/10.4254/wjh.v9.i16.715
82. Takaki A, Kawai D, Yamamoto K (2014) Molecular mechanisms and new treatment strategies for non-alcoholic steatohepatitis (NASH). Int J Mol Sci 15(5):7352–7379. https://doi.org/10.3390/ijms15057352
83. Kurumbail RG, Calabrese MF (2016) Structure and regulation of AMPK. EXS 107:3–22. https://doi.org/10.1007/978-3-319-43589-3_1
84. Kawaguchi T, Ueno T, Nogata Y, Hayakawa M, Koga H, Torimura T (2017) Wheat-bran autolytic peptides containing a branched-chain amino acid attenuate non-alcoholic steatohepatitis via the suppression of oxidative stress and the upregulation of AMPK/ACC in high-fat diet-fed mice. Int J Mol Med 39(2):407–414. https://doi.org/10.3892/ijmm.2016.2831
85. Balandrán-Quintana RR, Mercado-Ruiz JN, Mendoza-Wilson AM (2015) Wheat bran proteins: a review of their uses and potential. Food Rev Int 31(3):279–293. https://doi.org/10.1080/87559129.2015.1015137
86. Fellers D, Sinkey V, Shepherd A, Pence J (1966) Solubilization and recovery of protein from wheat millfeeds. Cereal Chem 43:1–13
87. Woerman J, Satterlee L (1974) Extraction and nutritive quality of wheat protein concentrate. Food Technol 28:50–52
88. Saunders R, Betschart A, Edwards R, Kohler G (1975) Nutritive assessment and potential food applications of protein concentrates prepared by alkaline extraction of wheat millfeeds. In: 9th national conference on wheat utilization research, Agricultural Research Service. United States Department of Agriculture, Seattle, pp 9–22
89. Oszmianski J, Lee CY (1990) Inhibition of polyphenol oxidase activity and browning by honey. J Agric Food Chem 38(10):1892–1895. https://doi.org/10.1021/jf00100a002
90. Ates S, Pekyardimci S, Cokmus C (2001) Partial characterization of a peptide from honey that inhibits mushroom polyphenol oxidase. J Food Biochem 25(2):127–137. https://doi.org/10.1111/j.1745-4514.2001.tb00729.x
91. Puangphet A, Tiyaboonchai W, Thongsook T (2015) Inhibitory effect of sericin hydrolysate on polyphenol oxidase and browning of fresh-cut products. Int Food Res J 22(22):1623–1630
92. Kubglomsong S, Theerakulkait C (2014) Effect of rice bran protein extract on enzymatic browning inhibition in vegetable and fruit puree. Kasetsart J 48:205–2013
93. Altunkaya A (2011) Effect of whey protein concentrate on phenolic profile and browning of fresh-cut lettuce (*Lactuca sativa*). Food Chem 128:754–760
94. Araji S, Grammer TA, Gertzen R, Anderson SD, Mikulic-Petkovsek M, Veberic R, Phu ML, Solar A, Leslie CA, Dandekar AM, Escobar MA (2014) Novel roles for the polyphenol oxidase enzyme in secondary metabolism and the regulation of cell death in walnut. Plant Physiol 164(3):1191–1203. https://doi.org/10.1104/pp.113.228593
95. Ali HM, El-Gizawy AM, El-Bassiouny REI, Saleh MA (2015) Browning inhibition mechanisms by cysteine, ascorbic acid and citric acid, and identifying PPO-catechol-cysteine

reaction products. J Food Sci Technol 52(6):3651–3659. https://doi.org/10.1007/s13197-014-1437-0
96. Ortíz-Estrada AM, Mercado-Ruiz JN, García-Robles JM, Islas-Rubio AR, Mendoza-Wilson AM, Balandrán-Quintana RR (2012) Wheat bran globulins: competitive inhibitors of mushroom tyrosinase. Food Sci Biotechnol 21(3):633–635. https://doi.org/10.1007/s10068-012-0082-5
97. García-Molina F, Hiner ANP, Fenoll LG, Rodríguez-Lopez JN, García-Ruiz PA, García-Cánovas F, Tudela J (2005) Mushroom tyrosinase: catalase activity, inhibition, and suicide inactivation. J Agric Food Chem 53(9):3702–3709. https://doi.org/10.1021/jf048340h
98. Seo SY, Sharma VK, Sharma N (2003) Mushroom tyrosinase: recent prospects. J Agric Food Chem 51(10):2837–2853. https://doi.org/10.1021/jf020826f
99. Lohcharoenkal W, Wang L, Chen YC, Rojanasakul Y (2014) Protein nanoparticles as drug delivery carriers for cancer therapy. Biomed Res Int 2014:180549. https://doi.org/10.1155/2014/180549
100. Elzoghby AO, Elgohary MM, Kamel NM (2015) Implications of protein- and peptide-based nanoparticles as potential vehicles for anticancer drugs. Adv Protein Chem Struct Biol 98:169–221. https://doi.org/10.1016/bs.apcsb.2014.12.002
101. Wan ZL, Guo J, Yang XQ (2015) Plant protein-based delivery systems for bioactive ingredients in foods. Food Funct 6(9):2876–2889. https://doi.org/10.1039/c5fo00050e
102. Jafari SM, McClements DJ (2017) Nanotechnology approaches for increasing nutrient bioavailability. Adv Food Nutr Res 81:1–30. https://doi.org/10.1016/bs.afnr.2016.12.008
103. Kumari A, Singla R, Guliani A, Yadav SK (2014) Nanoencapsulation for drug delivery. EXCLI J 13:265–286
104. Chaquilla-Quilca G, Balandran-Quintana RR, Azamar-Barrios JA, Ramos-Clamont Montfort G, Mendoza-Wilson AM, Mercado-Ruiz JN, Madera-Santana TJ, Lopez-Franco YL, Luna-Valdez JG (2016) Synthesis of tubular nanostructures from wheat bran albumins during proteolysis with V8 protease in the presence of calcium ions. Food Chem 200:16–23. https://doi.org/10.1016/j.foodchem.2016.01.005
105. Luna-Valdez JG, Balandrán-Quintana RR, Azamar-Barrios JA, Ramos Clamont-Montfort G, Mendoza-Wilson AM, Mercado-Ruiz JN, Madera-Santana TJ, Rascon-Chu A, Chaquilla-Quilca G (2017) Structural and physicochemical characterization of nanoparticles synthesized from an aqueous extract of wheat bran by a cold-set gelation/desolvation approach. Food Hydrocoll 62:165–173. https://doi.org/10.1016/j.foodhyd.2016.07.034
106. Zhang J, Liang L, Tian Z, Chen L, Subirade M (2012) Preparation and in vitro evaluation of calcium-induced soy protein isolate nanoparticles and their formation mechanism study. Food Chem 133(2):390–399
107. Barbut S, Foegeding EA (1993) Ca^{2+}-induced gelation of pre-heated whey protein isolate. J Food Sci 58:867–871. https://doi.org/10.1111/j.1365-2621.1993.tb09379.x
108. Roff CF, Foegeding EA (1996) Dicationic-induced gelation of pre-denatured whey protein isolate. Food Hydrocoll 10(2):193–198
109. Luna-Valdez JG (2017) Formación y caracterización de nanopartículas de la fracción de albúminas de salvado de trigo por el método de desolvatación. Doctoral dissertation, Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo

Antihypertensive Peptides from Animal Proteins

13

Z. F. Bhat, Susan Mason, James D. Morton, Alaa El-Din A. Bekhit, and Hina F. Bhat

Contents

Z. F. Bhat (✉)
Division of Livestock Products Technology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, Jammu and Kashmir, India
e-mail: zuhaibbhat@yahoo.co.in

S. Mason · J. D. Morton
Department of Wine Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand
e-mail: Sue.Mason@lincolnuni.ac.nz; James.Morton@lincolnuni.ac.nz

A. E.-D. A. Bekhit
Department of Food Sciences, University of Otago, Dunedin, New Zealand
e-mail: aladin.bekhit@otago.ac.nz

H. F. Bhat
Division of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu and Kashmir, India
e-mail: bhat.hina@rediffmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_18

Abstract

Hypertension is considered a major health problem throughout the world among adults, adolescents, as well as children and several preventive and therapeutic interventions are available. In addition to the pharmaceutical drugs and lifestyle changes, significant milestones have been achieved in the past decades in the identification of bioactive peptides from animal proteins with useful antihypertensive activities. The antihypertensive properties of these peptides are attributed to several mechanisms ranging from mineral-binding, opioid-like and antithrombotic properties to inhibition of ACE (angiotensin-converting enzyme). ACE-inhibitory peptides are the most widely studied bioactive peptides with promising potential in hypertension management. In addition to milk and dairy products, which are the major sources of antihypertensive peptides, a remarkable increase has been observed in the documentation of peptides from other animal proteins, such as meat, with demonstrated in vitro and in vivo antihypertensive properties. Numerous opportunities exist in the global market for the development of novel food products and additives based on these antihypertensive peptides for the dietary management of hypertension. This chapter reviews the antihypertensive peptides derived from meat proteins and examines their possible role as a functional ingredient in foods for the management of hypertension.

Keywords

Antihypertensive peptides · ACE-inhibitory peptides · Muscle proteins · Connective tissue proteins · Aging · Meat products

1 Introduction

Being the largest cause of death and leading cause of disability worldwide, cardiovascular diseases are responsible for 17.3 million deaths per year globally [1] and by 2030 this toll is expected to increase to more than 23.6 million deaths worldwide [2]. Global deaths caused by cardiovascular diseases grew by 41% from 1990 to 2013 [3] and are considered to be the cause of one third of female deaths worldwide [4].

Despite of all prevention efforts and recent therapeutic advances, health issues associated with atherosclerotic cardiovascular diseases are increasing [5].

For all racial and ethnic groups, hypertension is a leading independent risk factor for cardiovascular diseases [6] and is thought to be the major preventable cause of premature death throughout the world [7]. Being the leading cause of mortality and morbidity among human adults globally [8, 9], epidemiologic data indicates that approximately 40% of the human population aged above 25 years are affected by hypertension [10] and has been held responsible for 10.4 million deaths per year worldwide [11]. It is the major cause of death among women compared with other metabolic and lifestyle risk factors [12]. Out of the annual 17 million deaths associated with cardiovascular diseases, more than half are caused by hypertension [13]. In the last few decades, childhood hypertension is constantly increasing and has become a major health problem in children [14].

With at least a diastolic blood pressure of 90 mm Hg and a systolic blood pressure of 140 mm Hg [15], the prevalence of hypertension is massive as more than 1 billion people have hypertension worldwide and 80 million people are affected in the USA alone [2, 16]. The number of the adult patients with hypertension has increased from 594 million in 1975 to 1.13 billion in 2015 [17]. Based on the definition used, the childhood hypertension has been recorded to exceed 30% in some reports [14]. Almost 90–95% of all the hypertensive patients belong to the most common class of high blood pressure known as "essential hypertension" which is recognized by an increase in a patient's blood pressure due to an unknown cause as no clear etiology is identified. It seems to be caused by a complex interaction between environmental factors and genetic predisposition. Essential hypertension is regarded as a controllable risk factor of cardiovascular disease as it can be improved with lifestyle choices like eating healthy foods, regular physical activity, decreasing sodium intake, quit smoking, reducing alcohol consumption, and reducing the stress level [18].

2 Management of Hypertension

2.1 Pharmacological Management

A range of pharmacologically active drugs are available on the market for the treatment of hypertension which include calcium channel blockers, ACE (angiotensin converting enzyme) inhibitors, adrenergic inhibitors (such as α- and β-blockers), diuretics, Ang II (angiotensin II) receptor blockers, direct vasodilators, renin inhibitors, and mineralocorticoid receptor antagonists. Drugs that inhibit renin-angiotensin system, which is a significant blood pressure regulator, are extensively used for pharmacological management of hypertension. Hypertension can be safely treated in most patients by using pharmacological drugs; however, these drugs have frequent side effects which may notably affect the quality of life and is a major burden on global healthcare costs.

2.2 Changes in Lifestyle

Lifestyle choices and dietary recommendations are considered utmost important in the management of hypertension. Unhealthy lifestyle and food eating habits are considered to contribute strongly to the persistence and onset of arterial hypertension [19]. The fast pace of modern times has increased preferences for processed and fast foods due to convenience that has contributed significantly to the spread of noncommunicable diseases like hypertension [20]. A substantial body of evidence has demonstrated that certain individual dietary elements and dietary patterns play a significant role in the development of hypertension [21]. Also, there is convincing scientific evidence that the consumption of food rich in both energy and dietary sodium is strongly associated with the development of hypertension [20, 22]. This has compelled many multinational food companies to reformulate their products towards healthier and better nutritional versions [23].

Several reports on hypertension recommend early intervention for pre-hypertension. In addition to the pharmacological drugs, changes in lifestyle including weight loss, reducing sodium intake, limiting alcohol consumption, increasing potassium intake, avoiding smoking, and regular physical activity are advised for both preventing the hypertension over the life span and for management of blood pressure in hypertensive subjects [21, 24, 25]. Dietary strategies to control the blood pressure in hypertensive patients and to lower the risk of hypertension in healthy persons include diets low in sodium and rich in vegetables, fruits, and low-fat milk products [21, 25].

2.3 Functional Foods and Food-Derived Peptides

Over the last few decades, efforts have been made consistently to produce designer and functional foods that contain certain components with blood pressure lowering properties. These foods are intended to supplement or provide an alternative to the pharmaceutical management of hypertension. Any food ingredient or additive with antihypertensive properties, including food-derived peptides, could contribute to the treatment or prevention of hypertension. Peptides with well-established in vitro ACE-inhibitory activities may exert in vivo hypotensive effects if they reach the target site in an active state [26, 27]. Fu et al. [28] suggested that daily consumption of aged beef with naturally developed antihypertensive peptides may play a vital role in maintaining normal blood pressure and indicated the possible use of these peptides for the development of functional beef products, like patties, with antihypertensive properties. Unlike synthetic ACE inhibitor drugs (such as captopril, benazepril, perindopril, enalapril, trandolapril, and quinapril) which have frequent side effects such as cough, hypotension, headaches, skin rashes, fatigue, fetal disorder and so on, food-derived ACE inhibitors are believed to be stable and have minimal side effects [29]. Very few studies have been conducted on the side effects of food-derived ACE-inhibitory peptides to date; however, it is believed if these natural peptides would cause any side effects, those would be milder [30, 31].

3 Antihypertensive Peptides

Latent within the parent protein, bioactive peptides are specific stretches or fragments of food proteins which can be released during food processing like enzymatic hydrolysis or fermentation or during digestion inside the gut and have some physiological benefits to human health beyond their nutritional capabilities. These short sequence fragments are characterized with a low molecular weight and approximately 2–30 amino acid residues in length [32]. Released in vivo or in vitro from food proteins, these peptides have the ability to affect various systems of body like cardiovascular, digestive, immune, endocrine, and nervous system and attribute various health effects including hypotensive, antimicrobial, cholesterol-lowering, opioid, antioxidant, antithrombotic, immunomodulatory, cytomodulatory, and antigenotoxic activity.

Among the various bioactivities, antihypertensive property is widely studied and several peptides from numerous sources have been reported. Proteins of animal origin like meat, milk, whey, egg, blood, and fish proteins have been reported to contain potential antihypertensive peptide sequences in their primary structure which could become active when released during enzymatic or microbial hydrolysis [33]. Table 1 presents some of the identified peptide sequences with antihypertensive properties from different animal proteins. Potentially bioactive ACE-inhibitory peptides are normally short di- or tri-peptides as sequence length plays an important role in the antihypertensive property of a peptide which could easily enter and bind the deep-seated active site of ACE [58, 59]. However, peptide sequences of up to 14 amino acids have been reported to possess antihypertensive properties [60]. These peptides usually contain aromatic or hydrophobic residues at their ultimate and penultimate positions, with proline being particularly favorable residue in both these positions [49, 59, 61, 62]. Besides the sequence length, the potency of the ACE-inhibitory peptides is also determined by the three C-terminal residues, with aromatic residues being most favored [62]. Several studies have suggested that ACE-inhibitory peptides may also possess antioxidant properties due to the shared requirements for both properties in terms of structure and length of peptide [63, 64]. The antihypertensive peptides have attracted global interest for the possible use of their therapeutic potential for the development of antihypertensive foods for dietary management of hypertension. Several new products have already struck the market with antihypertensive claims (such as Ameal S, Danaten, and Evolus) and several are under process of development and validation. Results of extensive in vivo clinical trials are used to support the health claims of these products.

4 Mechanisms of Antihypertensive and ACE-Inhibitory Peptides

Regulation of blood pressure in human body is a complex process involving several metabolic pathways that makes the pathophysiology of hypertension highly complex. It involves the interaction of genetic, environmental, and several other dietary

Table 1 Peptide sequences with antihypertensive properties from different animal proteins

Peptide sequence	IC_{50} (μM)	Protein source (protease used)	Reference
MEVFVP	79.0	Flounder fish (*Paralichthys olivaceus*) muscle (Kojizyme, papain, pepsin, trypsin)	Ko et al. [34]
VSQLTR	105		
LHP	1.6	Camel milk proteins (α-amylase, pepsin, pancreatic and bile fluids)	Tagliazucchi et al. [35]
IY	2.1		
AI	3.4		
IPP	5.0		
VY	7.1		
LY	18.0		
TF	18.0		
FPGPIPK, IPPK, IVPN, QPPQ	–	Buffalo skim milk (papain, pepsin, trypsin)	Mahmoud et al. [36]
RVCL	175	Lizard fish (*Saurida elongata*) muscle (neutral protease)	Wu et al. [37]
LAFNPTQLEGQCHV	–	Sheep cheese whey (β-lactoglobulin) (protease from *Bacillus* sp. P7.)	Corrêa et al. [38]
SWVE	33.88	Egg white proteins (protease from *C. ficifolia* fruit)	Pokora et al. [39]
DILN	73.44		
GASSGMPG	6.90	Pacific cod skin (pepsin, papain, trypsin, neutrase, alcalase, chymotrypsin)	Ngo et al. [40]
LAYA	14.5		
GLPLNLP	18.7	Chum salmon (*Oncorhynchus keta*) skin (α-chymotrypsin, trypsin, papain)	Lee et al. [41]
PLYV	0.05		
DPHI	0.02		
AER	0.11	Leatherjacket (*Meuchenia sp.*) muscle (papain, bromelain, flavourzyme)	Salampessy et al. [42]
EQIDNLQ	0.24		
WDDME	0.01 (g/L)		
VPP, IPP	–	Emmental and gouda cheese	
VPP, IPP, RYLG, RYLGY, HLPLP, AYFYPEL, LHLPLP	–	Cheddar cheese	Stuknytė et al. [43]
IQW	1.40	Egg white protein (ovotransferrin) (pepsin, thermolysin)	Majumder et al. [44, 45]
LKP	2.80		
GL, GI, AI, VP, AY, GLN, ALN, AEK	–	Skimmed goat milk (simulated gastric, pancreatic and bile fluids)	Tagliazucchi et al. [46]
YQEPVLGPVRGPFPIIV	–	Bovine caseins (proteolytic extract from *M. pomifera* latex)	Corrons et al. [47]
PEQSLACQCL QSLVYPFTGPI ARHPHPHLSFM	–	Goat milk (β-lactoglobulin, β-casein, κ-casein) (pepsin)	Ibrahim et al. [48]

(*continued*)

Table 1 (continued)

Peptide sequence	IC_{50} (μM)	Protein source (protease used)	Reference
KHP	>800	Sodium caseinate and purified α-casein (*Aspergillus niger*-derived prolyl endoproteinase)	Norris et al. [49]
NP	>800		
ITP	10.0		
WIQP	14.2		
VLSRYP	36.7	Milk fermented with *Kluyveromyces marxianus* Z17	Li et al. [50]
LRFF	116.9		
PFPEVFGK	108	Bovine αS1-casein (C12 antihypertensive peptide)	Paul et al. [51]
ESLSRLLG	46.7	Ostrich (*Struthio camelus*) egg white proteins (pepsin, pancreatin)	Asoodeh et al. [52]
YQDPRLGPTGELDPATQPI-	14.53	Koumiss (fermented mare's milk)	Chen et al. [53]
VAVHNPVIV			
PKDLREN	9.82		
LLLAHLL	5.19		
NHRNRMMDHVH	13.42		
VLGPVRGPFP	137	β-casein (milk fermented with *E. faecalis* strains CECT 5727, 5728, 5826, and/or 5827)	Quiro et al. [54]
VVVPPF	>1500		
LHLPLP	5.5		
LTQTPVVVPPF	>1500		
VRGPFPIIV	599		
LHLPLPL	425		
LVYPFPGPIPNSLPQNIPP	5.2		
VLGPVRGPFPIIV	>700		
TGPIPN	316	Goat milk – β-casein (*Subtilisin alcalase*)	Geerlings et al. [55]
SLPQ	330		
SQPK	354		
RYLGY	0.71	Skim milk – casein-derived peptides (porcine pepsin A)	Contreras et al. [56]
AYFYPEL	6.58		
YQKFPQY	20.08		
FRADHPFL	3.2	Egg white protein hydrolysates (BC pepsin 1:3000)	Garcés-Rimón et al. [57]
RADHPFL	6.2		
YAEERYPIL	4.7		
YRGGLEPINF	>1000		
ESIINF	>1000		
RDILNQ	435.7		
IVF	33.9		
YQIGL	173.8		
SALAM	229.1		
FSL	172.9		

and physiological factors such as long-term high sodium intake levels, increased sympathetic nervous system activity, increased RAS activity, low dietary calcium and potassium intake, endothelial dysfunction, altered cellular ion channel, vessel resistance variations due to vascular inflammation, and elevated activity of vascular growth factors [65, 66]. The most widely studied pathways, with regard to food-derived antihypertensive peptides, involve those reported to inhibit angiotensin converting enzyme (ACE) in vitro. ACE is a key enzyme that regulates the blood pressure of the body through the renin-angiotensin system. In addition to ACE-inhibitory mechanism, food-derived peptides may help in lowering the blood pressure through mechanisms that target rennin activity, calcium channels, arginine-nitric oxide pathway, endothelin system function, Angiotensin (Ang) receptors, vascular inflammation and oxidative stress, vascular remodeling, and sympathetic nervous system [65, 67, 68]. As the peptides can trigger signaling processes by binding the receptors in the gut, they may not need to be absorbed; however, many peptides exert their antihypertensive effects only when present in relevant amounts in the vascular system.

ACE inhibition is a better physiological target for treatment of clinical hypertension due to its association with two types of blood pressure systems, the rennin-angiotensin system (RAS) and kinin-nitric oxide system (KNOS). The angiotensin converting enzyme (ACE), a heavily glycosylated membrane-bound zinc metalloprotease, is a principle enzyme in RAS that controls the amount of fluids in the body and thus regulates the blood pressure. Angiotensinogen is a prohormone synthesized in liver which is cleaved by rennin produced by kidney to produce a decapeptide Ang I (angiotensin I). Ang I is further cleaved by ACE to produce octapeptide Ang II (angiotensin II) which is a potent vasoconstrictor and acts on vascular smooth muscles. Further, ACE also causes inactivation of bradykinin and kallidin, the vasodilatory peptides of KNOS. Therefore, by blocking the formation of Ang II and by reducing the degradation of bradykinin and kallidin, ACE inhibitors can exert antihypertensive effects. Although, ACE inhibitor pharmaceutical drugs are very much successful in reducing the blood pressure, food-derived antihypertensive peptides are free from any associated side effects and are believed to be safer than pharmaceutical drugs [65].

5 Production of Antihypertensive Peptides

Protein hydrolysates could be produced from protein sources by employing several methods like enzymatic hydrolysis by using proteolytic enzymes, chemical hydrolysis process, and microbial fermentation [69]. Hydrolysates with ACE-inhibitory properties have been generated from several kinds of proteins including proteins of animal origin, like fish protein [70], meat protein [71, 72], milk protein [73], egg protein [44], microbial proteins, like *Saccharomyces cerevisiae* protein [74], or plant proteins, like sorghum grain protein [75]. These peptide hydrolysates could be prepared economically from animal wastes and by-products that are rich in protein and are generally processed into low-cost end products like meat meal, blood meal, and so on [76].

5.1 Chemical Hydrolysis

Solutions of alkalis and acids could be used to cleave the peptide bonds of protein substrates to produce different peptides. There is less control over the hydrolysis which results into hydrolysates of different peptide profiles with reduced bioactivities and nutritional qualities. Higher variations in the end product and use of organic solvents limit the high-end use of these hydrolysates.

5.2 Enzymatic Hydrolysis

Exogenous proteolytic enzymes are widely used to produce peptides of several bioactivities by hydrolyzing various food protein substrates. Novel peptides with higher antihypertensive properties have been discovered by using proteases from various sources like alcalase, pepsin, flavourzyme, trypsin, actinidin, and papain which are commercially available or by using some noncommercially available proteases, such as *Virgibacillus halodenitrificans* SK1–3-7 protease [77]. Due to the milder conditions and better control over the hydrolysis, enzymatic hydrolysis is considered a better method for the production of bioactive peptides. The amino acid composition of the hydrolysates obtained is similar to that of the protein substrates with few modifications depending on the type of enzymes employed for the hydrolysis. This method is suitable for the production of hydrolysates for food and pharmaceutical industries due to noninvolvement of any toxic chemicals or organic solvents [78]. Protein hydrolysates of specific bioactivities could be produced by controlling the conditions using specific enzymes in comparison to chemical hydrolysis method. Protein hydrolysates of different peptide profiles could be obtained by using different enzymes and enzyme:substrate ratio.

5.3 Microbial Fermentation

Several microorganisms that are involved in the fermentation processes of foods have the proteolytic capabilities and could produce protein hydrolysates with bioactive peptides. The most common examples of these microorganisms are the ones involved in the fermentation of dairy products [79]. The best known hypotensive peptides IPP (Ile-Pro-Pr) and VPP (Val-Pro-Pro) were first isolated and identified from fermented milk [76]. Several studies have been conducted for the production of protein hydrolysates by utilization of microbial fermentation [80, 81]. Antioxidative and antimicrobial peptides were produced from tannery fleshings by fermentation using lactic acid bacteria [82]. *Bacillus pumilus* A1, a keratolytic bacterium, has been reported to produce wool waste protein hydrolysates with antioxidative properties [80]. *Bacillus subtilis* A26, another proteolytic bacterium, has been reported to produce protein hydrolysates through fermentation of several fish proteins [81].

5.4 Recombinant DNA Technology

Over the years, many novel genetic engineering-based techniques have been developed for the production of peptides at industrial scale. Recombinant DNA technology is a preferred choice for the production of relatively large peptides consisting of up to several hundred amino acids. This technology is well suited for the production of large quantities of peptides from very inexpensive starting materials; however, it typically requires a long and expensive research and development phase. There are certain challenges in the production of peptides by these methods like degradation of short hypotensive peptides by peptidases or proteases and the threat that expression products may be harmful to the host. These shortcomings have been taken care of by producing the peptides as a part of fusion proteins and afterwards separating the target peptides by using enzymes and purifying them, though that adds to the cost of production. Peptides with sequences FFVAPFPEVFGK, GHIATFQER, HVLPVP, and HHL with antihypertensive properties have been successfully expressed in E. coli [83]. A multimer, a precursor of 11 different hypotensive peptides, was expressed and shown to release peptides during simulated gastrointestinal digestion [84].

5.5 Synthetic Antihypertensive Peptides

Peptides with antihypertensive properties could be conveniently synthesized for therapeutic management of hypertension and is the most popular method of production of peptides in a laboratory [85, 86]. The choice of the method depends on the length and quantity of the desired product. While liquid-phase synthesis is a classical approach for large-scale production of peptides for industrial purposes, the solid-phase approach is now the standard method of production of peptides at the laboratory scale and is most powerful method for synthesis of peptides composed of about 10 to over 100 residues [85]. Peptides with several bioactivities have been synthesized for their therapeutic applications and are currently being used for their therapeutic contribution to the treatment of diabetes, obesity, immunosuppression and gastrointestinal, cardiovascular, osteoporotic, antibacterial, or oncologic diseases [87]. Synthesizing peptides or modifying peptides to improve their therapeutic value is a cost-effective and convenient method of production; however, use of synthetic peptides for the therapeutic purposes cause several and severe side effects [88] which reduces their superiority over therapeutic drugs.

6 Antihypertensive Peptides as Functional Food Ingredients

The search for functional foods with antihypertensive properties has increased as hypertension continues to increase worldwide. Several milk products like Calpis (Calpis Co., Tokyo, Japan), Evolus (Valio, Helsinki, Finland), Ameal (Calpis Co., Tokyo, Japan), and Danaten (Danone, Paris, France) with scientifically proven health

claims are available in the market and exclusive rights of these manufacturers to use these functional ingredients has proven to be a crucial factor in the ultimate success of these products in the market [89]. Table 2 presents some of the commercially available food products with antihypertensive claims. Using antihypertensive peptides as functional ingredients for the development of food products needs certain considerations including characterization of their physicochemical and sensorial properties. Figure 1 shows some of the essential steps in the development of meat peptide-based functional foods with antihypertensive properties. One of the challenges of using peptides as ingredients in food products is the bitter taste of these peptides composed mainly of hydrophobic amino acids which are responsible for both bitterness and antihypertensive properties [83]. However, several masking methods could preferably be used to improve the acceptability. An optimal method for the production of peptides by hydrolysis is required which could generate the output sufficient to meet the industrial scale. The peptides need to retain their antihypertensive properties during the entire stages of industrial level processing and remain stable during long-term storage. Little information is available in the literature about the effects of various processing methods on the activities of antihypertensive peptides. Research is required in some areas like analyzing the interaction of food components and peptides during processing, storage, and digestion.

Dehydration of peptides by using spray drying method has been reported to induce changes in peptide composition and causing nonenzymatic browning and a reduction in amino acid content [83]. Contreras et al. [90] studied the possibility of production of two antihypertensive peptides viz. Ala-Tyr-Phe-Tyr-Pro-Glu-Leu and Arg-Tyr-Leu-Gly-Tyr in a casein hydrolysate at an industrial scale. The peptides were also incorporated into yoghurt and evaluated for stability during various processing conditions and during storage under refrigerated conditions. The peptides retained their ACE-inhibitory properties in vivo in spontaneously hypertensive rats as well as under in vitro conditions. Four synthetic peptides (DFHINQ, GFHI, FHG, and GLSDGEWQ) with ACE-inhibitory properties have been reported to retain stability in water during heat treatment [91].

Another important challenge associated with antihypertensive food products is the bioavailability of peptides which is responsible for differences between in vitro and in vivo activities of peptides. The capacity of peptides to reach the target organ in active form determines the physiological effect of the peptides. The final desired activity of peptides depends on a series of processes involved in the oral administration of peptides. These include the effect of GIT enzymes, brush border peptidases, active intracellular peptidases during absorption through intestinal barrier, and enzymes in the blood once they make it to the circulation [92, 93]. These processes are more likely to affect the sequences which are responsible for the antihypertensive properties of the peptides before they will elicit their response in the target organ. Thus more scientists are interested in evaluating the different factors affecting the bioavailability of different sequences responsible for hypotensive properties. Different studies have been conducted on the effect of digestion on the hypotensive peptides by using in vitro gastrointestinal simulation models. Several studies have also analyzed the intestinal absorption by using in vitro intestinal epithelial

Table 2 Commercially available products with antihypertensive claims

Product	Company	Peptides	Product type
Ameal S 120, Ameal S, Ameal BP	Calpis Co. (Tokyo, Japan)	VPP, IPP	Cultured milk, tablets, capsules, component
Calpis	Calpis Co. (Tokyo, Japan)	VPP, IPP	Uncarbonated sweetened fermented milk beverage
Evolus	Valio (Helsinki, Finland)	VPP, IPP	Calcium-enriched fermented milk
Vasotensin	Metagenics, USA	LKPNM, LKP	Tablets (bonito protein hydrolyzate)
Peptide C12, C12 Peption	DMV International, The Netherlands	FFVAPFPEVFGK	Ingredient (casein protein hydrolyzate)
Tensiocontrol	Bioactor, The Netherlands	RADHPFL, IVF, YAEERYPIL	Ingredient (egg protein hydrolyzate)
Biozate	Davisco Foods International, USA	Whey peptides	Purified hydrolyzed whey protein (powder form)
Lowpept	Innaves Biotech, Spain	RYLGY, AYFYPEL	Tablets, ingredient (powdered casein hydrolyzate)
Casein DP Peptio	Kanebo, Japan	FFVAPFPEVFGK	Soft drink (casein protein hydrolyzate)
Danaten	Danone (Paris, France)	Tripeptides	Fermented milk drink (*Lactobacillus helveticus* DN-119 90)

simulations involving the use of monolayer of intestinal cell lines. While evaluating the effectiveness of hypotensive peptides, improvement in the bioavailability is considered as an important goal.

7 Antihypertensive Peptides from Meat Proteins

With increasing awareness and concern about the health and safety, the quest to replace synthetic drugs with natural compounds is of great interest. Antihypertensive peptides from food proteins could provide an excellent alternative to synthetic drugs [94] which is a major target for the treatment and prevention of clinical hypertension [48]. Several human and animal studies have demonstrated the blood pressure lowering capabilities of the food-derived peptides through ACE inhibition [95, 96]. Proteins from meat and meat by-products are considered as an excellent source of peptides (Fig. 2) with both in vitro and in vivo ACE-inhibitory properties [97]. In the last few decades, peptides derived from meat proteins from several sources, such as chicken muscle [98], porcine muscle [99], and beef muscle [100], have been found to reduce blood pressure through ACE inhibition [101]. Peptides with ACE-

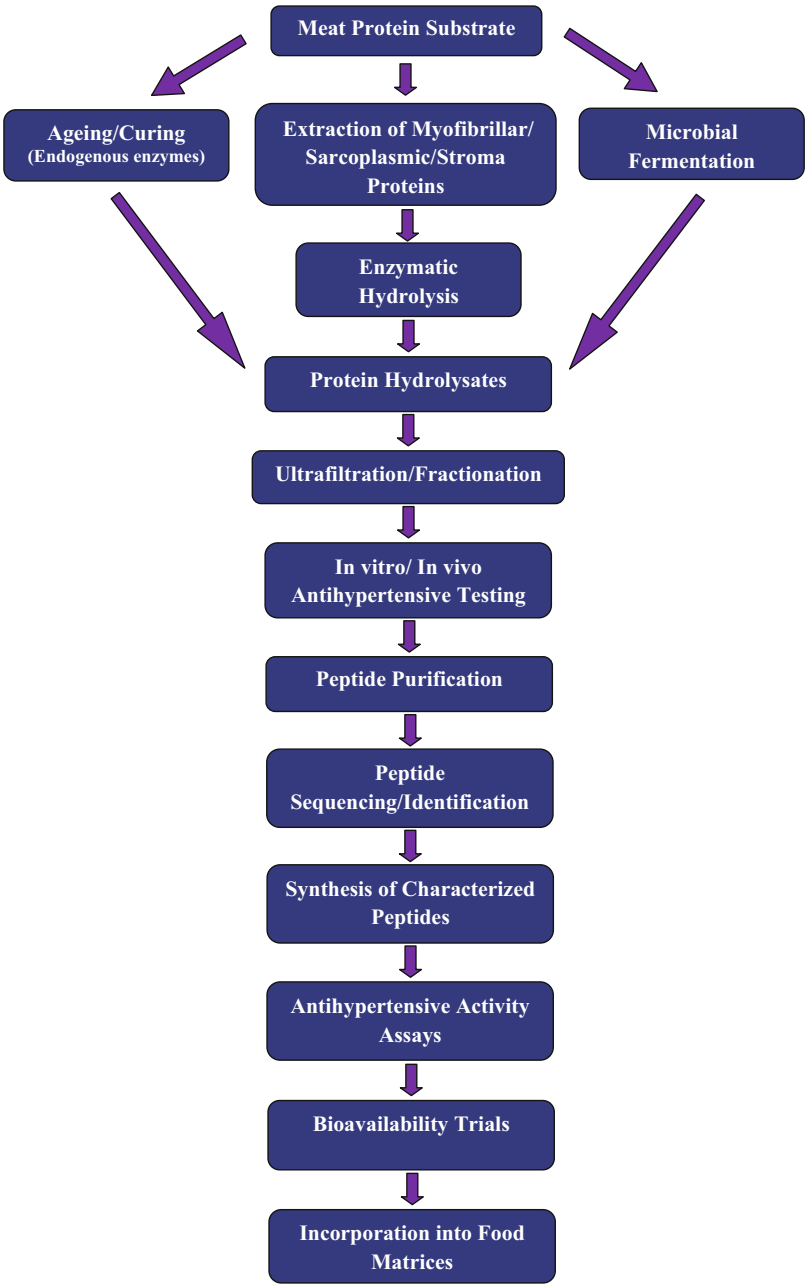


Fig. 1 Schematic diagram for the production of functional foods with antihypertensive properties

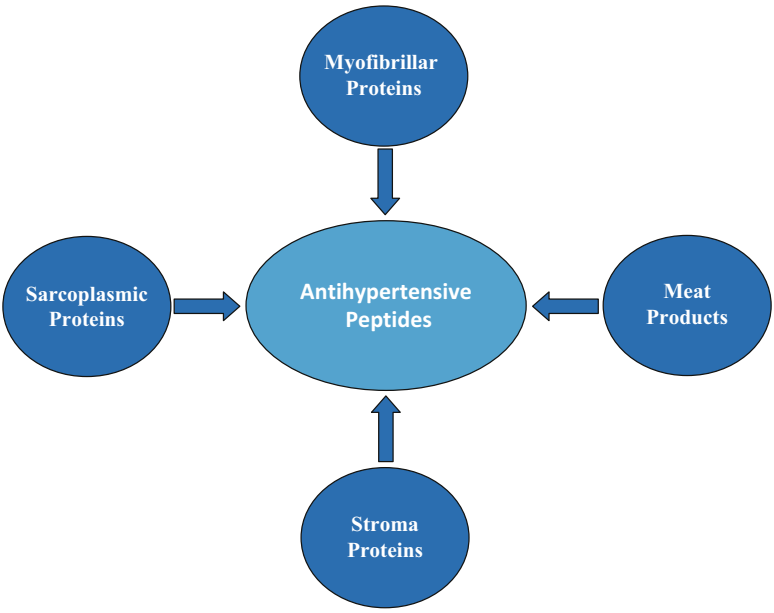


Fig. 2 Different meat protein substrates for the production of antihypertensive peptides

inhibitory properties have been reported from several skeletal muscle proteins namely myosin, actin, tropomyosin, troponin, and collagen [85]. ACE-inhibitory potential of these peptides is measured in vitro by IC_{50} value and in vivo by using spontaneously hypertensive rats as an accepted animal model for human essential hypertension [102–104]. Results from several studies have demonstrated that meat protein hydrolysates may represent the source of natural peptides capable to reduce ACE-I activity.

7.1 Myofibrillar Proteins

7.1.1 Contractile Proteins

Among all the meat protein-derived bioactive peptides, ACE-inhibitory peptides are most extensively studied [102, 105]. ACE-inhibitory peptides are commonly produced from hydrolysis of skeletal muscle and connective tissue proteins of farm animals like pig and poultry [106–108]. A large number of peptides with ACE-inhibitory properties have already been reported from the enzymatic hydrolysates of various meat proteins [27, 102, 105, 109]. Two ACE-inhibitory pentapeptides sequenced as MNPPK and ITTNP were identified from the hydrolysates of pig skeletal muscle proteins and were named as myopentapeptide A and B, respectively [108]. These pentapeptides, corresponding to position 79–83 and 306–310 on the myosin heavy chain, respectively, were shown to have ACE-inhibitory properties in

spontaneously hypertensive rats [110]. Six other peptides viz. PPK, MNP, NPP, ITT, TNP, and TTN sharing the sequence parts of these two myopentapeptides also showed the antihypertensive properties. An octapeptide VKKVLGNP corresponding to position 47–54 on myosin light chain with temporary ACE-inhibitory properties was identified from the myosin protein hydrolysates [99]. One more peptide sequenced as KRVITY isolated from pig skeletal myosin B treated with pepsin and VKAGF isolated from porcine actin were reported to have significant blood pressure lowering properties in vivo in rats [111, 112]. With IC_{50} value of 6.1 μM, KRVIQY was reported to retain its ACE-inhibitory activity after heating the myosin B at 98 °C for 10 min prior to hydrolysis by pepsin, suggesting the bioavailability of the peptide even after cooking.

Seven ACE-inhibitory peptides with sequence LKP, LKA, LAP, IKW, FKGRYYP, FQKPKR, and IVGRPRHQG were isolated from chicken muscle proteins by thermolysin treatment [98]. Although, IC_{50} value of these peptides ranged from 0.21 μM to 14 μM, however, the heptapeptide FKGRYYP with IC_{50} value of 0.55 μM failed to produce antihypertensive activity in vivo in spontaneously hypertensive rats. This indicated that in vitro ACE-inhibitory activity of the peptides may not necessarily correlate with their in vivo ACE-inhibitory activity. Intracellular peptidases or enzymes in the GIT or blood vascular system may have degraded the peptide affecting its antihypertensive properties in vivo. Further, modification of the peptides in the liver may also affect their in vivo antihypertensive potential [102]. ACE-inhibitory properties of chicken breast muscle extract were reported in vitro as well as in vivo in spontaneously hypertensive rats [104]. There was a significant increase in the ACE-inhibitory activity of the extracts treated with an Aspergillus protease and gastric proteases (trypsin, chymotrypsin, and intestinal juice). All the three peptides identified with ACE-inhibitory properties possessed a common sequence Gly-X-X-Gly-X-X-Gly-X-X, and the peptide with sequence Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe showed the strongest ACE-inhibitory activity with IC_{50} value of 42.4 μM. This peptide had a high affinity for ACE and inhibited the enzyme in a noncompetitive manner. The Phe residue at the C-terminus position and an aromatic residue at the antepenultimate position were reported to play an important role in the inhibitory activity [113] supporting the theory of presence of three C-terminal hydrophobic residues for the ACE-inhibitory activity of a peptide. Binding of a peptide to ACE is strongly influenced by this C-terminal tripeptide sequence and is required for interaction with three hydrophobic subunits located on the active site of ACE [114]. Main inhibitors of the enzyme ACE show hydrophobic residues or proline, lysine, or arginine as C-terminal amino acids [115–117].

Two novel ACE-inhibitory peptides MNVKHWPWMK and VTVNPYKWLP corresponding to positions 825–834 and 125–135 of myosin heavy chain, respectively, were identified from chicken leg meat hydrolysate digested with pepsin. With IC_{50} values of 228 μM and 5.5 μM, respectively, the peptides were suggested to be a good starting substance for designing food supplements for hypertensive patients [118]. Gu et al. [119] studied the production of ACE-inhibitory peptides from muscles of black-bone silky fowl (*Gallus gallus domesticus Brisson*) hydrolyzed

with Alcalase 2.4 L (2.4 AU/g) and papain (320,000 U/g). A total of 29 peptide sequences were identified and based on the ACE-inhibitory properties of different fractions and prior literature, 11 peptide sequences with probable ACE-inhibitory properties were selected, synthesized, and further analyzed for ACE-inhibitory properties. Finally, two novel peptides Leu-Glu-Arg (IC_{50} = 45.62 ± 2.40 μM) and Gly-Ala-Gly-Pro (IC_{50} = 253.07 ± 6.66 μM) with strong ACE-inhibitory properties were found.

Beef myofibrillar protein extract was used to produce ACE-inhibitory peptides by using five commercially available food-grade microbial protease preparations viz. HT proteolytic (HT; 2 mg/mL) protease, acidic fungal protease (AFP; 20 mg/mL), fungal protease 31,000 (F31 K; 10 mg/mL), fungal protease 60,000 (F60 K 4 mg/mL), and fungal protease II (FPII; 4 mg/mL) [32]. The small peptide pool obtained from hydrolysate by using gel permeation chromatography (nominally <15 residue peptides) was reported to exhibit 89.0 ± 2.9% ACE-inhibitory activity. Castellano et al. [79] studied the production of ACE-inhibitory peptides from porcine myofibrillar proteins hydrolyzed by *Lactobacillus sakei* CRL1862 and *Lactobacillus curvatus* CRL705 isolated from traditional Argentinean sausages at 30 °C for 36 h. Meat-borne *Lactobacilli* were able to produce peptides with ACE-inhibitory properties and a total of 50 and 18 peptides were characterized from *L. curvatus* CRL705 and *L. sakei* CRL1862 protein hydrolysates, respectively.

7.1.2 Regulatory and Cytoskeletal Proteins

ACE-inhibitory peptides have been reported not only from muscle contractile proteins but also from regulatory and cytoskeletal proteins like troponin, tropomyosin, nebulin, and titin. An ACE-inhibitory peptide with sequence RMLGQTPTK was isolated from porcine troponin-C hydrolyzed with pepsin and showed high resistance to digestive enzymes, suggesting its possible in vivo ACE-inhibitory activity [120]. Two novel ACE-inhibitory peptides with sequence Lys-Arg-Gln-Lys-Tyr-Asp-Ile (KRQKYDI) and Glu-Lys-Glu-Arg-Glu-Arg-Gln (EKERERQ) were isolated from porcine skeletal muscle troponin treated with pepsin with proven in vivo antihypertensive properties in spontaneously hypertensive rats [103]. The IC_{50} value of KRQKYDI and EKERERQ was reported to be 26.2 μM and 552.5 μM, respectively, and the peptide KRQKYDI showed the strongest ACE-inhibitory activity among all previously reported troponin peptides.

Nearly 22 peptides, including the novel peptides MYPGIA and VIPEL, were reported from porcine *Longissimus dorsi* muscle hydrolyzed by sequential action of pepsin and pancreatin [121]. Among these, two pentapeptides with sequence KAPVA (IC_{50} 46.56 μM) and PTPVP (IC_{50} 256.41 μM) corresponding to position 4784–4788 and 4216–4220 on titin, respectively, and tripeptide RPR (IC_{50} 382 μM) corresponding to position 1263–1265 on nebulin were also reported. Strong ACE-inhibitory activity of these peptides (KAPVA, PTPVP, and RPR) was demonstrated in vivo in spontaneously hypertensive rats after oral administration [122]. Although, RPR showed far less ACE-inhibitory activity than KAPVA in vitro, it exhibited comparable ACE-inhibitory activity to that of KAPVA in vivo. Higher in vivo

activity of peptides which show weaker in vitro activity might be explained by their higher affinity to the target tissues or the possibility of involvement of a mechanism other than inhibition of ACE [102].

7.2 Sarcoplasmic Proteins

An ACE-inhibitory hexapeptide (VLAQYK) with an IC_{50} value of 32.06 μM was identified from enzymatic hydrolysates of sarcoplasmic protein extracts from beef *Biceps femoris* [123]. The peptide exhibited strong ACE-inhibitory properties in vivo in spontaneously hypertensive rats and also lowered blood total- and LDL-cholesterol concentrations [124]. Based on its strong ACE-inhibitory properties in vitro as well as in vivo, the authors proposed the use of this hexapeptide in clinical applications and for development of functional foods. Four ACE-inhibitory peptides identified as FHG, GFHI, DFHING, and GLSDGEWQ were produced from beef sarcoplasmic proteins hydrolyzed by incubating with commercial enzymes thermolysin + proteinase A (pH 7.5/37 °C), proteinase K (pH 7.5/37 °C), trypsin (pH 7.6/25 °C), tyrosinase (pH 6.5/25 °C), papain (pH 6.2/25 °C), pepsin (pH 3.0/37 °C), and protease (pH 7.5/37 °C) for 8 h [100]. The measured IC_{50} values of FHG, GFHI, DFHING, and GLSDGEWQ against ACE were 52.9, 117, 64.3, and 50.5 μg/ml, respectively.

Bernardini et al. [125] studied the possibility of production of ACE-inhibitory peptides from sarcoplasmic proteins hydrolyzed from beef low-value brisket cuts (*Pectoralis profundus*) with papain for 24 h. The 3 kDa peptidic fractions showed higher ACE-inhibitory activity than 10 kDa fractions which was in agreement with previous studies suggesting that peptide sequences with ACE-inhibitory properties are generally composed of small number of residues and are less than 3 kDa [50]. The authors suggested the possible use of these ACE-inhibitory brisket hydrolysates for the development of meat products, like patties, with antihypertensive properties. Hydrolysates from different sources like milk and soy have already been incorporated into the meat products like beef and pork patties [126–128]. A unique heptapeptide with sequence FISNHAY was generated from porcine sarcoplasmic proteins by *L. sakei* CRL1862 in fraction 27 which showed highest ACE-inhibitory activity. This peptide had, rather than a proline residue, aromatic (tyrosine) and aliphatic (alanine) residues at the ultimate and penultimate C-terminal positions, respectively [79].

7.3 Connective Tissue and Other Proteins

In comparison to other meat proteins, bovine elastin and collagen possess highest frequency of peptide sequences with different bioactivities [129]. Peptides derived from hydrolysis of bovine connective tissues have been reported to have strong ACE-inhibitory properties [130–132]. Peptides with strong ACE and renin-inhibitory activities were identified from collagen extracted from nuchal ligament of

bovine carcasses (GPRGF) and from cooked *semitendinosus* muscle (SPLPPPE, EGPQGPPGPVG, and PGLIGARGPPGP) [28, 132]. Two ACE-inhibitory peptides were produced and identified from collagen obtained from bovine Achilles tendon hydrolyzed with bacterial collagenase. Both the peptides sequenced as AKGANGAPGIAGAPGFPGARGPSGPQGPSGPP and PAGNPGADGQP-GAKGANGAP retained 80% of the ACE-inhibitory properties after treatment with common GIT enzymes [133]. Ryder et al. [32] studied the production of ACE-inhibitory peptides from beef connective tissue protein extract by using five microbial protease preparations. The small peptide fraction obtained from the hydrolysates showed 90.9 ± 1.0% ACE-inhibitory activity.

O'Keeffe et al. [134] studied the ACE-inhibitory activity of porcine skin gelatin hydrolyzed by *Aspergillus niger* prolyl endoproteinase for 4 h. Peptides were identified by using UPLC-ESI-MS/MS and 166 sequences were identified, Met-Gly-Pro being the most potent ACE-inhibitory peptide. Some of the peptides identified in the hydrolysate like Gln-Phe, Tyr-Pro, Leu-Ala, Val-Pro, Ile-Pro, Ile-Ala, and Gly-Pro were previously identified as strong ACE-inhibitors. Among these peptides Val-Pro, Ile-Pro, Ile-Ala, and Gly-Pro were suggested to be resistant to GIT enzymes following an in silico GI digest using PeptideCutter. The hydrolysate showed strong blood pressure lowering properties in spontaneously hypertensive rats which was attributed to the abundance of peptides with C-terminal Pro residue. The in vivo antihypertensive properties of hydrolysates obtained from gelatin from different sources have been reported by several workers [135–141]. Short peptides, such as Pro-Hyp, released from gelatin have been reported to cross the intestinal barrier and enter the blood vascular system after oral ingestion of hydrolysates by mice [142] and humans [143].

Inoue et al. [144] produced hydrolysates with ACE-inhibitory properties from porcine liver with in vivo blood pressure lowering properties in spontaneously hypertensive rats. Lafargaa et al. [72] studied the release of ACE-inhibitory peptides from porcine and bovine meat proteins including collagen, hemoglobin, and serum albumin, commonly found in meat by-products such as blood, bone, and low-value meat cuts, using enzymes papain, pepsin, bromelain, ficain, and thermolysin. PeptideCutter and BIOPEP were used for in silico cleavage of the proteins to predict the release of over 30,000 peptides. Four novel ACE-inhibitory peptides viz. Asp-Phe-Tyr, Ala-Pro-Pro-His, Ile-Ile-Tyr, and Ile-Phe-Tyr which were not present in existing databases were identified. Sequence Glu-Tyr, an ACE-inhibitory dipeptide corresponding to position 23–24 of bovine hemoglobin subunit alpha, was identified which was previously reported from shark meat [145] and several natural products including milk and fish [145, 146]. Fu et al. [131] identified two collagen peptides with sequence GPRGF and VGPV from bovine connective tissue and were demonstrated to be transported across Caco-2 cells, a monolayer of human intestinal cell lines, suggesting the possible bioavailability of the peptides and likelihood to exhibit the ACE-inhibition activity in vivo. Two peptides identified from the porcine hemoglobin with sequence GFPTTKTYFPHF, corresponding to 34–46 fragment of the α-chain, and VVYPWT, corresponding to 34–39 fragment of the β-chain, were reported to have ACE-inhibitory properties, showing IC_{50} values of 4.92 μM and 6.02 μM, respectively [147].

Saiga et al. [141] studied the ACE-inhibitory properties of hydrolysates obtained from collagen extracted from chicken legs. The hydrolysate produced with an *Aspergillus* species-derived enzyme showed the highest activity ($IC_{50} = 260$ μg/mL) with a strong antihypertensive effect observed in spontaneously hypertensive rats administered with low fraction. Peptides with a sequence Gly-Ala-Hyp-Gly-Leu-Hyp-Gly-Pro exhibited highest ACE-inhibitory activity ($IC_{50} = 29$ μM). Two ACE-inhibitory peptides with sequence GPL and GPV and IC_{50} values of 2.55 μM and 4.67 μM, respectively, were purified from bovine skin gelatin hydrolysate [148].

7.4 Effects of Aging and Cooking of Meat

Encrypted in the meat proteins are several ACE-I inhibitory sequences which are released during natural processes, like aging, dry curing, and fermentation, which naturally induce proteolysis of both sarcoplasmic and myofibrillar proteins in the muscles [149]. Fu et al. [28] studied the endogenous generation of peptides in beef *Longissimus thoracis* (LT) and s*emitendinosus* (ST) muscles during 20 days postmortem aging and reported the presence of a number of peptides with ACE-inhibitory properties. The activity was highest on day 10 in cooked ST (63 °C) which decreased at day 20, suggesting the presence of potent peptides on day 10 which became accessible by cooking. Highest ACE-inhibitory activity was exhibited by collagen-derived peptide sequences SPLPPPE, EGPQGPPGPVG, and PGLIGARGPPGP. After 20 days postmortem, more peptides were identified due to extensive degradation of proteins. Degradation of potent peptides by the endogenous enzymes may be the reason for the decrease in the ACE-inhibitory activity at day 20 [150]. The authors also reported for the first time the release of low molecular weight peptides (< 3 kDa) from aged beef with renin-inhibitory activity. The highest concentration of low molecular weight peptides was observed in cooked beef aged for 20 days and also exhibited highest renin-inhibitory properties. These results suggest that generation of peptides and their related bioactivities depends on aging time and cooking [151, 152] and may be ascribed to the release of certain active peptides during different phases of aged or cooked beef [28]. Although, calpains, which are mainly responsible for proteolysis, get inactivated at 55 °C [151], other endogenous proteases like cathepsins, metalloproteinase, and collagenase may still cause proteolysis in beef during cooking, increasing the concentration of small peptides [28, 153].

Liu et al. [154] studied the endogenous release of ACE-inhibitory peptides in duck meat during postmortem aging and reported that the small peptides (<5 kDa) produced due to the degradation of muscle proteins exhibited no ACE-inhibitory activity. Endogenous enzymes, unlike exogenous enzymes, work in uncontrollable manner and might only release a limited number of peptides [72] which might be produced in too little quantities to induce ACE-inhibitory activity [154].

Sangsawad et al. [155] studied the effect of cooking methods on the production of ACE-inhibitory peptides derived from simulated in vitro gastrointestinal digestion of *Korat* crossbred and commercial broiler chicken breast. The highest ACE-inhibitory

activity was exhibited by *Korat* crossbred chicken digestas heated at 70 °C for 0.5 h while the lowest inhibitory activity was shown by the digestas of *Korat* crossbred breast meat heated at 121 °C for 1 h. In general, ACE-inhibitory activity of the digestas also varied with the protein digestibility. The highest digestibility and ACE-inhibitory activity was reported in samples cooked by mild thermal treatment. The digestas heated at 70 °C for 0.5 h had highest proportion of small peptides (<1 kDa) whereas the digestas heated at 121 °C for 1 h had highest proportion of large peptides (>5 kDa). Peptide size has been reported to play an important role in ACE-inhibitory activity with most of the peptides possessing 2–20 amino acids [30, 156]. Larger peptides have difficulty in binding to the active site of ACE, resulting in decreased inhibitory activity [157]. This may partly explain the lower ACE-inhibitory activity of the digestas treated by high thermal treatment. High thermal treatment (>100 °C) has also been reported to induce several changes in proteins, like aggregation, cross-linking, and oxidative modifications [158, 159] which reduce their susceptibility to proteases, resulting in larger size peptides. Novel peptide sequences KPLLCS, ELFTT, and KPLL with potent ACE-inhibitory properties were identified with sequences homologous to that of myosin. The IC_{50} values of KPLLCS, ELFTT, and KPLL were 0.37, 6.35, and 11.98 μM, respectively. Peptides ELFTT and KPLL were reported to be resistant to in vitro plasmin hydrolysis for up to 60 min while KPLLCS was hydrolyzed by plasmin after 15 min, suggesting the resistance of these peptides to the blood proteases. Peptides need to resist the blood protease digestion after absorption to exert the ACE-inhibitory activity in vivo (Table 3).

7.5 Meat Products

Meat is considered as a good source of peptides with different bioactivities [120, 122, 162]; however, dry-cured ham was the only product among the processed meat products that was reported to be a natural source of peptides with antihypertensive properties [163, 164]. Table 4 presents the identified peptide sequences with antihypertensive properties from different meat products. Seven dipeptides sequenced as RP, KA, AA, GP, AR, GR, and RR with ACE-inhibitory properties were produced by the action of dipeptidyl peptidases purified from porcine skeletal muscle [169]. Since dipeptidyl peptidases remain active during the whole processing period of dry-cured meat products, or at least a great part of it, the authors hypothesized that their proteolytic action could contribute to the generation of peptides with ACE-inhibitory activity in cured meat products. Vaštag et al. [170] also suggested the possibility of production of meat protein hydrolysates with ACE-inhibitory properties during ripening process of *Petrovská Kolbása*, a traditional dry fermented sausage. Later three novel peptides possessing in vivo ACE-inhibitory properties were identified from Spanish dry-cured ham [153, 163] and were reported to be highly stable during in vitro gastrointestinal digestion and heat treatment [165]. AAATP was the identified peptide sequence with most potent ACE-inhibitory activity and effectively decreased systolic blood pressure in spontaneously hypertensive rats [153]. ASGPINFT and DVITGA were other sequences that yielded a moderate ACE

Table 3 Peptide sequences with antihypertensive properties from different meat proteins

Peptide sequence	Proteins	Authors
LKP, LKA, LAP, IKW, FKGRYYP, FQKPKR, IVGRPRHQG	Chicken muscle proteins	Fujita et al. [98]
GPL, GPV	Bovine skin proteins	Kim et al. [148]
MNPPK, ITTNP, MNP, NPP, PPK, ITT, TTN, TNP	Porcine skeletal muscle proteins	Arihara et al. [108]
VKKVLGNP, TNP, NPP, MNP, ITTNP, MNPPK, PPK, TTN, KRQKYDI	Porcine skeletal muscle proteins	Nakashima et al. [110]
RMLGQTPTK, RMLGQTP	Porcine troponin C	Katayama et al. [120]
GFXGTXGLXGF	Chicken breast muscle proteins	Saiga et al. [104]
RMLGQTPTK, RMLGQTP, RMLGQ, RML, GQ, TP, TK	Porcine troponin	Katayama et al. [160]
VLAQYK	Beef muscle proteins	Jang and Lee [123]
GFXGTXGLXGF, GAXGLXGP	Chicken collagen	Saiga et al. [113] Saiga et al. [141]
VKKVLGNP	Porcine skeletal muscle myosin	Katayama et al. [99]
KRQKYDI, EKERERQ	Porcine skeletal muscle troponin	Katayama et al. [103]
FHG, GFHI, DFHING, GLSDGEWQ	Beef sarcoplasmic proteins	Jang et al. [100]
KRVIQY, VKAGF	Porcine skeletal myosin and actin	Muguruma et al. [111]
KAPVA, PTPVP, ER, KLP, RPR, MYPGIA, VIPEL, VLPE RVAPEEHP, VAPEEHPT, LFDKPVSP, FDKPVSPL, ITTNPY, MLGQTP, DQVFPMNPPK, MMVPI, NIIPA	Porcine muscle proteins	Escudero et al. [121]
VTVNPYKWLP, MNVKHWPWMK	Chicken muscle proteins	Terashima et al. [118]
FISNHAY, AMQKIF, LKTAIQAAGYPDKV, SDHHIYL, IKNYPVVSIED, AAVYKALSDHHIY, MPQQIGVP, LSGGQSEEEASINL, TFSYGRALQA, VGGASLKPEF, SPLPVIPH, EVGGEALGRL, IKWGDAGATY, LEGKVLPGVDALS, LVGGASLKPEF	Porcine sarcoplasmic proteins	Castellanoa et al. [79]
LPP, APPH, NFY, IFY, IIY, LPP, IPP, IF, FY	Bovine and porcine muscle proteins	Lafargaa et al. [72]
FQPS	Kacang goat muscle proteins	Mirdhayati et al. [161]
KPLLCS, ELFTT, KPLL	Chicken breast proteins	Sangsawad et al. [155]
SPLPPPE, EGPQGPPGPVG, PGLIGARGPPGP	Beef collagen	Fu et al. [28]
MGP, QF, YP, LA,VP, IP, IA, GP	Porcine skin proteins	O'Keeffe et al. [134]

Table 4 Peptide sequences with antihypertensive properties from different meat products

Peptide sequence	Product	Authors
GNGGA, DVITGA, KDQGSYEDF, GVDNPGHPF, LNSLT, KAEEEYPDL, EEYPDL, ASGPINFT, AAATP	Spanish dry-cured ham	Escudero et al. [153]
AAPLAP, AAATP, KAAAAP, KPVAAP, IAGRP, KAAAATP, PTPVP, PAPPK, AMNPP, IKLPP, VPPAK, KPGRP, PSNPP, EAPPK, PAAPPK, KVLPG, TGLKP	Spanish dry-cured ham	Escudero et al. [165]
PPK, PAP, AAP	*Iberian* dry-cured ham	Mora et al. [166]
LGL, ALM, SFVTT, GVVPL, NSIM	*Parma* dry-cured ham	Dellafiora et al. [167]
RVAPEEHPT, VAPEEHPT, IGGSI, LFDKPVSPL, LAPST, PGIAD, IVAPG, PSIV, DLTDY, SYELPDGQ, IDDL, LKGADPEDVITGA, TVKDLQHRL	*Parma* dry-cured ham	Paolella et al. [168]
(R)AVFPSIVGRPR(H), (R)LDLAGRDLTDYLMK(I), (R)AVFPSIVGRPR(H), (F)PSIVGRPR(H), (V)FPSIVGRPR(H), (R)FGVTITVHPEPR(V), (R)MDVSITGEPRPV(A), (R)AVFPSIVGRPR(H), (V)TVKEDQVFPMNPPKFDKIED(M), (R)AVFPSIVGRPR(H), (F)PSIVGRPR(H), (V)FPSIVGRPR(H), (K)AGDNIKVEIPVLGRPKPT(V), (L)NVLGRPGPPVGPI(K), (R)LNVLGRPGPPVGPI(K), (R)MDVSITGEPRPV(A), (R)AVFPSIVGRPR(H), (V)FPSIVGRPR(H), (W)AAFPPDVGGNVDYK(N), (W)AAFPPDVGGNVDYK(N), (V)TVKEDQVFPMNPPKFDKIED(M), etc.	*Bresaola*	Ferranti et al. [149]

inhibition. Escudero et al. [165] studied the stability of ACE-inhibitory peptides from Spanish dry-cured ham and reported that almost the same activity was retained after applying diverse heating (50–117 °C), times of processing (3–60 min), and simulated in vitro digestion with gastrointestinal proteases. Peptides AAPLAP, KAAAAP, KPVAAP, KAAAATP, and IAGRP were reported to be the most potent ACE-inhibitory peptides. Gallego et al. [171] studied the stability of three ACE-inhibitory peptides (KPVAAP, AAATP, and AAPLAP) naturally generated in dry-cured ham during transepithelial transport in a Caco-2 cell monolayer. The results showed that the peptides degraded during the assay, although, some of the KPVAAP peptides remained intact and was also absorbed through the intestinal barrier, suggesting the possible absorption and bioavailability of dry-cured ham peptides in the blood stream to exert an antihypertensive action.

Production of peptides in dry-cured meat products depends on certain factors such as activity of muscle endogenous enzymes, which is very much influenced by genetics and muscle-type, and processing conditions including added ingredients and ripening time [162]. Paolella et al. [168] studied the effect of maturation time of dry-cured *Parma* hams on the peptide profile obtained upon simulated gastrointestinal digestion. *Parma* ham is a well-known dry-cured meat product obtained from heavy pig thighs, mildly salted and aged for a long period. A total of 81 peptide sequences were identified in digested samples and most of the sequences mainly

originated from myofibrillar proteins (myosin and actin) and sarcoplasmic proteins (such as pyruvate kinase, creatine kinase, fructose bisphosphate aldolase, etc.). A higher amount of short sequences were found in the digesta with a total of 21 dipeptides and 11 tripeptides among the identified sequences. Several of the identified sequences were already known in the literature with demonstrated ACE-inhibitory properties. Dellafiora et al. [167] used hybrid in silico/in vitro approach for the identification of ACE-inhibitory peptides from dry-cured *Parma* hams. Strong ACE-inhibitory sequences, like LGL and SFVTT, were identified for the first time in *Parma* dry-cured ham. On the basis of in silico results, a total of 25 peptides were predicted as active, and five ACE-inhibitory peptides with sequence LGL, SFVTT, GVVPL, ALM, and NSIM with IC_{50} values of 145, 395, 956, >1100, and >1100 μM, respectively, were identified.

Mora et al. [166] evaluated the antihypertensive peptides generated naturally in *Iberian* dry-cured ham and also studied the differences in the generated peptides between traditional Spanish dry-cured (14 months of ripening) and *Iberian* dry-cured ham (24 months of ripening). The extract obtained from *Iberian* dry-cured ham was analyzed in vitro showing up to 97.7% of ACE-inhibition in some of the fractions. A significant decrease of 12 mm Hg in systolic blood pressure of spontaneously hypertensive rats was also observed after 8 h of ingestion. Pro-Pro-Lys, Pro-Ala-Pro, and Ala-Ala-Pro with strong ACE-inhibitory activities were the most repeated sequences among 2632 peptides identified in *Iberian* dry-cured ham. These sequences appeared a total of 322, 302, and 119 times, respectively, and were mainly derived from the proteins troponin-T, myosin-heavy and myosin-light chains. Mora et al. [172] characterized the peptide profile of three European hams viz. Spanish *Teruel*, Italian *Parma*, and Belgian dry-cured hams. The peptides generated in these types of hams were identified and quantified using a label-free methodology to assess main differences in proteolysis between them. Peptide fractions from Spanish *Teruel* exhibited highest ACE-inhibitory activity of 93% while those from Belgian and *Parma* hams had ACE-inhibitory activity of 76% and 70%, respectively.

The *Bresaola della Valtellina*, produced by salting and naturally curing specific cuts of lean bovine hind quarters, is a Protected Geographical Indication from Valtellina, Italy. There are other similar products which are traditionally produced in other places such as *Viande des Grison* or *Bunder fleish* in Switzerland and *Cecina* in Spain. Both myofibrillar and sarcoplasmic protein fractions of *Bresaola* get extensively hydrolyzed by endogenous proteases during ripening, releasing a large variety of peptides which could exert several biological activities if they survive the digestive process. With this aim, Ferranti et al. [149] attempted to identify the bovine muscle-derived proteins and peptides from two different *Bresaola* samples (with and without the Protected Geographical Indication label) which could survive a static in vitro model of digestion that included the sequential oral, gastric, and duodenal phases. More than 170 peptides were identified which were released during in vitro digestion from the major structural and sarcoplasmic muscle proteins. Most of the identified peptides had reported antihypertensive properties or were precursors of potentially antihypertensive sequences.

Pastırma, a Turkish dry-cured meat product produced from whole beef muscles, was analyzed for antihypertensive activity and was reported to possess strong ACE-inhibitory properties [71]. ACE-inhibitory activity of more than 86% was shown from fractions corresponding to molecular masses between 900 Da and 1500 Da. ACE-inhibitory peptides were suggested to be generated due to the proteolysis caused by endogenous enzymes and the *çemen* paste used in production.

Recently, Fernández et al. [116] studied the possibility of generation of ACE-I inhibitory properties in the dry-fermented sausage *salchichón* by using purified proteolytic enzyme EPg222 and starter culture (P200S34) composed of *Pediococcus acidilactici* (MS200) and *Staphylococcus vitulus* (RS34), separately and together, followed by ripening for 90 days. EPg222 protease purified from *Penicillium chrysogenum* Pg222, isolated from dry-cured meat products, has been reported to have high proteolytic activity against myofibrillar proteins under the conditions normally present in dry-cured meat products [173]. Batches inoculated with EPg222 were reported to have highest ACE-inhibitory activities at 63 days of ripening. The ACE-inhibitory activities remained stable in most cases even after in vitro simulated gastrointestinal digestion, suggesting the significance of use of the enzyme EPg222 in association with the starter culture P200S34 for the production of functional meat products with antihypertensive properties. Mejri et al. [174] studied the effect of different starter cultures and ripening times on the size, concentration, and antihypertensive capacities of peptides in dry-fermented camel sausages. Sausages were inoculated with isolated strains of *S. xylosus* and *L. plantarum* (batch A), *S. xylosus* and *L. pentosus* (batch B), and *S. carnosus* and *L. sakei* (batch C) and were ripened up to 28 days. Both peptide size and concentration were reported to be affected by the ripening time and the inoculated bacteria. The ripening process resulted in an increased antihypertensive capacity with highest bioactivities in the fractions with <3 kDa peptides. Low molecular weight peptides (< 3 kDa) have been reported to contribute to the development of flavor as well as antihypertensive activities in fermented meat products [166].

8 Conclusions

A large number of peptides with demonstrated in vivo and in vitro antihypertensive activities have been reported from different meat proteins. Endogenous generation of peptides with antihypertensive properties has also been reported during natural aging process increasing the value of meat as a functional food. Cured meat products like dry-cured ham and *pastırma* might be considered as natural sources of peptides with strong antihypertensive properties. Several meat-derived antihypertensive peptides have been shown to retain their activity and bioavailability even after digestion and cooking. Thus peptides derived from meat proteins provide an exciting option for the

development of functional foods with antihypertensive properties for preventive and curative management of hypertension.

References

1. Knowlin L, Reid T, Williams F, Cairns B, Charles A (2017) Burn mortality in patients with pre-existing cardiovascular disease. Burns 43:949–955. https://doi.org/10.1016/j.burns.2017.01.026
2. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB (2015) Heart disease and stroke statistics-2015 update: a report from the American heart association. Circulation 131:e29–322. https://doi.org/10.1161/CIR.0000000000000152
3. Roth GA, Moran AE, Barber R, Nguyen G, Feigin VL, Naghavi M, Mensah GA, Murray CJL (2015) Demographic and epidemiologic drivers of global cardiovascular mortality. N Engl J Med 372:1333–1341. https://doi.org/10.1056/NEJMoa1406656
4. Humphries KH, Izadnegahdar M, Sedlak T, Saw J, Johnston N, Schenck-Gustafsson K, Shah RU, Regitz-Zagrosek V, Grewal J, Vaccarino V, Wei J, Merz BCN (2017) Sex differences in cardiovascular disease-Impact on care and outcomes. Front Neuroendocrinol 46:46–47. https://doi.org/10.1016/j.atherosclerosis.2015.01.027
5. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvänne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F (2012) European guidelines on cardiovascular disease prevention in clinical practice (version 2012): The fifth joint task force of the European society of cardiology and other societies on cardiovascular disease prevention in clinical practice. Eur Heart J 33:1635–1701. https://doi.org/10.1093/eurheartj/ehs092
6. Rosendorff C, Lackland DT, Allison M, Aronow WS, Black HR, Blumenthal RS, Cannon CP, de Lemos JA, Elliott WJ, Findeiss L, Gersh BJ, Gore JM, Levy D, Long JB, O'Connor CM, O'Gara PT, Ogedegbe G, Oparil S, White WB (2015) Treatment of hypertension in patients with coronary artery disease: a scientific statement from the American heart association, American college of cardiology, and American society of hypertension. Circulation 131:e435–e470. https://doi.org/10.1161/CIR.0000000000000207
7. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, Chen J, He J (2016) Global disparities of hypertension prevalence and control: a systematic analysis of population-based studies from 90 countries. Circulation 134:441–450. https://doi.org/10.1161/circulationaha.115.018912
8. Gasowski J, Piotrowicz K (2017) Hypertension in the elderly: change of, or new implications within the existing, paradigm? Eur Geriatric Med. https://doi.org/10.1016/j.eurger.2017.05.002
9. Campbell NRC, Lackland DT, Niebylski ML (2014) High blood pressure: why prevention and control are urgent and important – a 2014 fact sheet from the world hypertension league and the international society of hypertension. J Clin Hypertens 16:551–553. https://doi.org/10.1111/jch.12372
10. Garfinkle MA (2017) Salt and essential hypertension: pathophysiology and implications for treatment. J Am Soc Hypertens 11:385–391. https://doi.org/10.1016/j.jash.2017.04.006

11. Campbell NR, Lackland DT, Lisheng L, Niebylski ML, Nilsson PM, Zhang XH (2015) Using the global burden of disease study to assist development of nation-specific fact sheets to promote prevention and control of hypertension and reduction in dietary salt: a resource from the world hypertension league. J Clin Hypertens 17:165–167. https://doi.org/10.1111/jch.12479
12. Danaei G, Ding EL, Mozaffarian D, Taylor B, Rehm J, Murray CJ, Ezzati M (2009) The preventable causes of death in the United States: comparative risk assessment of dietary, lifestyle, and metabolic risk factors. PLoS Med 6(4):e1000058. https://doi.org/10.1371/journal.pmed.1000058
13. IFPMA (2016) Hypertension: putting the pressure on the silent killer. https://www.ifpma.org/wp-content/uploads/2016/05/2016-Hypertension-putting-the-pressure-on-the-silent-killer.pdf
14. Karatzi K, Protogerou AD, Moschonis G, Tsirimiagou C, Androutsos O, Chrousos GP, Lionis C, Manios Y (2017) Prevalence of hypertension and hypertension phenotypes by age and gender among school children in Greece: the healthy growth study. Atherosclerosis 259:128–133. https://doi.org/10.1016/j.atherosclerosis.2017.01.027
15. Beckie TM (2017) Ethnic and racial disparities in hypertension management among women. Semin Perinatol. https://doi.org/10.1053/j.semperi.2017.04.004
16. Chobanian AV (2015) Time to reassess blood-pressure goals. N Engl J Med 373:2093–2095. https://doi.org/10.1056/nejmp1513290
17. O'Shea PM, Griffin TP, Fitzgibbon M (2017) Hypertension: the role of biochemistry in the diagnosis and management. Clin Chim Acta 465:131–143. https://doi.org/10.1016/j.cca.2016.12.014
18. Kearney PM, Whelton M, Reynolds K, Whelton PK, He J (2004) Worldwide prevalence of hypertension: a systematic review. J Hypertens 22:11–19. https://doi.org/10.1097/01.hjh.0000098149.7095679
19. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F, Redon J, Dominiczak A, Narkiewicz K, Nilsson PM, Burnier M, Viigimaa M, Ambrosioni E, Caufield M, Coca A, Olsen MH, Schmieder RE, Tsioufis C, van de Borne P, Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol C, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Sirnes PA, Tamargo JL, Tendera M, Torbicki A, Wijns W, Windecker S, Clement DL, Coca A, Gillebert TC, Tendera M, Rosei EA, Ambrosioni E, Anker SD, Bauersachs J, Hitij JB, Caulfield M, De Buyzere M, De Geest S, Derumeaux GA, Erdine S, Farsang C, Funck-Brentano C, Gerc V, Germano G, Gielen S, Haller H, Hoes AW, Jordan J, Kahan T, Komajda M, Lovic D, Mahrholdt H, Olsen MH, Ostergren J, Parati G, Perk J, Polonia J, Popescu BA, Reiner Z, Rydén L, Sirenko Y, Stanton A, Struijker-Boudier H, Tsioufis C, van de Borne P, Vlachopoulos C, Volpe M, Wood DA (2013) ESH/ESC guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European society of hypertension (ESH) and of the European society of cardiology (ESC). Eur Heart J 34:2159–2219. https://doi.org/10.1093/eurheartj/eht151
20. Heredia-Blonval K, Blanco-Metzler A, Montero-Campos M, Dunford EK (2014) The salt content of products from popular fast-food chains in Costa Rica. Appetite 83:173–177. https://doi.org/10.1016/j.appet.2014.08.027
21. Bazzano LA, Green T, Harrison TN, Reynolds K (2013) Dietary approaches to prevent hypertension. Curr Hypertens Rep 15:694–702. https://doi.org/10.1007/s11906-013-0390-z
22. Campbell N, Jilliian J, Campbell T (2012) Sodium consumption: and individual's choice? Int J Hypertens 2012:1–6. https://doi.org/10.1155/2012/860954
23. Dunford E (2012) International collaborative project to compare and track the nutritional composition of fast foods. BMC Public Health 12:559. https://doi.org/10.1186/1471-2458-12-559
24. McDonough AA, Veiras LC, Guevara CA, Ralph DL (2017) Cardiovascular benefits associated with higher dietary K vs. lower dietary Na evidence from population and mechanistic

studies. Am J Physiol Endocrinol Metab 312:E348. https://doi.org/10.1152/ajpendo.00453.2016
25. Wu L, Sun D, He Y (2016a) Fruit and vegetables consumption and incident hypertension: dose–response meta-analysis of prospective cohort studies. J Human Hypertens 30:573–580. https://doi.org/10.1038/jhh.2016.44
26. Aluko RE (2015) Antihypertensive peptides from food proteins. Annual Rev Food Sci Technol 6:235–262. https://doi.org/10.1146/annurev-food-022814-015520
27. Vercruysse L, Van Camp J, Smagghe G (2005) ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscle protein: a review. J Agric Food Chem 53:8106–8115. https://doi.org/10.1021/jf0508908
28. Fu Y, Young JF, Therkildsen M (2017) Bioactive peptides in beef: endogenous generation through postmortem aging. Meat Sci 123:134–142. https://doi.org/10.1016/j.meatsci.2016.09.015
29. Lee SY, Hur SJ (2017) Antihypertensive peptides from animal products, marine organisms, and plants. Food Chem 228:506–517. https://doi.org/10.1016/j.foodchem.2017.02.039
30. Norris R, Fitzgerald RJ (2013) Antihypertensive peptides from food proteins. In: Hernandez-Ledesma B, Hsieh CC (eds) Bioactive food peptides in health and disease. InTech, Rijeka. https://doi.org/10.5772/51710
31. Bah CSF, Bekhit AEDA, Carne A, McConnell MA (2013) Slaughterhouse blood: an emerging source of bioactive compounds. Compr Rev Food Sci Food Saf 12:314–331. https://doi.org/10.1111/1541-4337.12013
32. Ryder K, Bekhit AED, McConnell M, Carne A (2016) Towards generation of bioactive peptides from meat industry waste proteins: generation of peptides using commercial microbial proteases. Food Chem 208:42–50. https://doi.org/10.1016/j.foodchem.2016.03.121
33. Murray BA, FitzGerald RJ (2007) Angiotensin converting enzyme inhibitory peptides derived from proteins: biochemistry, bioactivity, and production. Curr Pharm Des 13:773–791
34. Ko JY, Kang N, Lee JH, Kim JS, Kim WS, Park SJ, Yong-Tae Kim YT, Jeo YJ (2016) Angiotensin I-converting enzyme inhibitory peptides from an enzymatic hydrolysate of flounder fish (*Paralichthys olivaceus*) muscle as a potent anti-hypertensive agent. Process Biochem 51:535–541. https://doi.org/10.1016/j.procbio.2016.01.009
35. Tagliazucchi D, Shamsia S, Helal A, Conte A (2016) Release of angiotensin converting enzyme-inhibitory peptides during *in vitro* gastro-intestinal digestion of camel milk. Int Dairy J 56:119–128. https://doi.org/10.1016/j.idairyj.2016.01.009
36. Mahmoud AH, Jeanette O, Cristian DG, Ali O, Essam H (2017) Angiotensin I-converting enzyme inhibitory activity and antioxidant capacity of bioactive peptides derived from enzymatic hydrolysis of buffalo milk proteins. Int Dairy J 66:91–98. https://doi.org/10.1016/j.idairyj.2016.11.006
37. Wu S, Feng X, Lan X, Xu Y, Liao D (2015) Purification and identification of angiotensin-I converting enzyme (ACE) inhibitory peptide from lizard fish (*Saurida elongata*) hydrolysate. J Funct Foods 13:295–299. https://doi.org/10.1016/j.jff.2014.12.051
38. Corrêa APF, Daroit DJ, Fontoura R, Meira SMM, Segalin J, Brandelli A (2014) Hydrolysates of sheep cheese whey as a source of bioactive peptides with antioxidant and angiotensin-converting enzyme inhibitory activities. Peptides 61:48–55. https://doi.org/10.1016/j.peptides.2014.09.001
39. Pokora M, Zambrowicz A, Da browska A, Eckert E, Setner B, Szołtysik M, Chrzanowska J (2014) An attractive way of egg white protein by-product use for producing of novel antihypertensive peptides. Food Chem 151:500–505. https://doi.org/10.1016/j.foodchem.2013.11.111
40. Ngo D-H, Vo T-S, Ryu B-M, Kim S-K (2016) Angiotensin-I-converting enzyme (ACE) inhibitory peptides from Pacific cod skin gelatine using ultrafiltration membranes. Process Biochem 51:1622–1628. https://doi.org/10.1016/j.procbio.2016.07.006
41. Lee JK, Jeon JK, Byun HG (2014) Antihypertensive effect of novel angiotensin I converting enzyme inhibitory peptide from chum salmon (*Oncorhynchusketa*) skin in spontaneously hypertensive rats. J Funct Foods 7:381–389. https://doi.org/10.1016/j.jff.2014.01.021

42. Salampessy J, Reddy N, Phillips M, Kailasapathy K (2017) Isolation and characterization of nutraceutically potential ACE-Inhibitory peptides from leatherjacket (*Meuchenia sp*.) protein hydrolysates. LWT-Food Sci Technol 80:430–436. https://doi.org/10.1016/j.lwt.2017.03.004
43. Stuknytè M, Cattaneo S, Masotti F, De Noni I (2015) Occurrence and fate of ACE-inhibitor peptides in cheeses and in their digestates following *in vitro* static gastrointestinal digestion. Food Chem 168:27–33. https://doi.org/10.1016/j.foodchem.2014.07.045
44. Majumder K, Chakrabarti S, Morton JS, Panahi S, Kaufman S, Davidge ST, Jianping WU (2015) Egg-derived ACE-inhibitory peptides IQW and LKP reduce blood pressure in spontaneously hypertensive rats. J Funct Foods 13:50–60. https://doi.org/10.1016/j.jff.2014.12.028
45. Majumder K, Jianping W (2010) A new approach for identification of novel antihypertensive peptides from egg proteins by QSAR and bioinformatics. Food Res Int 43:1371–1378. https://doi.org/10.1016/j.foodres.2010.04.027
46. Tagliazucchi D, Shamsia S, Helal A, Conte A (2017) Angiotensin-converting enzyme inhibitory peptides from goats' milk released by *in vitro* gastro-intestinal digestion. Int Dairy J 71:6–16. https://doi.org/10.1016/j.idairyj.2017.03.001
47. Corrons MA, Liggieri CS, Trejo SA, Bruno MA (2017) ACE-inhibitory peptides from bovine caseins released with peptidases from *Maclura pomifera* latex. Food Res Int 93:8–15. https://doi.org/10.1016/j.foodres.2017.01.003
48. Ibrahim HR, Ahmed AS, Miyata T (2017) Novel angiotensin-converting enzyme inhibitory peptides from caseins and whey proteins of goat milk. J Adv Res 8(1):63–71. https://doi.org/10.1016/j.jare.2016.12.002
49. Norris R, O'Keeffe MB, Poyarkov A, FitzGerald RJ (2015) Peptide identification and angiotensin converting enzyme (ACE) inhibitory activity in prolyl endoproteinase digests of bovine α_s-casein. Food Chem 188:210–217. https://doi.org/10.1016/j.foodchem.2015.04.130
50. Li Y, Sadiq FA, Liu T-J, Chen J-C, He G-Q (2015) Purification and identification of novel peptides with inhibitory effect against angiotensin I-converting enzyme and optimization of process conditions in milk fermented with the yeast *Kluyveromyces marxianus*. J Funct Foods 16:278–288. https://doi.org/10.1016/j.jff.2015.04.043
51. Paul M, Phillips JG, Renye JA Jr (2016) Measuring the angiotensin-converting enzyme inhibitory activity of an 8-amino acid (8mer) fragment of the C12 antihypertensive peptide. J Dairy Sci 99:3263–3266. https://doi.org/10.3168/jds.2015-10437
52. Asoodeh A, Homayouni-Tabrizi M, Shabestarian H, Emtenani S, Emtenani S (2016) Biochemical characterization of a novel antioxidant and angiotensin I-converting enzyme inhibitory peptide from *Struthio camelus* egg white protein hydrolysis. J Food Drug Analysis 24:332–342. https://doi.org/10.1016/j.jfda.2015.11.010
53. Chen Y, Wang Z, Chen X, Liu Y, Zhang H, Sun T (2010) Identification of angiotensin I-converting enzyme inhibitory peptides from koumiss, a traditional fermented mare's milk. J Dairy Sci 93:884–892. https://doi.org/10.3168/jds.2009-2672
54. Quiro A, Ramos M, Muguerza B, Delgado MA, Miguel M, Aleixandre A, Recio I (2007) Identification of novel antihypertensive peptides in milk fermented with *Enterococcus faecalis*. Int Dairy J 17:33–41. https://doi.org/10.1016/j.idairyj.2005.12.011
55. Geerlings A, Villar IC, Zarco FH, Sanchez M, Vera RA, Gomez Z, Boza J, Duarte J (2006) Identification and characterization of novel angiotensin-converting enzyme inhibitors obtained from goat milk. J Dairy Sci 89:3326–3335. https://doi.org/10.3168/jds.S0022-0302(06)72369-4
56. Contreras MDM, Carrón R, Montero MJ, Ramos M, Recio I (2009) Novel casein-derived peptides with antihypertensive activity. Int Dairy J 19:566–573. https://doi.org/10.1016/j.idairyj.2009.05.004
57. Garcés-Rimón M, López-Expósito I, López-Fandiño R, Miguel M (2016) Egg white hydrolysates with *in vitro* biological multiactivities to control complications associated with the metabolic syndrome. Eur Food Res Technol 242:61–69. https://doi.org/10.1007/s00217-015-2518-7
58. Sarmadi BH, Ismail A (2010) Antioxidative peptides from food proteins: a review. Peptides 31:1949–1956. https://doi.org/10.1016/j.peptides.2010.06.020
59. Wu J, Aluko RE, Nakai S (2006) Structural requirements of angiotensin I-converting enzyme inhibitory peptides: Quantitative structure - Activity relationship study of di- and tripeptides. J Agric Food Chem 54:732–738. https://doi.org/10.1021/jf0512631

60. Ferreira LAF, Galle A, Raida M, Schrader M, Lebrun I, Habermehl G (1998) Isolation, analysis and properties of three bradykinin-potentiating peptides (BPP-II, BPPIII, and BPP-V) From *Bothrops neuwiedi* venom. J Protein Chem 17:285–289
61. Norris R, Casey F, FitzGerald RJ, Shields D, Mooney C (2012) Predictive modelling of angiotensin converting enzyme inhibitory dipeptides. Food Chem 133:1349–1354. https://doi.org/10.1016/j.foodchem.2012.02.023
62. Gobbetti M, Minervini F, Rizzello CG (2004) Angiotensin I-converting enzyme-inhibitory and antimicrobial bioactive peptides. Int J Dairy Technol 57:173–188. https://doi.org/10.1111/j.1471-0307.2004.00139
63. De Gobba C, Tompa G, Otte J (2014) Bioactive peptides from caseins released by cold active proteolytic enzymes from *Arsukibacterium ikkense*. Food Chem 165:205–215. https://doi.org/10.1016/j.foodchem.2014.05.082
64. Hernández-Ledesma B, Miralles B, Amigo L, Ramos M, Recio I (2005) Identification of antioxidant and ACE-inhibitory peptides in fermented milk. J Sci Food Agric 85:1041–1048. https://doi.org/10.1002/jsfa.2063
65. Wu J, Liao W, Udenigwe CC (2017) Revisiting the mechanisms of ACE inhibitory peptides from food proteins. Trends Food Sci Technol. https://doi.org/10.1016/j.tifs.2017.07.011
66. Hall JE, Granger JP, do Carmo JM, da Silva AA, Dubinion J, George E, Hamza S, Speed J, Hall ME (2012) Hypertension: physiology and pathophysiology. J Compr Physiol 2:2393–2442. https://doi.org/10.1002/cphy.c110058.
67. Udenigwe CC, Mohan A (2014) Mechanisms of food protein-derived antihypertensive peptides other than ACE inhibition. J Funct Foods 8:45–52. https://doi.org/10.1016/j.jff.2014.03.002
68. Majumder K, Wu J (2014) Molecular targets of antihypertensive peptides: understanding the mechanisms of action based on the pathophysiology of hypertension. Int J Mol Sci 16 (1):256–283. https://doi.org/10.3390/ijms16010256
69. Saadi S, Saari N, Anwar F, Hamid AA, Ghazali MH (2015) Recent advances in food biopeptides: production, biological functionalities and therapeutic applications. Biotechnol Adv 33:80–116. https://doi.org/10.1016/j.biotechadv.2014.12.00
70. Elavarasan K, Shamasundar BA, Badii F, Howell N (2016) Angiotensin I-converting enzyme (ACE) inhibitory activity and structural properties of oven and freeze-dried protein hydrolysate from fresh water fish (*Cirrhinus mrigala*). Food Chem 206:210–216. https://doi.org/10.1016/j.foodchem.2016.03.047
71. Deniz E, Mora L, Aristoy MC, Candoğan K, Toldrá F (2016) Free amino acids and bioactive peptides profile of *Pastırma* during its processing. Food Res Int 89:194–201. https://doi.org/10.1016/j.foodres.2016.07.025
72. Lafargaa T, O'Connorb P, Hayes M (2014) Identification of novel dipeptidyl peptidase-IV and angiotensin-I-converting enzyme inhibitory peptides from meat proteins using *in silico* analysis. Peptides 59:53–62. https://doi.org/10.1016/j.peptides.2014.07.005
73. Nejati F, Rizzello CG, Di Cagno R, Sheikh-Zeinoddin M, Diviccaro A, Minervini F, Gobbetti M (2013) Manufacture of a functional fermented milk enriched of angiotensin-I-converting enzyme (ACE)-inhibitory peptides and g-amino butyric acid (GABA). LWT-Food Sci Technol 51:183–189. https://doi.org/10.1016/j.lwt.2012.09.017
74. Mirzaei M, Mirdamadi S, Ehsani MR, Aminlari M, Hosseini E (2015) Purification and identification of antioxidant and ACE-inhibitory peptide from *Saccharomyces cerevisiae* protein hydrolysate. J Functional Foods 19:259–268. https://doi.org/10.1016/j.jff.2015.09.031
75. Wu Q, Du J, Jia J, Kuang C (2016) Production of ACE inhibitory peptides from sweet sorghum grain protein using alcalase: hydrolysis kinetic, purification and molecular docking study. Food Chem 199:140–149. https://doi.org/10.1016/j.foodchem.2015.12.012
76. Nasri M (2017) Protein hydrolysates and biopeptides: production, biological activities, and applications in foods and health benefits. A review. In: Toldra, F. (Ed.), Advances in food and nutrition research, vol 81. ISSN 1043–4526, Elsevier Publications. https://doi.org/10.1016/bs.afnr.2016.10.003
77. Toopcham T, Roytrakul S, Yongsawatdigul J (2015) Characterization and identification of angiotensin I-converting enzyme (ACE) inhibitory peptides derived from tilapia using

Virgibacillus halodenitrificans SK1-3-7 proteinases. J Funct Foods 14:435–444. https://doi.org/10.1016/j.foodchem.2016.09.183
78. Kim SK, Wijesekara I (2010) Development and biological activities of marine-derived bioactive peptides: a review. J Funct Foods 2:1–9. https://doi.org/10.1016/j.jff.2010.01.003
79. Castellano P, Aristoy MC, Sentandreu MA, Vignolo G, Toldra F (2013) Peptides with angiotensin I-converting enzyme (ACE) inhibitory activity generated from porcine skeletal muscle proteins by the action of meat-borne lactobacillus. J Proteome 89:183–190. https://doi.org/10.1016/j.jprot.2013.06.023
80. Fakhfakh N, Ktari N, Siala R, Nasri M (2013) Wool-waste valorization: production of protein hydrolysates with high antioxidative potential by fermentation with a new keratinolytic bacterium, *Bacillus pumilus* A1. J Applied Microbiol 115:424–433. https://doi.org/10.1111/jam.12246
81. Jemil I, Jridi M, Nasri R, Ktari N, Slama-Ben Salem RB, Mehiri M, Hajji M, Nasri M (2014) Functional, antioxidant and antibacterial properties of protein hydrolysates prepared from fish meat fermented by *Bacillus subtilis*. Process Biochem 49:963–972. https://doi.org/10.1016/j.procbio.2014.03.004
82. Balakrishnan B, Prasad B, Rai AK, Narayan B (2011) *In vitro* antioxidant and antibacterial properties of hydrolysed proteins of delimed tannery fleshings: comparison of acid hydrolysis and fermentation methods. Biodegradation 22:287–295. https://doi.org/10.1007/s10532-010-9398-0
83. Hernández-Ledesma B, Contreras MDM, Recio I (2011) Antihypertensive peptides: production, bioavailability and incorporation into foods. Adv Colloid Interf Sci 165:23–35. https://doi.org/10.1016/j.cis.2010.11.001
84. Rao S, Su Y, Junhua Li XZ, Yang Y (2009) Design and expression of recombinant antihypertensive peptide multimer gene in *Escherichia coli* BL21. J Microbiol Biotechnol 19(12):1620–1627. https://doi.org/10.4014/jmb.0905.05055
85. Bhat ZF, Kumar S, Bhat HF (2017) Antihypertensive peptides of animal origin: a review. Crit Rev Food Sci Nutr 57:566–578. https://doi.org/10.1080/10408398.2014.898241
86. Dziuba B, Dziuba M (2014) Milk proteins-derived bioactive peptides in dairy products: molecular, biological and methodological aspects. Acta Sci Pol Technol Aliment 13(1):5–25. 10.17306/J.AFS.2014.1.1
87. Fosgerau K, Hoffmann T (2015) Peptide therapeutics: current status and future directions. Drug Discov Today 20(1):122–128. https://doi.org/10.1016/j.drudis.2014.10.003
88. Mora L, Gallego M, Reig M, Toldra F (2017) Challenges in the quantitation of naturally generated bioactive peptides in processed meats. Trends Food Sci Technol. https://doi.org/10.1016/j.tifs.2017.04.011
89. Khan RS, Grigor J, Winger R, Win A (2013) Functional food product development-opportunities and challenges for food manufacturers. Trends Food Sci Technol 30:27–23. https://doi.org/10.1016/j.tifs.2012.11.004
90. Contreras MM, Sevilla MA, Monroy-Ruix J, Amigo L, Gómez-Sala B, Molina E, Ramos M, Recio I (2011) Food-grade production of an antihypertensive casein hydrolysate and resistance of active peptides to drying and storage. Int Dairy J 21:470–476. https://doi.org/10.1016/j.idairyj.2011.02.004
91. Jang A, Jo C, Lee M (2007) Storage stability of the synthetic angiotensin converting enzyme (ACE) inhibitory peptides separated from beef sarcoplasmic protein extracts at different pH, temperature, and gastric digestion. Food Sci Biotechnol 16:572
92. Bouglé D, Bouhallab S (2017) Dietary bioactive peptides: human studies. Crit Rev Food Sci Nutr 57:335–343. https://doi.org/10.1080/10408398.2013.873766
93. Leo FD, Panarese S, Gallerani R, Ceci LR (2009) Angiotensin converting enzyme (ACE) inhibitory peptides: production and implementation of functional food. Curr Pharm Des 15:3622–3643. https://doi.org/10.2174/138161209789271834
94. Daskaya-Dikmen C, Yucetepe A, Karbancioglu-Guler F, Daskaya H, Ozcelik B (2017) Angiotensin-I-converting enzyme (ace)-inhibitory peptides from plants. Forum Nutr 9(4):316. https://doi.org/10.3390/nu9040316

95. Balti R, Bougatef A, Sila A, Guillochon D, Dhulster P, Nedjar-Arroume N (2015) Nine novel angiotensin I-converting enzyme (ACE) inhibitory peptides from cuttlefish (*Sepia officinalis*) muscle protein hydrolysates and antihypertensive effect of the potent active peptide in spontaneously hypertensive rats. Food Chem 170:519–525. https://doi.org/10.1016/j.foodchem.2013.03.091
96. Turpeinen AM, Kumpu M, Seppo RL, Kautiainen T, Jauhiainen H, Korpela VR (2009) Antihypertensive and cholesterol-lowering effects of a spread containing bioactive peptides IPP and VPP and plant sterols. J Funct Foods 1:260–265. https://doi.org/10.1016/j.jff.2009.03.001
97. Lafarga T, Hayes M (2014) Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. Meat Sci 98:227–239. https://doi.org/10.1016/j.meatsci.2014.05.036
98. Fujita H, Yokohama K, Yoshikawa M (2000) Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. J Food Chem 65:564–569. https://doi.org/10.1111/j.1365-2621.2000.tb16049.x
99. Katayama K, Jamhari Mori T, Kawahara S, Miake K, Kodama Y, Sugiyama M, Kawamura Y, Nakayama T, Muruyama M, Muguruma M (2007) Angiotensin-I converting enzyme inhibitory peptide derived from porcine skeletal muscle myosin and its antihypertensive activity in spontaneously hypertensive rats. J Food Sci 9:S702–S706. https://doi.org/10.1111/j.1750-3841.2007.00571.x
100. Jang A, Jo C, Kang KS, Lee M (2008) Antimicrobial and human cancer cell cytotoxic effect of synthetic angiotensin-converting enzyme (ACE) inhibitory peptides. Food Chem 107:327–336. https://doi.org/10.1016/j.foodchem.2007.08.036
101. Ahhmed AM, Muguruma M (2010) A review of meat protein hydrolysates and hypertension. Meat Sci 86(1):110–118. https://doi.org/10.1016/j.meatsci.2010.04.032
102. Iwaniak A, Minkiewicz P, Darewicz M (2014) Food originating ACE inhibitors, including antihypertensive peptides, as preventive food components in blood pressure reduction. Compr Rev Food Sci Food Saf 13:114–134. https://doi.org/10.1111/1541-4337.12051
103. Katayama K, Anggraeni HE, Mori T, Ahhmed AM, Kawahara S, Sugiyama M, Nakayama T, Maruyama M, Muguruma M (2008) Porcine skeletal muscle troponin is a good source of peptides with angiotensin-I converting enzyme inhibitory activity and antihypertensive effects in spontaneously hypertensive rats. J Agric Food Chem 56:355–360. https://doi.org/10.1021/jf071408j
104. Saiga A, Okumura T, Makihara T, Katsuta S, Shimizu T, Yamada R, Nishimura TF (2003) Angiotensin I-converting enzyme inhibitory peptides in a hydrolyzed chicken breast muscle extract. J Agric Food Chem 51:1741–1745. https://doi.org/10.1021/jf020604h
105. Stadnik J, Kęska P (2015) Meat and fermented meat products as a source of bioactive peptides. Acta Sci Pol Technol Aliment 14:181–190. https://doi.org/10.17306/J.AFS.2015.3.19.
106. Gomez-Guillen MC, Gimenez B, Lopez-Caballero ME, Montero MP (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocol 25:1813–1827. https://doi.org/10.1016/j.foodhyd.2011.02.007
107. Atsuta SHK, Himizu TSS, Amada RYY, Ishimura TON (2003) Angiotensin I-converting enzyme inhibitory peptides in a hydrolyzed chicken breast muscle extract. Blood Press 18:1741–1745. https://doi.org/10.1021/jf020604h
108. Arihara K, Nakashima Y, Mukai T, Ishikawa S, Itoh M (2001) Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. Meat Sci 57:319–324. https://doi.org/10.1016/S0309-1740(00)00108-X
109. Arihara K (2006) Strategies for designing novel functional meat products. Meat Sci 74:219–229. https://doi.org/10.1016/j.meatsci.2006.04.028
110. Nakashima Y, Arihara K, Sasaki A, Mio H, Ishikawa S, Itoh M (2002) Antihypertensive activities of peptides derived from porcine skeletal muscle myosin in spontaneously hypertensive rats. J Food Sci 67:434–437. https://doi.org/10.1111/j.1365-2621.2002.tb11424.x

111. Muguruma M, Ahhmed AM, Katayama K, Kawahara S, Maruyama M, Nakamura T (2009) Identification of pro-drug type ACE inhibitory peptide sourced from porcine myosin B: evaluation of its antihypertensive effects *in vivo*. Food Chem 114:516–522. https://doi.org/10.1016/j.foodchem.2008.09.081
112. Ukeda H, Matsuda H, Osajima K, Matsufuji H, Matsui T, Osajima Y (1992) Peptides from peptidic hydrolysate of heated sardine meat that inhibit angiotensin I-converting enzyme. Nippon Nogei Kaishi 66:25–29
113. Saiga A, Okumura T, Makihara T, Katsuda S-I, Morimatsu F, Nishimura T (2006) Action mechanism of an angiotensin I-converting enzyme inhibitory peptide derived from chicken breast muscle. J Agric Food Chem 54:942–945. https://doi.org/10.1021/jf0508201
114. Ryan JT, Ross RP, Bolton D, Fitzgerald GF, Stanton C (2011) Bioactive peptides from muscle sources: meat and fish. Forum Nutr 3(9):765–791. https://doi.org/10.3390/nu3090765
115. Toldrá F, Reig M, Aristoy MC, Mora L (2017) Generation of bioactive peptides during food processing. Food Chem. https://doi.org/10.1016/j.foodchem.2017.06.119
116. Fernández M, Benito MH, Martín A, Casquete R, Córdoba JJ, Córdoba MG (2016) Influence of starter culture and a protease on the generation of ACE-inhibitory and antioxidant bioactive nitrogen compounds in Iberian dry-fermented sausage '*salchichón*'. Heliyon 2(3):e00093. https://doi.org/10.1016/j.heliyon.2016.e00093
117. Gu Y, Majumder K, Wu J (2011) QSAR-aided in silico approach in evaluation of food proteins as precursors of ACE inhibitory peptides. Food Res Int 44:2465–2474. https://doi.org/10.1016/j.foodres.2011.01.051
118. Terashima M, Baba T, Ikemoto N, Katayama M, Morimoto T, Matsumura S (2010) Novel angiotensin-converting enzyme (ACE) inhibitory peptides derived from boneless chicken leg meat. J Agric Food Chem 58(12):7432–7436. https://doi.org/10.1021/jf100977z
119. R-Z G, Liu WY, Lin F, Jin ZT, Chen L, Yi WX, Lu J, Cai MY (2012) Antioxidant and angiotensin I-converting enzyme inhibitory properties of oligopeptides derived from black-bone silky fowl (*Gallus gallus domesticus Brisson*) muscle. Food Res Int 49:326–333. https://doi.org/10.1016/j.foodres.2012.07.009
120. Katayama K, Tomatsu M, Fuchu H, Sugiyama M, Kawahara S, Yamauchi K, Muguruma M (2003) Purification and characterization of an angiotensin I-converting enzyme inhibitory peptide derived from porcine troponin C. Anim Sci J 74:53–58. https://doi.org/10.1046/j.1344-3941.2003.00086.x
121. Escudero E, Sentandreu MA, Arihara K, Toldrá F (2010) Angiotensin I-converting enzyme inhibitory peptides generated from *in vitro* gastrointestinal digestion of pork meat. J Agric Food Chem 58:2895–2901. https://doi.org/10.1021/jf904204n.
122. Escudero E, Toldrá F, Sentandreu MA, Nishimura H, Arihara K (2012b) Antihypertensive activity of peptides identified in the in vitro gastrointestinal digest of pork meat. Meat Sci 91:382–384. https://doi.org/10.1016/j.meatsci.2012.02.007
123. Jang A, Lee M (2005) Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. Meat Sci 69:653–661. https://doi.org/10.1016/j.meatsci.2004.10.014
124. Jang A, Cho YJ, Lee JI, Shin JH, Kim IS, Lee M (2004) The effect of beef peptide on blood pressure and serum lipid concentration of spontaneously hypertensive rat (SHR). J Anim Sci Technol 46:107–114. https://doi.org/10.5187/JAST.2004.46.1.107
125. Bernardini RD, Mullen AM, Bolton D, Kerry J, O'Neill E, Hayes M (2012) Assessment of the angiotensin-I-converting enzyme (ACE-I) inhibitory and antioxidant activities of hydrolysates of bovine brisket sarcoplasmic proteins produced by papain and characterisation of associated bioactive peptidic fractions. Meat Sci 90:226–235. https://doi.org/10.1016/j.meatsci.2011.07.008
126. Sakanaka S, Tachibana Y, Ishihara N, Juneja LR (2005) Antioxidant properties of casein calcium peptides and their effects on lipid oxidation in beef homogenates. Food Chem 53:464–468. https://doi.org/10.1021/jf0487699
127. Díaz M, Decker EA (2004) Antioxidant mechanisms of caseinophosphopeptidases and casein hydrolysates and their application in ground beef. J Agric Food Chem 52:8208–8213. https://doi.org/10.1021/jf048869e

128. Peña-Ramos EA, Xiong YL (2003) Whey and soy hydrolysates inhibit lipid oxidation in cooked pork patties. Meat Sci 64:259–263. https://doi.org/10.1016/S0309-1740(02)00187-0
129. Minkiewicz P, Dziuba J, Michalska J (2011) Bovine meat proteins as potential precursors of biologically active peptides – a computational study based on the BIOPEP database. Food Sci Technol Int 17:39–45. https://doi.org/10.1177/1082013210368461.
130. Fu Y, Young JF, Løkke MM, Lametsch R, Aluko RE, Therkildsen M (2016) Revalorisation of bovine collagen as a potential precursor of angiotensin I-converting enzyme (ACE) inhibitory peptides based on in silico and in vitro protein digestions. J Funct Foods 24:196–206. https://doi.org/10.1016/j.jff.2016.03.026
131. Fu Y, Young JF, Rasmussen MK, Dalsgaard TK, Lametsch R, Aluko RE, Therkildsen M (2016) Angiotensin I-Converting enzyme–inhibitory peptides from bovine collagen: insights into inhibitory mechanism and transepithelial transport. Food Res Int 89:373–381. https://doi.org/10.1016/j.foodres.2016.08.037
132. Fu Y, Young JF, Dalsgaard TK, Therkildsen M (2015) Separation of angiotensin I converting enzyme inhibitory peptides from bovine connective tissue and their stability towards temperature, pH and digestive enzymes. Int J Food Sci Technol 50:1234–1243. https://doi.org/10.1111/ijfs.12771
133. Banerjee P, Shanthi C (2012) Isolation of novel bioactive regions from bovine Achilles tendon collagen having angiotensin I-converting enzyme-inhibitory properties. Process Biochem 47:2335–2346. https://doi.org/10.1016/j.procbio.2012.09.012
134. O'Keeffe MB, Norris R, Alashi MA, Aluko RE, FitzGerald RJ (2017) Peptide identification in a porcine gelatin prolyl endoproteinase hydrolysate with angiotensin converting enzyme (ACE) inhibitory and hypotensive activity. J Funct Foods 34:77–88. https://doi.org/10.1016/j.jff.2017.04.018
135. Ngo DH, Kang KH, Ryu B, Vo TS, Jung WK, Byun HG, Kim SK (2015) Angiotensin-I converting enzyme inhibitory peptides from antihypertensive skate (*Okamejei kenojei*) skin gelatin hydrolysate in spontaneously hypertensive rats. Food Chem 174:37–43. https://doi.org/10.1016/j.foodchem.2014.11.013
136. Lin L, Lv S, Li B (2012) Angiotensin-I-converting enzyme (ACE)-inhibitory and antihypertensive properties of squid skin gelatin hydrolysates. Food Chem 131:225–230. https://doi.org/10.1016/j.foodchem.2011.08.064
137. Zhuang Y, Sun L, Zhang Y, Liu G (2012) Antihypertensive effect of long-term oral administration of jellyfish (*Rhopilema esculentum*) collagen peptides on renovascular hypertension. Mar Drugs 10:417. https://doi.org/10.3390/md10020417.
138. Herregods G, Van Camp J, Morel N, Ghesquière B, Gevaert K, Vercruysse L, Smagghe G (2011) Angiotensin I-converting enzyme inhibitory activity of gelatin hydrolysates and identification of bioactive peptides. J Agric Food Chem 59:552–558. https://doi.org/10.1021/jf1037823
139. Ichimura T, Yamanaka A, Otsuka T, Yamashita E, Maruyama S (2009) Antihypertensive effect of enzymatic hydrolysate of collagen and Gly-Pro in spontaneously hypertensive rats. Biosci Biotechnol Biochem 73:2317–2319. https://doi.org/10.1271/bbb.90197
140. Faria M, da Costa EL, Gontijo JAR, Netto FM (2008) Evaluation of the hypotensive potential of bovine and porcine collagen hydrolysates. J Med Food 11:560–567. https://doi.org/10.1089/jmf.2007.0573.
141. Saiga A, Iwai K, Hayakawa T, Takahata Y, Kitamura S, Nishimura T, Morimatsu F (2008) Angiotensin I-converting enzyme-inhibitory peptides obtained from chicken collagen hydrolysate. J Agric Food Chem 56:9586–9591. https://doi.org/10.1021/jf072669w
142. Taga Y, Kusubata M, Ogawa-Goto K, Hattori S (2016) Efficient absorption of X-hydroxyproline (Hyp)-Gly after oral administration of a novel gelatine hydrolysate prepared using ginger protease. J Agric Food Chem 64:2962–2970. https://doi.org/10.1021/acs.jafc.6b00609
143. Iwai K, Hasegawa T, Taguchi Y, Morimatsu F, Sato K, Nakamura Y, Ohtsuki K (2005) Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. J Agric Food Chem 53:6531–6536. https://doi.org/10.1021/acs.jafc.6b00609
144. Inoue N, Hamasaki A, Hidaka S, Miura N, Fukahori M, Maruyama M, Kawahara S, Ohta K, Muguruma M (2013) Analysis of the components of porcine liver hydrolysate

and examination of the antioxidant activity and angiotensin converting enzyme (ACE)-inhibiting activity. Yakugaku Zasshi 133(1):107–115. https://doi.org/10.1248/yakushi.y110184
145. Wu H, He HL, Chen XL, Sun CY, Zhang YZ, Zhou BC (2008) Purification and identification of novel angiotensin-I-converting enzyme inhibitory peptides from shark meat hydrolysate. Process Biochem 43:457–461. https://doi.org/10.1016/j.procbio.2008.01.018
146. Van Platerink CJ, Janssen HGM, Haverkamp J (2008) Application of at-line two-dimensional liquid chromatography-mass spectrometry for identification of small hydrophilic angiotensin I-inhibiting peptides in milk hydrolysates. Anal Bioanal Chem 391:299–307. https://doi.org/10.1007/s00216-008-1990-3
147. Yu Y, Hu J, Miyaguchi Y, Bai X, Du Y, Lin B (2006) Isolation and characterisation of angiotensin I-converting enzyme inhibitory peptides derived from porcine haemoglobin. Peptides 27:2950–2956. https://doi.org/10.1016/j.peptides.2006.05.025
148. Kim SK, Byun HG, Park PJ, Shahidi F (2001) Angiotensin I converting enzyme inhibitory peptides purified from bovine skin gelatin hydrolysate. J Agric Food Chem 49:2992–2997
149. Ferranti P, Nitride C, Nicolai MA, Mamone G, Picariello G, Bordoni A, Valli V, Nunzio MD, Babini E, Marcolini E, Capozzi F (2014) *In vitro* digestion of *Bresaola* proteins and release of potential bioactive peptides. Food Res Int 63:157–169. https://doi.org/10.1016/j.foodres.2014.02.008
150. Gallego M, Mora L, Fraser PD, Aristoy MC, Toldrá F (2014) Degradation of LIM domain-binding protein three during processing of Spanish dry-cured ham. Food Chem 149:121–128. https://doi.org/10.1016/j.foodchem.2013.10.076
151. Christensen L, Ertbjerg P, Løje H, Risbo J, van den Berg FW, Christensen M (2013) Relationship between meat toughness and properties of connective tissue from cows and young bulls heat treated at low temperatures for prolonged times. Meat Sci 93:787–795. https://doi.org/10.1016/j.meatsci.2012.12.001
152. Fogle DR, Plimpton RF, Ockerman HW, Jarenback L, Persson T (1982) Tenderization of beef: effect of enzyme, enzyme level, and cooking method. J Food Sci 47:1113–1118
153. Escudero E, Mora L, Fraser PD, Aristoy MC, Arihara K, Toldrá F (2013) Purification and identification of antihypertensive peptides in Spanish dry-cured ham. J Proteome 78:499–507. https://doi.org/10.1016/j.jprot.2012.10.019
154. Liu D, Chen X, Huang J, Huang M, Zhou G (2017) Generation of bioactive peptides from duck meat during post-mortem aging. Food Chem 237:408–415. https://doi.org/10.1016/j.foodchem.2017.05.094
155. Sangsawad P, Roytrakul S, Yongsawatdigul J (2017) Angiotensin converting enzyme (ACE) inhibitory peptides derived from the simulated *in vitro* gastrointestinal digestion of cooked chicken breast. J Funct Foods 29:77–83. https://doi.org/10.1016/j.jff.2016.12.005
156. Darewicz M, Borawska J, Vegarud GE, Minkiewicz P, Iwaniak A (2014) Angiotensin I-converting enzyme (ACE) inhibitory activity and ACE inhibitory peptides of salmon (*Salmo salar*) protein hydrolysates obtained by human and porcine gastrointestinal enzymes. Int J Mol Sci 15:14077–14101. https://doi.org/10.3390/ijms150814077
157. Natesh R, Schwager SL, Sturrock ED, Acharya KR (2003) Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. Nature 421:551–554. https://doi.org/10.1038/nature01370
158. Soladoye O, Juárez M, Aalhus J, Shand P, Estévez M (2015) Protein oxidation in processed meat: mechanisms and potential implications on human health. Compr Rev Food Sci Food Saf 14:106–122. https://doi.org/10.1111/1541-4337.12127
159. Estévez M (2011) Protein carbonyls in meat systems: a review. Meat Sci 89:259–279. https://doi.org/10.1016/j.meatsci.2011.04.025
160. Katayama K, Tomatsu M, Kawahara S, Yamauchi K, Fuchu H, Kodama Y, Kawamura Y, Muguruma M (2004) Inhibitory profile of nanopeptide derived from porcine troponin C against angiotensin I-converting enzyme. J Agric Food Chem 52:771–775. https://doi.org/10.1021/jf0350865

161. Mirdhayati I, Hermanianto J, Wijaya CH, Sajuthi D, Arihara K (2016) Angiotensin converting enzyme (ACE) inhibitory and antihypertensive activities of protein hydrolysate from meat of Kacang goat (*Capra aegagrus hircus*). J Sci Food Agric 96:3536–3542. https://doi.org/10.1002/jsfa.7538
162. Mora L, Aristoy M, Toldrá F (2016) Bioactive peptides in foods. In: Caballero B, Finglas PM, Toldrá F (eds) Encyclopedia of food and health, vol vol 1. Academic Press/Elsevier Science Ltd, London, pp 395–400
163. Escudero E, Aristoy MC, Nishimura H, Arihara K, Toldrá F (2012) Antihypertensive effect and antioxidant activity of peptide fractions extracted from Spanish dry cured ham. Meat Sci 91:306–311. https://doi.org/10.1016/j.meatsci.2012.02.008
164. Arihara K, Ohata M (2008) Bioactive compounds in meat. In: Meat biotechnology, Springer, pp 231–249, Toldrá, F (Edn), ISBN: 978-0-387-79381-8 (Print), 978-0-387-79382-5 (Online).
165. Escudero E, Mora L, Toldrá F (2014) Stability of ACE inhibitory ham peptides against heat treatment and in vitro digestion. Food Chem 161:305–311. https://doi.org/10.1016/j.foodchem.2014.03.117
166. Mora L, Escudero E, Arihara K, Toldrá F (2015) Antihypertensive effect of peptides naturally generated during Iberian dry-cured ham processing. Food Res Int 78:71–78. https://doi.org/10.1016/j.foodres.2015.11.005
167. Dellafiora L, Paolella S, Dall'Asta C, Dossena A, Cozzini P, Galaverna G (2015) Hybrid *in silico*/*in vitro* approach for the identification of angiotensin I converting enzyme inhibitory peptides from *Parma* dry-cured ham. J Agric Food Chem 63(28):6366–6375. https://doi.org/10.1021/acs.jafc.5b02303
168. Paolella S, Falavigna C, Faccini A, Virgili R, Sforza S, Dall'Asta C, Dossena A, Galaverna G (2015) Effect of dry-cured ham maturation time on simulated gastrointestinal digestion: characterization of the released peptide fraction. Food Res Int 67:136–144. https://doi.org/10.1016/j.foodres.2014.10.026
169. Sentandreu MA, Toldrá F (2007) Evaluation of ACE inhibitory activity of dipeptides generated by the action of porcine muscle dipeptidyl peptidases. Food Chem 102:511–515. https://doi.org/10.1016/j.foodchem.2006.04.018
170. Vaštag Ž, Popović L, Popović S, Petrović L, Peričin D (2010) Antioxidant and angiotensin-I converting enzyme inhibitory activity in the water-soluble protein extract from Petrovac Sausage (*Petrovská Kolbasá*). Food Control 21:1298–1302. https://doi.org/10.1016/j.foodcont.2010.03.004
171. Gallego M, Grootaert C, Mora L, Aristoy MC, Camp JV, Toldrá F (2016) Transepithelial transport of dry-cured ham peptides with ACE inhibitory activity through a Caco-2 cell monolayer. J Funct Foods 21:388–395. https://doi.org/10.1016/j.jff.2015.11.046
172. Mora L, Escudero E, Toldrá F (2016) Characterization of the peptide profile in Spanish *Teruel*, Italian *Parma* and Belgian dry-cured hams and its potential bioactivity. Food Res Int 89:638–646. https://doi.org/10.1016/j.foodres.2016.09.016
173. Benito MJ, Connerton IF, Cordoba JJ (2006) Genetic characterization and expression of the novel fungal protease: EPg222 active in dry-cured meat products. Appl Microbiol Biot 73:356–365. https://doi.org/10.1007/s00253-006-0498-z
174. Mejri L, Romy VV, Hassouna MN, Marina ML, García MC (2017) Identification of peptides with antioxidant and antihypertensive capacities by RP-HPLC-Q-TOF-MS in dry fermented camel sausages inoculated with different starter cultures and ripening times. Food Res Int. https://doi.org/10.1016/j.foodres.2017.07.072

Bioactive Peptides from Fish Protein By-Products

14

Aurélien V. Le Gouic, Pádraigín A. Harnedy, and Richard J. FitzGerald

Contents

Abstract

The interest in fish processing by-products and underutilized catch for the production of biofunctional food ingredients has increased in the last number of decades. These marine-derived components contain a significant quantity of protein, which is normally processed into low-value products such as animal feed, fishmeal, and fertilizer. However, due to the global demand for high-quality protein and the need for sustainable production and processing of landed material, the valorization of proteins and other nutrients from fish processing by-products has significantly increased. Fish processing by-products contain significant

A. V. Le Gouic · P. A. Harnedy · R. J. FitzGerald (✉)
Department of Biological Sciences, University of Limerick, Limerick, Ireland
e-mail: aurelien.legouic@ul.ie; padraigin.harnedy@ul.ie; dick.fitzgerald@ul.ie

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_29

quantities of high-quality protein, which can be exploited as sources of essential nitrogenous nutrients and biologically active peptides. Bioactive peptides, including those from fish processing by-products, have been reported to possess the ability to beneficially modulate physiological processes associated with non-communicable diseases. These short peptides, which are encrypted within the primary sequence of the parent protein and are released during food processing or gastrointestinal digestion, could have a role in the prevention and management of these diseases. This chapter reviews the recent literature on the processing and utilization of proteins and protein hydrolysates from fish processing by-products and underutilized fish species with a particular focus on their bioactive properties and peptide sequences.

Keywords
By-products · Proteins · Hydrolysates · Biofunctional properties · Fish · Peptides · Noncommunicable diseases

1 Introduction

The ocean covers about 70% of the total earth's surface and contains approximately 60% of all fish species. The other 40% are found in fresh water which comprises 1% of the earth's surface. The fishing industry represents an important economic activity for many countries around the world. In 2003, marine fisheries supported 260 million jobs directly and indirectly [1]. The demand for fish and shellfish has increased throughout the world. The amount of farmed fish doubled in the last decade while that of captured fish has tended to stabilize. According to the Food and Agriculture Organization (FAO), global fish production in 2016 was estimated to be 174.1 million tonnes (mt) by both capture and aquaculture with 152.8 mt being used for human food consumption and 21.3 mt for nonfood uses [2]. It has been estimated that greater than 60% (by weight) of the fish which are processed are represented by by-products, i.e., head, skin, bones, fins, trimmings, viscera, blood, and roe [3]. The large quantity of fish by-products generated represents a potentially significant source of pollution in developing and developed countries. Since 2014, the European Commission, under Directive No 1392/2014 [4], regulates the discard of all fish caught regardless of whether they are regulated by quota, smaller than the minimum size and either dead or alive, and now imposes an obligation to land all catch.

Fish processing by-products and underutilized catch contain a significant quantity of protein which is normally processed into low-value products such as animal feed, fish meal, and fertilizer [2]. However, given the increased global demand for high-quality protein and the requirement for sustainable production and processing, there is an increasing interest in the extraction and valorization of proteins and other nutrients from fish processing by-products. The proteins within fish processing by-products represent a source of high-quality protein which can be exploited for the provision of essential nitrogenous nutrients. Therefore, various biotechnological

approaches have, and are, being employed to extract valuable nutrients and bioactive compounds targeted at enhancing human health. Bioactive compounds have a role in the management and in the protection against a range of chronic noncommunicable diseases (NCDs). Furthermore, by-product proteins may be used to generate biologically active peptides (BAPs). BAPs have the ability to modulate physiological processes and thereby have a role in the prevention and management of disease.

Protein hydrolysates are obtained following the enzymatic conversion of intact proteins into peptides. These protein fragments usually contain no more than 20 amino acids. A large range of fish protein hydrolysates, generated using food-grade proteolytic preparations, such as trypsin, Alcalase® 2.4L, Flavourzyme® 500L/1000L, Corolase® PP, and Promod® 144MG, have been reported in the literature [5–7]. These hydrolysates, due to their physicochemical properties, are a source of amino acids [8] which have applications in human and animal nutrition, as well as in the pharmaceutical and cosmetic industries. Due to their good nutritional composition, amino acid profile, and bioactive properties, fish protein hydrolysates have many commercial applications [9].

This chapter reviews the recent literature on the processing and utilization of proteins and protein hydrolysates from fish processing by-products and underutilized fish species with a particular reference on their bioactive properties and peptide sequences.

2 Fish Protein Composition

Fish consumption is linked with many health benefits due to their high protein levels and also due to their content of unsaturated fatty acids, vitamins, and minerals [10]. Fish proteins are a particularly rich source of the essential amino acids valine and lysine [11].

The chemical composition of foods has an important role in the supply of essential nutrients for the maintenance of human health. The chemical composition of fish by-products, i.e., levels of protein, ash, and lipid, differs significantly between species.

Fish processing by-products are designated as parts of the fish which are not generally used for human consumption, e.g., head, skin, and viscera. However, the nutrients therein are recoverable and can be utilized after further processing. These components may represent between 30% and 60% by weight of the starting material. Consequently, these fish processing by-products represent a rich source of biofunctional materials such as vitamins, minerals, polysaccharides, polyunsaturated fatty acids (PUFA), enzymes, collagen, gelatin, and bioactive peptides with valuable nutraceutical, pharmaceutical, and cosmeceutical applications [12].

Proteins are characterized by their amino acid sequence and secondary structure and also by their tertiary structure, which may be highly ordered. Each type of protein has a unique structure that determines its function in the organism in addition to its technofunctional properties when utilized as a food source/ingredient [13]. The nutritional value of food proteins is determined on the basis of their essential amino

Table 1 Essential amino acid content of reference food proteins, fish proteins/protein hydrolysates, and human daily requirements

Amino acid	Sodium caseinate[a] (mg/g protein)	Egg white[a] (mg/g protein)	Tilapia fillets[b] (mg/g protein)	Tuna viscera hydrolysates[c] (mg/g protein)	Amino acid requirements (mg/kg/d)[a] age category (years)		
					1–2	3–18	>18
Thr	40.5	45.3	27.6	59.0	24	18–17	15
Met	32.0	68.4	42.0	–	22	17–16	15
Val	56.4	73.0	66.2	89.3	36	29–28	26
Ile	45.9	55.9	62.4	69.3	27	22–21	20
Leu	88.9	93.6	103.2	77.0	54	44–42	39
Phe	101.4	110.4	49.8	51.6	40	30–28	25
His	25.4	26.3	20.2	84.5	15	12–11	10
Lys	77.5	76.0	97.5	18.7	44	35–33	30
Trp	10.4	17.6	5.2	–	6	4.8–4.4	4.0

[a]FAO/WHO/UNU (2007)
[b]Vidotti et al. [14]
[c]Villamil et al. [15]

acid content. Table 1 summarized the essential amino acid content of foodstuffs compared to the daily requirement. In comparison to plant proteins, proteins derived from animal sources (because of their high content of essential amino acids) are considered nutritionally superior. Of these, egg white and milk proteins (casein) are usually used as reference proteins for determination of protein quality. Proteins derived from meat and poultry muscle are also considered as a source of high-quality protein. It has been shown that the nutritional value of most fish proteins is equal to or better than casein and the quality of fish proteins may exceed that of terrestrial animal meat [16].

Myofibrillar proteins are structural proteins responsible for movement by their capacity for contraction. They represent 65–75% of the total fish muscle, while sarcoplasmic proteins (soluble proteins) represent 20–35% [17]. Myofibrillar proteins are mainly composed of actin and myosin. The motility of fish is also stabilized and regulated by other structural proteins including titin, nebulin, α-actinin, tropomyosin, and troponin (T, I, and C). The proportion and presence of these structural proteins depend on the fish species. Myosin is the major protein in fish muscle (40%); however, it is a protein easily destabilized when heated, especially the myosin from cold-water fish species. It is a large protein, with a molecular mass of 470 kDa [18], and has an unusual structure as it has both fibrous and globular properties whereas most food protein ingredients, such as proteins from egg, soy, and milk, have globular structures and have a lower molecular mass. Myosin is composed of two heavy chains of 220 kDa and two pairs of different light chains (LCs), ranging from 17 to 22 kDa [19]. The myosin molecule is approximately 160 nm in length. The heavy chains interact via two domains: a globular domain called “head” and a fibrous domain called “tail.” Actin accounts for 15–30% of the myofibrillar proteins. The monomer form of actin is a globular protein (G-actin) of

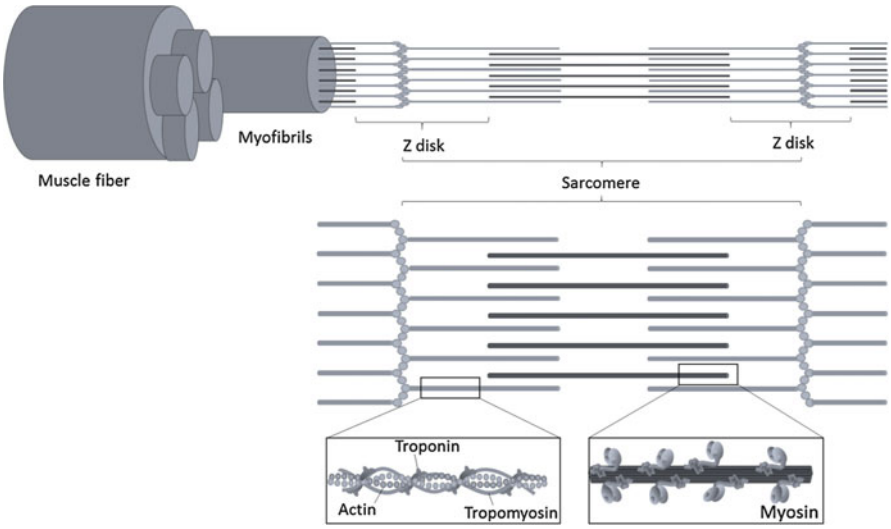


Fig. 1 Diagrammatic representation of the actin-myosin complex. (Modified from Gordon et al. [20])

43 kDa; however, globular actin monomers polymerize to form filaments of fibrous actin (F-actin). Other small proteins associated with either actin or myosin include titin, nebulin, tropomyosin, troponin, C-proteins, and M-proteins [18]. These proteins play an important role in the stabilization of the muscle myofibril basic structure termed the sarcomere (Fig. 1).

The denaturation of myofibrillar proteins by heat, chemical, and enzymatic treatment leads to the generation of a gel [21]. The gelation properties of fish myofibrillar proteins are exploited in the food industry to produce surimi or surimi-like products. The connective tissue proteins, or stromal proteins, provide the structural elements for connective tissue. The main protein in this group is collagen which in general represents approximatively 3% of the total protein in fish muscle and is present in the skin, bone, myocommata, and swim bladder [22]. However, some species, especially *Chondrichthyes* species, can contain a significantly higher amount of connective tissue (10%) compared with a value of 17% found in mammalian species [23].

3 Processing of Fish Proteins

3.1 Protein Extraction

To date, several methods have been used for the extraction of proteins from various fish species. The specifics of these methods vary depending on the parameters employed, e.g., pH, temperature, agitation time, homogenization,

weight-to-volume ratio, and the number of sequential extractions. The main approach used to recover proteins from fish is the so-called pH shift process. This technique employs homogenization of the fish sample in an acid (pH < 5.0) or alkaline (pH > 9.0) solution. The protein extract is then separated from the solid components by centrifugation where solids such as bones, scales, flesh, and skin are removed. Depending on the sample, a mechanical disruption process may also be employed prior to the homogenization step to disrupt the cells [24, 25]. The acid- or alkaline-solubilized protein is then precipitated by adjusting the pH to its isoelectric point. The precipitate is separated by centrifugation and a protein isolate is then obtained.

A second approach used for protein extraction is known as the surimi process. The production of surimi consists of protein recovery from fish mince by a series of sequential steps. Washing with cold water is essential to remove water-soluble, sarcoplasmic proteins and impurities such as the skin, bones, scales, and connective tissue which can reduce the gelation ability and ultimately reduce product quality. The number of successive washes and the volume of water required vary between fish species, the freshness of the starting fish mince, and the quality of the surimi required. It is common to use 0.1–0.3% (w/v) NaCl in the washing solution to facilitate removal of water during the subsequent processing steps. Before the dewatering step, the washed minced fish still contains a large quantity of impurities, which often consist of tissues from the skin and/or internal belly flap that need to be removed. A purification step is required to obtain a product with good organoleptic quality, appearance, and product safety. The water in the surimi base obtained can be removed by a screw press, and the water content is reduced from 91% to 80–84% (w/w) [26]. This method of extraction of proteins from fish is less successful than the pH shift method as sarcoplasmic proteins are lost during the process, leading to a reduced yield. A general flow diagram for these two processes is provided in Fig. 2.

Enzyme-assisted extraction of food proteins using food-grade proteolytic preparations relies on the intrinsic properties of enzymes, i.e., high specificity and selectivity. The disadvantages of enzyme-assisted extraction are the high cost which may be an issue in the case of production at commercial scale and the inability of some enzymes to disrupt the cell membrane which may lead to low extraction yield. A new approach to overcome these issues involves the use of microwave irradiation during enzyme-assisted extraction. The procedure known as microwave-assisted enzymatic extraction (MAEE) is recognized as having the potential to increase the yield of protein extracted and, in some cases, the bioactive properties of the resulting product. Nguyen et al. (2017) recently studied the generation of fish protein hydrolysates by MAEE and the pH shift method from rainbow trout (*Oncorhynchus mykiss*) using the proteolytic enzyme Alcalase 2.4L® [27]. The MAEE approach was reported to improve the technofunctional and bioactive properties of the hydrolysates when compared to those generated using the pH shift method.

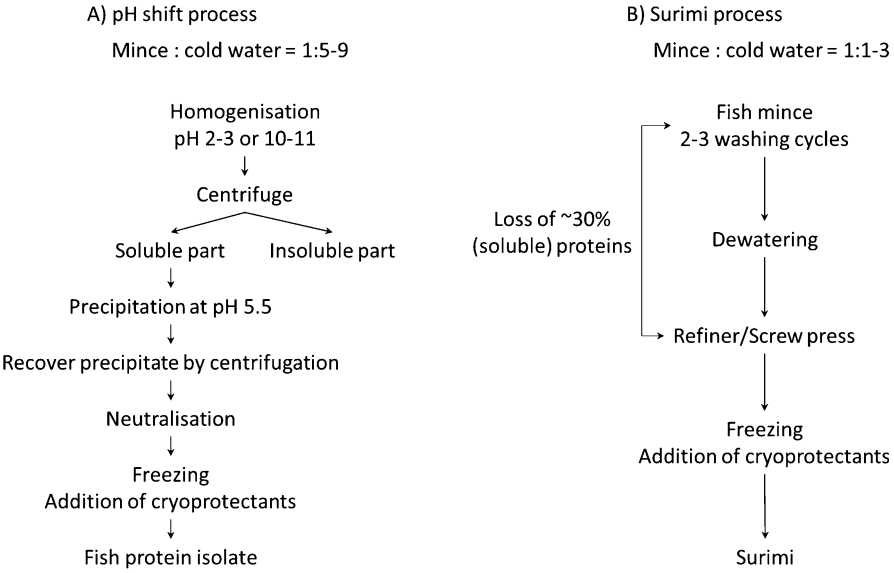


Fig. 2 Flow diagram representing the sequential steps involved in the pH shift (**a**) and surimi process (**b**) for the extraction of proteins from fish. (Modified from Kristinsson et al. [24])

3.2 Enzymatic Hydrolysis

Enzymatic modification is a method which has been used to treat food proteins to improve their technofunctional, physicochemical, bioactive, and organoleptic properties without alteration of their nutritive value [28]. As the degree of hydrolysis of the protein affects the bioavailability and the bioactivity of the peptides generated, the appropriate choice of the hydrolysis conditions (e.g., temperature, pH, enzyme preparation, and enzyme to substrate ratio) is critical [29, 30]. Proteolytic enzymes can be classified according to different characteristics, e.g., proteolytic enzymes can be endo- or exo-acting proteinases/peptidases. For example, endoproteinases cleave peptide bonds within the protein and release peptides or shorter fragments, while exopeptidases remove single amino acid residues or small peptides from either the C-terminus (carboxypeptidases) or N-terminus (aminopeptidases) by cleaving the terminal peptide bonds [31].

It has been shown that enzymatically modified food proteins have improved technofunctional as well as bioactive properties compared with intact proteins [6, 32]. The generation of lower molecular mass peptides with reduced secondary structures can improve the solubility, turbidity, gelling, emulsifying, and the foaming properties as well as heat and pH stability of proteins [33]. The use of fish protein hydrolysates for their technofunctional properties has been extensively reviewed [34–36]. The modification observed in the technofunctional properties of food

proteins following hydrolysis is highly dependent on the hydrolysis conditions, such as pH, incubation temperature, and duration of hydrolysis. Furthermore, the specificity of the enzyme used for the process and the operating parameters are responsible for the extent of hydrolysis and the peptide profile obtained. Control of the hydrolysis process is even more important when working with animal products since certain animal parts may contain endogenous proteinases [37] or even enzyme inhibitors [38]. These endogenous enzymes are mainly located in the gastrointestinal tract and are associated with the fish viscera; however, some hydrolytic enzymes, such as cathepsins, collagenases, alkaline proteases, and calcium-dependent proteinases, are located in the muscle tissue itself [39]. This endogenous proteolytic activity can increase the extent of hydrolysis independently from the exogenously added proteolytic preparations. Fortunately, the endogenous proteolytic activity in fish is generally not as high as that found in the muscle of terrestrial animals (bovine, porcine, and caprine).

A number of food-grade proteolytic preparations exist on the market. These arise from microbial, plant, and animal sources. Among the enzyme preparations available for the generation of protein hydrolysates from fish processing by-products, the most commonly used are Alcalase (*Bacillus licheniformis*), Neutrase (*Bacillus amyloliquefaciens*), Flavourzyme (*Aspergillus oryzae*), collagenase (*Clostridium histolyticum*), Pronase E (*Streptomyces griseus*), papain (*Carica papaya*), and bromelain (*Ananas comosus*) and digestive enzymes from bovine and porcine gastrointestinal tracts (e.g., pepsin, trypsin, and chymotrypsin). Moreover, fermentation and autolysis are processes which may be employed for the production of peptides by the action of the proteolytic enzymes from the product itself or from the action of the intrinsic microorganisms present. Furthermore, proteolytic enzymes have been purified from different fish species, especially from viscera for use during fish protein hydrolysate manufacture [15].

In some regions of the world, the endogenous enzymes from marine sources are used to improve the shelf life as well as the bioactive and technofunctional properties of proteins from fish and shellfish. Several examples of fermented fish, shellfish, and seafood exist for application as flavoring agents and food supplements with bioactive properties, such as Kapi, a fermented shrimp paste from Thailand, and Bakasang, a fermented fish sauce from Indonesia [40–44]. However, the high salt content and low pH and the presence of undesirable contaminants such as halophilic microorganisms and biogenic amines (histamine) represent some important issues for consumer safety linked to the consumption of these products. Therefore, the use of enzymatic hydrolysis with exogenously added food-grade proteolytic preparations when carried out in a controlled environment with the use of thermal treatment for enzyme inactivation and bacterial reduction represents a feasible approach for the generation of bioactive peptides from fish by-products for human consumption. Furthermore, it has been reported that the utilization of proteolytic enzymes releases a higher quantity of bioactive peptides with lower molecular masses than when fermentation processes are employed [45]. Additionally, enzymatic hydrolysis requires significantly less time to reach the desired degree of hydrolysis than fermentation processes. For example, the production of fish protein hydrolysates

takes between 4 and 6 h to reach the desired degree of hydrolysis [46], while it may take weeks or months for the fermentation process to reach the equivalent extent of hydrolysis [47].

4 Characterization of Fish Peptides

Fish proteins contain peptide sequences encrypted within their primary structure. Some of these peptides have the potential to beneficially modulate some metabolic pathways and consequently may play a role in disease prevention and health enhancement. Bioactive peptides are released from proteins during normal gastrointestinal digestion which occurs in the digestive tract or during food processing with the use of proteolytic enzymes (hydrolysis) or microorganisms (fermentation) [48]. The biological activity of food-derived peptides mainly depends on their structural properties such as molecular mass and the physicochemical characteristics of the amino acids within the sequence [49]. The biological activity of peptides present in protein hydrolysates is highly dependent on the hydrolysis conditions (pH, time, temperature), the enzymes used, and the enzyme-to-substrate ratio applied [50, 51]. Therefore, careful control of these conditions during the generation of bioactive peptides is essential to optimize bioactive properties and, therefore, their ability to enhance human health.

Type 2 diabetes mellitus (T2DM) and cardiovascular diseases are two of the main NCDs responsible for more than 0.6 and 3.9 million deaths, respectively, in Europe per annum [52]. Marine by-product-derived proteins represent a source of peptides that may have the ability to modulate specific biomarkers associated with these diseases, and therefore they have the potential for incorporation into functional food or nutraceutical products for the prevention and management of these conditions. Many studies have been conducted using several different food sources, such as bovine and camel milk, cereals, insects, and marine sources, to generate bioactive peptides with *in vitro* antioxidant, cardioprotective, antidiabetic, and appetite suppressant properties [53–58]. These include peptides with the ability to modulate specific pathways linked with blood pressure control and T2DM. This includes modulation of the renin-angiotensin system (inhibition of renin and angiotensin-converting enzyme (ACE)), stimulation of the incretin system (inhibition of dipeptidyl peptidase-IV (DPP-IV), and stimulation of the secretion of glucagon-like peptide (GLP-1)), as well as stimulation of the secretion of intestinal cholecystokinin, which is linked to appetite suppression *in vivo* [59–62]. Food protein-derived peptides have been shown to reduce oxidative stress associated with inflammation and tissue damage *in vivo*, which are complications generally linked to cardiovascular disease (ischemia), diabetes (diabetic food ulcer), and many other diseases such as neurodegenerative diseases and cancer [63]. The main bioactivities currently investigated for peptides from fish by-products are based on the regulation of oxidative stress and cardiovascular disease (Table 2). These include antioxidant, ACE inhibitory, renin inhibitory, and anticoagulant activities. Several reports suggest that protein hydrolysates generated from fish can modulate the immune response and inhibit

Table 2 Biological activities of protein hydrolysates and specific peptide sequences arising from fish processing by-products and underutilized fish species

Species	Source	Enzyme	Biological activity	Peptide(s) sequence	Potency	Ref
Atlantic salmon (*Salmo salar*)	Trimmings	Corolase PP	ACE inhibitory DPP-IV inhibitory Antioxidant	GGPAGPAV[1] GPVA[2] PP[3] GF[4]	[1]ACE IC_{50} = 673.16 μM, DPP-IV IC_{50} = 8139.11 μM, ORAC = 5.47 μmol TE/μmol peptide [2]ACE IC_{50} = 445.61 μM, DPP-IV IC_{50} = 264.74 μM, ORAC = 9.48 μmol TE/μmol peptide [3]ACE IC_{50} = 1912.46 μM, DPP-IV IC_{50} = 4343.48 μM, ORAC = 12.48 μmol TE/μmol peptide [4]ACE IC_{50} = 178.14 μM, DPP-IV IC_{50} = 1547.15 μM, ORAC = 19.74 μmol TE/μmol peptide	[64]
	Skin	Flavourzyme	DPP-IV inhibitory	GPAE[1] GPGA[2]	[1]IC_{50} = 49.6 μM [2]IC_{50} = 41.9 μM	[65]
	Skin collagen	Alcalase 2.4L, papain	ACE inhibitory	AP[1] VR[2]	[1]IC_{50} = 0.005 mg/mL [2]IC_{50} = 0.014 mg/mL	[66]
	Pectoral fin	Pepsin	Anti-inflammatory	PAY	NO inhibition (0.75 mM) = 63.80% iNOS Inhibition (0.75 mM) = 75.00% PGE_2 inhibition (0.75 mM) = 45.33% COX-2 inhibition (0.75 mM) = 48.00% TNF-α inhibition (0.75 mM) = 58.28%	[67]

					IL-6 inhibition (0.75 mM) = 43.48% IL-1β inhibition (0.75 mM) = 64.89%	
Alaska Pollack (*Theragra chalcogramma*)	Backbone	Pepsin	Calcium binding	VLSGGTTMAMYTLV		[68]
	Frame	Trypsin	Immunomodulatory	WT[1] NGLAP[2] NGMTY[3]	[1]Lymphocyte proliferation (20 μg/mL) = 31.35% [2]Lymphocyte proliferation (20 μg/mL) = 32.96% [3]Lymphocyte proliferation (20 μg/mL) = 35.92%	[69]
	Skin collagen	Trypsin	Metal chelating (Ca, Fe, Cu)	GPAGPHGPPG	11.52 nmol Ca/μmol peptide 1.71 nmol Fe/μmol peptide 0.43 nmol Cu/μmol peptide	[70]
Amur sturgeon (*Acipenser schrenckii*)	Skin gelatin	Alcalase 2.4L	Antioxidative Cryoprotective	PAGT	EC_{50} DPPH radical = 5.38 mg/mL EC_{50} ABTS radical = 0.008 mg/mL EC_{50} hydroxyl radical = 0.89 mg/mL	[71]
Bluefin tuna (*Thunnus thynnus*)	Frame	Pepsin	ACE inhibitory	GDLGKTTTSNWSPP	ACE IC_{50} = 11.28 μM	[72]
	Backbone	Pepsin	Antioxidant	APTBP	EC_{50} DPPH radical = 0.72 mg/mL EC_{50} hydroxyl radical = 0.21 mg/mL EC_{50} superoxide radical = 0.61 mg/mL	[73]

(*continued*)

Table 2 (continued)

Species	Source	Enzyme	Biological activity	Peptide(s) sequence	Potency	Ref
Bluefin leatherjacket (*Navodon septentrionalis*)	Skin	Papain	Antioxidant	FIGP	EC_{50} DPPH radical = 0.118 mg/mL EC_{50} hydroxyl radical = 0.073 mg/mL EC_{50} oxygen radical = 0.311 mg/mL	[74]
Blue whiting (*Micromesistius poutassou*)	Whole fish	Protamex, Flavourzyme 500L	Antioxidant	–		[75]
	Mince	Endopeptidase from *Bacillus amyloliquefaciens* and *B. licheniformis*	DPP-IV inhibitory CKK stimulation	–	CKK release (1.0% hydrolysate) = 122.03 pM	[76]
	Mince	Alcalase 2.4L/ Flavourzyme 500L	DPP-IV inhibitory Insulin secretion GLP-1 secretion	–	DPP-IV IC_{50} = 1.49 mg/mL	[77]
Boarfish (*Capros aper*)	Mince	Protease AP	ACE inhibitory	–	ACE inhibition (1 mg/mL) = 85.8%	[25]
Half-fin anchovy (*Setipinna taty*)	Whole fish	Pepsin	Antiproliferative Antioxidant	–	IC_{50} DU-145 cell = 41.67 mg/mL EC_{50} DPPH radical = 4.46 μg/mL	[78]
Leatherjacket (*Meuschenia* sp.)	Mince	Papain Bromelain Flavourzyme 500L	ACE inhibitory	EPLYV[1] DPHI[2] AER[3] EQIDNLQ[4] WDDME[5]	[1]IC_{50} = 118 μM [2]IC_{50} = 48.7 μM [3]IC_{50} = 420 μM [4]IC_{50} = 270 μM [5]IC_{50} = 31.6 μM	[29]
Loach (*Misgurnus anguillicaudatus*)	Whole fish	Papain	Antioxidant Antiproliferative	–	IC_{50} MCF-7 = 16 mg/mL IC_{50} Caco-2 = 10 mg/mL IC_{50} Hep-G2 = 13 mg/mL	[79]

Longtail tuna (*Thunnus tonggol*)	Dark muscle by-product	Papain Protease XXIII	Antiproliferative	LPHVLTPEAGAT[1] PTAEGGVYMVT[2]	[1]IC_{50} MCF-7 = 8.1 μM [2]IC_{50} MCF-7 = 8.8 μM	[80]
	Whole fish	Protease XXIII	DPP-IV inhibitory	PGVGGPLGPIGPCYE[1] CAYQWQRPVDRIR[2] PACGGFWISGRPG[3]	[1]IC_{50} = 116.1 μM [2]IC_{50} = 78.0 μM [3]IC_{50} = 96.4 μM	[81]
Olive flounder (*Paralichthys olivaceus)*	Mince	Pepsin	ACE inhibitory	MEVFVP[1] VSQLTR[2]	[1]IC_{50} = 79 μM SBP_{6h} (40 mg/kg) = 44.25 mmHg [2]IC_{50} = 105 μM SBP_{6h} (40 mg/kg) = 34.25 mmHg	[82]
Pangasius catfish (*Pangasius sutchi*)	Skin[1] and bone[2] gelatin	Alcalase 2.4L	ACE inhibitory	–	[1]IC_{50} = 3.2 μg/mL [2]IC_{50} = 1.3 μg/mL	[83]
Rockfish (*Sebastes hubbsi*)	Skin gelatin	Flavourzyme	Antioxidant ACE inhibitory	–	ACE IC_{50} = 0.82 mg/mL DPPH scavenging (4 mg/mL) = 45.8% Superoxide scavenging (4 mg/mL) = 67.8% Hydroxyl scavenging (4 mg/mL) = 94.7% Alkyl scavenging (4 mg/mL) = 64.8%	[84]
Rohu (*Labeo rohita*)	Roe	Pepsin	Immunomodulatory	–	Increasing of peritoneal macrophage, NK cell activity, and T-cell subpopulation, enhancing mucosal immunity (S-IgA) and antibody production (IgA)	[85]

(*continued*)

Table 2 (continued)

Species	Source	Enzyme	Biological activity	Peptide(s) sequence	Potency	Ref
	Roe	Protease N	Antiproliferative	–	IC_{50} Ca9-22 = 0.85 mg/mL	[86]
Sandfish (*Arctoscopus japonicus*)	Meat[1] and roe[2]	Alcalase 2.4L Collupulin MG	Anti-inflammatory	–	[1]NO scavenging (0.1 mg/mL) = 18.43% [2]NO scavenging (0.1 mg/mL) = 52.35%	[87]
Seabass (*Lates calcarifer*)	Skin	Alcalase 2.4L[1] Protease from hepatopancreas of Pacific white shrimp[2]	Antioxidant Metal chelating (Fe)	–	[1]DPPH = 6.77 µmol TE/g dw ABTS = 65.48 µmol TE/g dw FRAP = 2.57 µmol TE/g dw Fe^{2+} chelating = 1.98 µmol EDTA/g dw [2]DPPH = 6.30 µmol TE/g dw ABTS = 59.35 µmol TE/g dw FRAP = 2.65 µmol TE/g dw Fe^{2+} chelating = 3.43 µmol EDTA/g dw	[88]
Skate (*Okamejei kenojei*)	Skin gelatin	Alcalase 2.4L	ACE inhibitory	MVGSAPGVL[1] LGPLGHQ[2]	[1]IC_{50} = 3.09 µM [2]IC_{50} = 4.22 µM	[89]
Small red scorpionfish (*Scorpaena notata*)	Viscera	Crude enzyme from *Trichoderma harzianum*	Antimicrobial (MIC)	FPIGMGHGSRPA	*Bacillus cereus* = 0.6 mg/mL *Bacillus subtilis* = 0.42 mg/mL *Staphylococcus aureus* = 0.5 mg/mL *Salmonella* sp. = 0.72 mg/mL *Listeria innocua* = 0.49 mg/mL *Escherichia coli* = 0.8 mg/mL	[90]

Unicorn leatherjacket (*Aluterus monoceros*)	Skin	Glycyl endopeptidase from papaya	Antioxidant	EPGPVG[1] LPGPAG[2] LDGPVG[3] EGPLG[4]	[1]ABTS scavenging = 1.25 μmol TE/g peptide [2]ABTS scavenging = 1.22 μmol TE/g peptide [3]ABTS scavenging = 1.36 μmol TE/g peptide [4]ABTS scavenging = 4.95 μmol TE/g peptide	[91]
Winter flounder (*Pleuronectes americanus*)	–	Synthetic peptide	Antiviral	–	EC_{50} HSV-1 = 83 μg	[92]
Yellowfin sole (*Limanda aspera*)	Frame	α-Chymotrypsin	Anticoagulant	TDGSEDYGILEIDSR	IC_{50} FX11a (1.0 μM) = 62.4%	[93]

A one-letter notation was used for amino acid sequences
IC_{50}, inhibitor concentration that inhibits enzyme activity/activated coagulation factor 11 (FX11a) by 50%
EC_{50}, effective concentration causing 50% of antioxidant/antiviral activity
Antioxidant value expressed in μmol Trolox equivalent (TE)/μmol of dry weight (dw)
MIC, minimum inhibitory concentration
SBP_{6h}, systolic blood pressure after 6 h of oral peptide administration

cancer development, as well as having ACE inhibitory, antihypertensive, anticoagulant, ion chelating, antioxidant, antimicrobial, antiviral, and appetite suppressant activities [87, 94, 95]. These characteristics of peptides generated within marine-derived protein hydrolysates are linked to their potential as ingredients or nutraceutical products for the management of symptoms related to NCDs such as diabetes, cardiovascular diseases, cancer, and chronic allergic diseases. It has been previously reported that peptides with low molecular mass, mainly di- and tripeptides, have potent bioactive properties [96, 97], while longer peptides, with more than 20 amino acid residues, have been associated with technofunctional property improvements [98]. The most common method used to separate peptides within protein hydrolysates is reversed-phase high-performance liquid chromatography (RP-HPLC) [99]. The techniques mainly used to separate peptides on the basis of molecular weight include sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and gel permeation high-performance liquid chromatography (GP-HPLC) [100]. Furthermore, characterization of the amino acid sequence of the peptides is generally carried out using liquid chromatography systems coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) [54, 97, 101].

5 Bioactive Properties

Globally, the total mortality linked to NCDs was 38 million in 2012 and is estimated to reach 52 million by 2030 [102]. As mentioned in previous sections, peptides derived from proteinaceous marine processing by-products have been shown to possess a range of bioactive properties. The potential health benefits of consuming fish protein hydrolysates/peptides for the control of NCDs and oxidative stress, allergenicity and inflammation, hypertension, and cancer will therefore be outlined.

5.1 Oxidative Stress

Oxidative stress is an important factor contributing to the development and the progression of NCDs. It is characterized by the generation of reactive oxygen species (ROS), including hydroxyl radicals ($^{\bullet}OH$), superoxide radicals ($O_2^{\bullet -}$), and non-radical hydrogen peroxides (H_2O_2). High ROS levels have been associated with the deleterious modification of nucleic acids (DNA and RNA), proteins, and lipids and have been implicated in accelerating cellular aging and several human conditions, such as atherosclerosis and neurodegenerative diseases [63]. In contrast, low ROS levels have beneficial physiological effects linked to the regulation of cell signaling, through the redox regulation of transcription factors, protein phosphorylation (kinase), and ion transfer [103]. The consumption of dietary components containing natural antioxidants (such as vitamin C, polyphenols, and carotenoids) has been shown to reduce oxidative stress by enhancing natural defenses [104]. On the other hand, oxidative stress can contribute to a reduction in the proliferation of cancer cells by inducing cell death. However, while cell death can be achieved by

radical-induced oxidative stress, some cancer cells have developed resistance which indicates that cells develop sophisticated adaptation responses to oxidative stress [105]. The peptides from fish processing by-product proteins having antioxidant activity have recently been reviewed [95]. For example, Glu-Leu-Phe-Glu-Pro-Arg, a hexapeptide generated by Alcalase hydrolysis of seabass (*Lates calcarifer*) skin gelatin, was shown to scavenge hydrogen peroxide [106]. Table 2 provides a list of fish protein by-product hydrolysates/peptides exhibiting antioxidant activity as reported in the literature.

The *in vitro* determination of antioxidant activity of peptides can be performed using various chemical reactions. The methods used to assess antioxidant capacity can be classified according to whether they assess the transfer of either hydrogen atoms (HAT) or electron (ET) [107]. The assays used to measure proton-donating ability are represented by the oxygen radical absorbance capacity (ORAC), hydroxyl radical, alkyl radical, and peroxide radical scavenging activity assays. The reported ORAC activity of peptides derived from fish processing by-products ranges from 5.47 to 19.74 μmol TE/μmol peptide (Table 2). Compared to milk protein-derived antioxidant peptides, the radical scavenging activity was approximately 2.1- to 7.5-fold higher than the hendecapeptide, Trp-Tyr-Ser-Leu-Ala-Met-Ala-Ala-Ser-Asp-Ile derived from a β-lactoglobulin A hydrolysate [108]. The ET-based assays used to determine the reducing capacity of an antioxidant are the Trolox equivalent antioxidant capacity (TEAC), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$), FRAP (ferric reducing antioxidant power), and $DPPH^{\bullet}$ (2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl radical) assays. The DPPH radical scavenging activity (EC_{50}, concentration causing 50% of antioxidant activity) of Phe-Ile-Gly-Pro, a peptide derived from a bluefin leatherjacket skin hydrolysate, was reported to be 0.118 mg/mL. Interestingly, Song et al. (2011) reported that a potent pepsin hydrolysate from half-fin anchovy (*Setipinna taty*) possessed a DPPH EC_{50} value of 4.46 μg/mL, while the synthetic antioxidant butylated hydroxytoluene (BHT) is reported to exhibit an EC_{50} value of 22.78 μg/mL [78]. The antioxidant potency of bioactive peptides has been attributed to the presence of specific amino acids therein especially histidine residues. This has been attributed to the chelating properties and the radical-trapping properties of the imidazole ring. Furthermore, the presence of hydrophobic amino acid residues in peptides has been associated with an increase in their accessibility to hydrophobic targets [109, 110].

5.2 Allergenicity and Inflammation

Allergy is a hypersensitivity response associated with an immune system reaction toward an allergen. The major types of allergies include life-threatening anaphylaxis; food, drug, and insect allergies; asthma; rhinitis; angioedema; eczema; urticaria; and eosinophilic disorders [111]. Allergic reactions/diseases can be caused by environmental factors, such as pollution, as well as genetic factors. The global prevalence of allergic diseases is increasing with 200–250 million people suffering from food allergy and about 300 million people suffering from asthma causing 250,000 deaths

annually [112]. The diagnosis of allergy is commonly performed using blood or cutaneous tests. The cutaneous (skin prick) test is carried out by introducing a series of punctures on a subject's skin which are loaded with different suspected allergens. The signs of inflammation, indicative of an allergenic response, are then observed. Inflammation is one of the first responses of the immune system to a challenge/infection [113]. The increase in blood flow into the tissue causes redness, swelling, heat, and pain. Inflammation occurs when damaged tissues or infected cells release eicosanoids and cytokines. Eicosanoids, which include prostaglandins, dilate blood vessels and increase the temperature locally, while leukotrienes recruit white blood cells (leukocytes). Cytokines, including interleukins and chemokines, are responsible for the recruitment and communication between leukocytes at the site of infection, while interferons shut down protein synthesis in infected cells [114]. Cytotoxic and growth factors can also be released by the damaged tissues. These molecules have the ability to recruit immune cells to the site of challenge/infection and contribute to the healing of damaged tissue following removal of the antigen [115].

The human immune system reacts against various chemical and biological threats by two separate but interconnected systems. The first defense system, called the innate immune system, consists of cells and proteins present in the circulation system, and it is activated in the presence of an exogenous threat. This system includes mucosal epithelial barriers, dendritic cells, and leukocytes [116]. The second system, called the adaptive immune system, is a specific system which involves B- and T-cells and the production of specific antibodies against the detected threat. The adaptive immune system is activated when the innate immune system is insufficiently effective or when it has been overcome [117]. Current treatments for allergies consist of the use of medication such as steroids, adrenalin, and antihistamines [118]. Immunotherapy is also available to treat some forms of allergy by injecting an inactive form of the allergen to stimulate the production of specific antibodies by the adaptive immune system [119]. Even though immunotherapy provides a longer-lasting effect, it also represents an expensive alternative to pharmacological solutions.

Immunomodulatory peptides can modulate immune functions by enhancing lymphocyte proliferation, antibody synthesis, and cytokine regulation [120]. Moreover, immunomodulatory peptides may have the ability to reduce allergic reactions and enhance the mucosal immune system in the gastrointestinal tract. Immunomodulatory peptides isolated from human milk, rice, and soybean tryptic hydrolysates act to stimulate the innate immune system [121–123]. A review of literature reveals that the mechanism of action of immunomodulatory peptides is relatively non-specific, and this may be the reason why the exact mechanism and the *in vivo* destination of these peptides are still unknown. Figure 3 depicts the main mechanisms of action of anti-inflammatory peptides. The details of the anti-inflammatory peptides isolated from fish by-products are reported in Table 2.

The main anti-inflammatory activity of bioactive peptides, as currently described in the literature, involves the up- and downregulation of signaling proteins (cytokines) such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) and

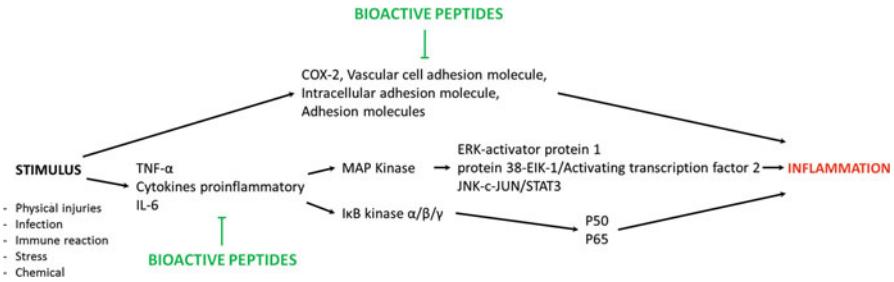


Fig. 3 Mechanisms of inflammation and potential sites of action of bioactive peptides. (Modified from Cicero et al. [124])

adhesion molecules. Modification of the expression and translation of these components has the ability to reduce inflammation. Chalamaiah et al. (2018) have recently reviewed the area of immunomodulatory peptides from food protein hydrolysates [125]. Subhan et al. (2017) demonstrated that peptides from fish scale collagen could downregulate the expression of pro-inflammatory cytokines *in vitro* [126]. This suggests that peptides isolated from fish scale collagen had a beneficial effect in the control of inflammatory diseases.

As shown in Table 2, a number of peptides/hydrolysates derived from fish processing by-products are reported to possess anti-inflammatory capacity. For example, the tripeptide Pro-Ala-Tyr derived from an Atlantic salmon (*Salmo salar*) pectoral fin peptic hydrolysate exhibits anti-inflammatory activity via the down-regulation of NO/iNOS and PGE_2/COX-2 pathways by 64–75% and 45–48%, respectively, compared to the control group. The inhibition of the production of pro-inflammatory cytokines, TNF-α, IL-6, and IL-1β, has been also reported (Table 2) by Anh et al. [67].

5.3 Hypertension

The literature reports that dietary protein intake can contribute to reducing high blood pressure, coronary heart disease, and other infarctions [127]. Blood pressure is determined by measuring two values: systolic blood pressure (SBP), which measures the pressure in blood vessels when the heart beats, and diastolic blood pressure, which measures the pressure in blood vessels when the heart is at rest. The normal values for systolic and diastolic blood pressures are 120 and 80 mmHg, respectively. A range of mechanisms are involved in the control of blood pressure, including the secretion of specific hormones, modulation of blood volume, and secretion of nitric oxide by endothelial cells and the renin-angiotensin-aldosterone system (Fig. 4). ACE, for example, catalyzes the conversion of angiotensin I to angiotensin II, a hormone which leads to vasoconstriction and an increase in blood pressure [128]. Furthermore, ACE degrades the vasodilator molecule bradykinin. Consequently,

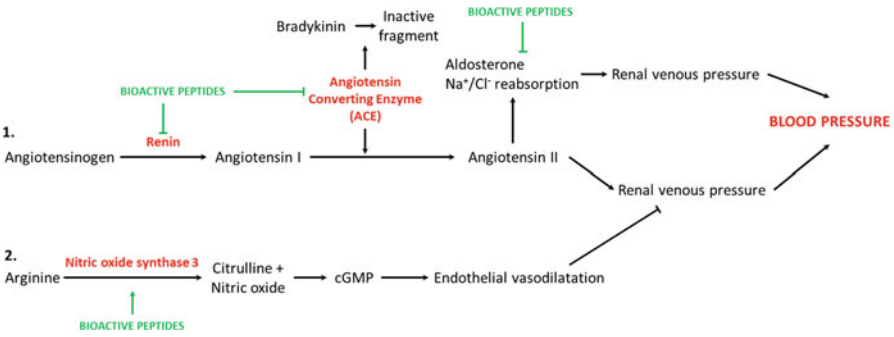


Fig. 4 Mechanisms of blood pressure control and the potential action of bioactive peptides. (Modified from Cicero et al. [124]) ⟞: direct inhibition, ⟶: direct stimulation

ACE inhibitory agents can lower hypertension. Many studies have focused on the ability of fish protein hydrolysates/peptides to inhibit ACE [66, 89]. ACE inhibitory peptides are generally short sequences. Moreover, structural studies of the ACE active site using X-ray crystallography show a lid-like extension formed by the amino-terminal helix (α 1-3) that partially covers the active channel and leaves an opening of almost 3 Å for substrate and inhibitor access [129]. Wu et al. (2006) performed an *in silico* analysis of the ACE inhibitory activity of long (4–10 amino acids) and short (2–3 amino acids) peptides. The importance of the type of amino acid residue in the peptide sequence for ACE inhibitory activity was predicted [130]. It was concluded that the optimum amino acid residues for potent ACE inhibition starting from the C-terminus were Tyr and Cys in the first position; Trp, Met, and His in the second position; Leu, Ile, Val, and Met in the third position; and Trp in the fourth position. Blood pressure is highly regulated *in vivo* and involves mechanisms other than modulation of ACE activity. It is likely that bioactive peptides derived from fish protein hydrolysates also beneficially modulate these systems.

Recently, research on nitric oxide synthase 3 (iNOS) suggested that stimulation of the production of nitric oxide (NO) in endothelial cells has a beneficial effect on blood pressure (Fig. 4) [131]. Ahn et al. (2015) isolated the tripeptide Pro-Ala-Tyr from an Atlantic salmon (*Salmo salar*) pectoral fin peptic hydrolysate and demonstrated that it possessed the ability to modulate the secretion of intracellular NO *in vitro* [67].

Several reports from *in vivo* studies using spontaneously hypertensive rats (SHRs) show hydrolysates/peptides derived from fish proteins having the ability to significantly reduce hypertension. Ko et al. (2016) identified ACE inhibitory peptides (Table 2) from hydrolysates of flounder fish (*Paralichthys olivaceus*) protein which were shown to have hypotensive effects *in vivo* [82]. The *in vitro* ACE IC_{50}'s for two identified hexapeptides, Met-Glu-Val-Phe-Val-Pro and Val-Ser-Gln-Leu-Thr-Arg, was 79 and 105 μM, respectively. These peptides were found to reduce SBP in SHRs after 6 h oral administration (Table 2). Interestingly, the reduction in SBP value obtained with the Val-Ser-Gln-Leu-Thr-Arg-treated group was similar to

the group treated with Captopril®, a synthetic drug inhibitor of ACE. As shown in Table 2, numerous peptides with ACE inhibitory activity have been derived from marine by-products. These include hydrolysates/peptides from Atlantic salmon, bluefin tuna, boarfish, leatherjacket, *Pangasius* catfish, rockfish, and skate.

5.4 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is characterized by insulin deficiency caused by pancreatic β-cell dysfunction and insulin resistance [132], which arises from the fact that the pancreatic cells produce and release insulin, but the quantity released is insufficient. All these complications lead to hyperglycemia and many side effects such as atherosclerosis leading to heart attack, stroke, and organ failure. Type 1 diabetes, also named "insulin-dependent diabetes," is a malfunction of the pancreatic cells that fail to produce and release insulin resulting from a cell-mediated autoimmune attack of pancreatic β-cells [133]. However, T2DM is the most common type of diabetes accounting for 90–95% of all diabetes cases [132]. The occurrence of T2DM has increased in developed countries and is associated with an unhealthy lifestyle and obesity which contributes to higher rates of morbidity as diabetic individuals generally have a higher risk of heart disease, kidney failure, blindness, and nerve and circulatory damage [132]. The global prevalence of diabetes was 415 million adults aged over 20 years in 2015 (8.8% of the adult population), and this is expected to increase to 642 million by 2040 (10.4% of the adult population) [134]. The countries with the highest incidences of diabetes in 2015 were China (109.6 million), India (69.2 million), the United States (29.3 million), Brazil (14.3 million), the Russian Federation (12.1 million), Mexico (11.5 million), Indonesia (10.0 million), Egypt (7.8 million), Japan (7.2 million), and Bangladesh (7.1 million). Several types of medication are currently employed for the management and control of T2DM. The most common treatments involve the use of Metformin® and Gliclazide® which decrease the release of hepatic glucose and increase insulin secretion, respectively. Both medications are on the World Health Organization list of essential medicines for the treatment of T2DM. Other treatments used in the management of the disease include the injection of glucagon-like peptide-1 analogues and the use of enzyme inhibitors which inhibit the activity of dipeptidyl peptidase-IV (DPP-IV), α-amylase, and α-glucosidase. These treatment approaches enhance the body's response to reduce postprandial serum glucose levels. The enzymes targeted for inhibition are linked to the reduction of glucagon release and the stimulation of insulin synthesis, glucose absorption, and metabolism, as well as appetite reduction [135–137]. DPP-IV degrades two incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). In the presence of glucose, the incretin system stimulates pancreatic β-cells to secrete insulin. Therefore, by inhibiting the action of DPP-IV, the incretin hormones are maintained at a stable level in the circulation and continue to stimulate insulin production [138]. Human *in vivo* studies have shown that the inhibition of DPP-IV leads to a reduction in glycated hemoglobin (hemoglobin A1c) [139] as well as an

increase in circulating GLP-1 [140]. Consequently, this represents clear evidence of the blood glucose-lowering effect of DPP-IV inhibitors. Although DPP-IV inhibitors and GLP-1 analogues are interesting targets for blood glucose regulation, they represent distinct drug classes with different mechanisms of action, route of administration, and clinical efficiency. However, some synthetic drugs with DPP-IV inhibitory activity are now being used for the treatment of T2DM, such as sitagliptin (Merck & Co. as Januvia®, FDA approved in 2006), vildagliptin (Novartis as Galvus®, EU approved in 2007), and alogliptin (Takeda Pharmaceutical Company as Nesina®, FDA approved in 2013). While these drugs have proven to be efficient in the management of T2DM, side effects including postprandial hypoglycemia, nasopharyngitis, headache, nausea, heart failure, hypersensitivity, skin reaction, joint pain, and adverse cardiovascular effects have been associated with their use [141]. Therefore, the use of natural sources to produce DPP-IV inhibitors is being explored in order to reduce the side effects of antidiabetic drugs. Protein hydrolysates derived from fish processing by-products have been reported to have *in vitro* DPP-IV inhibitory activity, and numerous DPP-IV inhibitory peptides have been identified. These include Gly-Gly-Pro-Ala-Gly-Pro-Ala-Val (624.7 Da) and Gly-Pro-Val-Ala (342.4 Da) which can inhibit DPP-IV by 50% at a concentration (IC_{50} value) of 0.26 and 8.14 $mg.mL^{-1}$, respectively [64]. Recent *in vivo* studies on protein hydrolysates derived from the underutilized fish blue whiting (*Micromesistius poutassou*) have shown the ability to lower glucose and increase insulin level in mice [77]. Another approach to regulate blood glucose is to stimulate the production of cholecystokinin (CKK) by enteroendocrine cells in the duodenum. The secretion of CCK is linked to gastric emptying, the stimulation of pancreatic secretion, and satiety [142]. Several *in vitro* and *in vivo* studies testing intact proteins and their hydrolysates or corresponding amino acid mixtures demonstrate this phenomenon. Sharara et al. (1993) have shown that protein intake stimulates CKK secretion postprandially in rats, whereas free amino acid intake had no effect [143]. Furthermore, Cudennec et al. (2008) have shown that protein hydrolysates derived from blue whiting can enhance the secretion of CKK and GLP-1 *in vitro* [144]. *In vivo* studies with the same hydrolysates showed an increase in CKK and GLP-1 levels in the plasma of rat [145] and in human [62]. Greco et al. (2017) in reviewing the effect of protein intake on appetite have shown that the effect observed depends on the protein source [146]. Madani et al. (2015) showed that feeding obese rats with sardine proteins resulted in reduced plasma glucose and reduced insulin resistance as well as higher plasma GLP-1 levels compared to the group fed with casein [147]. A list of DPP-IV inhibitory peptides and CKK-stimulating hydrolysates obtained from fish processing by-products and underutilized fish species is provided in Table 2. The *in vitro* DPP-IV inhibitory activity of two peptides, Gly-Pro-Ala-Glu and Gly-Pro-Gly-Ala, identified from Atlantic salmon skin hydrolysates had IC_{50} values of 49.6 and 41.9 μM, respectively. Furthermore, Gly-Pro-Val-Ala identified from an Atlantic salmon trimming protein hydrolysate possessed a DPP-IV IC_{50} value of 264.74 μM. It was noted that the differences in amino acid residues in the peptide sequence had a major impact on DPP-IV inhibitory activity. The peptide Ile-Pro-Ile which is a known DPP-IV inhibitor and found in many dietary proteins was reported to have an IC_{50}

value of 3.2 μM [148]. It is possible that fish processing by-products could be a potential source of peptides with similar or higher levels of DPP-IV inhibitory activity.

5.5 Cancer

Cell division is a normal physiological event that occurs in tissues. Cell proliferation and cell death are highly regulated processes. Certain mutations in cellular DNA destabilize this process and can ultimately lead to cancer. The process that transforms normal cells into cancer cells is called carcinogenesis. It is characterized by a series of changes at cellular and genetic level that reprogram the cell into an uncontrolled division process leading to the formation of a tumor. This malignant mass can remain at a particular site or spread throughout the body via an angiogenesis process and metastasis diffusion.

Apoptosis is a form of programmed cell death and is one of the main mechanisms used in cancer treatment. As apoptosis does not enhance immune response or produce inflammation, it is a better method of treatment compared to classic chemical chemotherapies. Therefore, selective induction and modulation of apoptotic pathways in cancer cells represent a promising approach for cancer therapy [149]. In mammals, two major apoptosis signaling pathways are involved in the activation of cysteine proteases (caspases), the extrinsic death receptor, and the intrinsic mitochondrial pathways [150]. These interlinked pathways involve pro- and anti-apoptotic molecules that can trigger or regulate apoptosis. Therefore, the development of antiproliferative peptides that specifically target these pathways has become an interesting strategy for the development of anticancer therapies.

A large diversity of peptides with anticancer activity have been extracted from various marine organisms, mainly sessile animals, such as sponges, molluscs, and tunicates, which synthesize potent cytotoxic compounds to protect themselves against predators. These compounds are currently being exploited for cancer therapy. However, reports on the antiproliferative activity of peptides derived from fish protein hydrolysates are limited. Chalamaiah et al. (2018) reviewed the area of anticancer peptides from food protein hydrolysates [125]. Several studies have reported that free amino acids have diverse effects on different cancer cells [151, 152]. Cys promoted the proliferation of gastric and breast cancer cells. Asp and Arg stimulated the growth of breast cancer cells, while Glu induced apoptosis in gastric cancer cells. Ala showed an *in vitro* antiproliferative activity against gastric and breast cancer cells, while Pro and Lys showed an antiproliferative activity against prostate cancer cells. These reports suggest that the presence of specific amino acids in peptide sequences could modulate their activity against different cancer cell metabolic pathways. Furthermore, two peptides, Leu-Pro-His-Val-Leu-Thr-Pro-Glu-Ala-Gly-Ala-Thr and Pro-Thr-Ala-Glu-Gly-Gly-Val-Tyr-Met-Val-Thr, isolated from tuna protein hydrolysates possessed antiproliferative EC_{50} values of 8.1 and 8.8 μM on the human breast cancer cell line MCF-7 *in vitro*, respectively [80]. A list of the antiproliferative peptides obtained from half-fin anchovy, loach, and rohu by-products is provided in Table 2.

To date, Alemán et al. (2011) have reported the highest antiproliferative EC_{50} value (0.13 mg/mL) for a squid gelatin hydrolysate using Esperase® on MCF-7 cell line [153].

5.6 Other Bioactivities

Other biological activities involving peptides which possess antiviral and antimicrobial activity have been reported in the Antarctic fish (*Pleuronectes americanus*) and small red scorpionfish (*Scorpaena notate*), respectively (Table 2). Mineral-binding peptides have been identified from Alaska pollock and seabass. Mineral-binding capacities are important in many metabolic processes, including nutrient absorption, cellular proliferation, energy production, and oxygen transport [68, 70, 88]. Anticoagulant activity had been reported in peptides extracted from yellowfin sole frame [93] (Table 2).

6 Bioavailability

As already outlined, bioactive peptides can be released from food proteins during food processing by fermentation or enzymatic hydrolysis as well as during normal gastrointestinal digestion. Research has proven that fish processing by-products are a potential source of bioactive peptides for the production of functional food ingredients [154]. However, more studies on the stability of the bioactive properties need to be carried out. In order to be active at the target site in the human body, the peptides must maintain their biological activity after passing through the gastrointestinal tract, be resistant to extreme pH values and the action of gastrointestinal enzymes. An *in vitro* simulated gastrointestinal digestion (SGID) approach is often carried out to determine the stability of bioactive peptides following *in vitro* incubation with gastrointestinal enzymes. The enzymes used are salivary amylase at pH 7.0 for the oral phase, pepsin at pH 3.0 to simulate the gastric system, and a pancreatic enzyme preparation composed of trypsin, chymotrypsin, elastase, lipase, and amylase at pH 7.0 to mimic the intestinal phase [155]. Hydrolysis of proteins by these enzymes can release bioactive peptides. This has been shown when using the SGID approach for the generation of bioactive peptides from different food sources such as cereals, dairy products, and fish [156–159]. Moreover, *in vitro* SGID has shown that the hydrolytic action of gastrointestinal enzymes has the potential to modulate the bioactive properties of peptides generated following hydrolysis using non-mammalian food-grade enzyme preparations [160]. For this reason, bioactive peptides may be tested using *in vitro* gastrointestinal digestion to assess their potential stability and bioavailability after ingestion. However, some bioactive peptides have shown resistance to further digestion by gastrointestinal enzymes, such as the ACE inhibitory peptide Leu-Leu-Pro from tilapia, which maintained its activity following incubation with pepsin, pancreatin, and α-chymotrypsin [161]. The permeability of biological membranes, which allow bioactive peptides to reach the circulation,

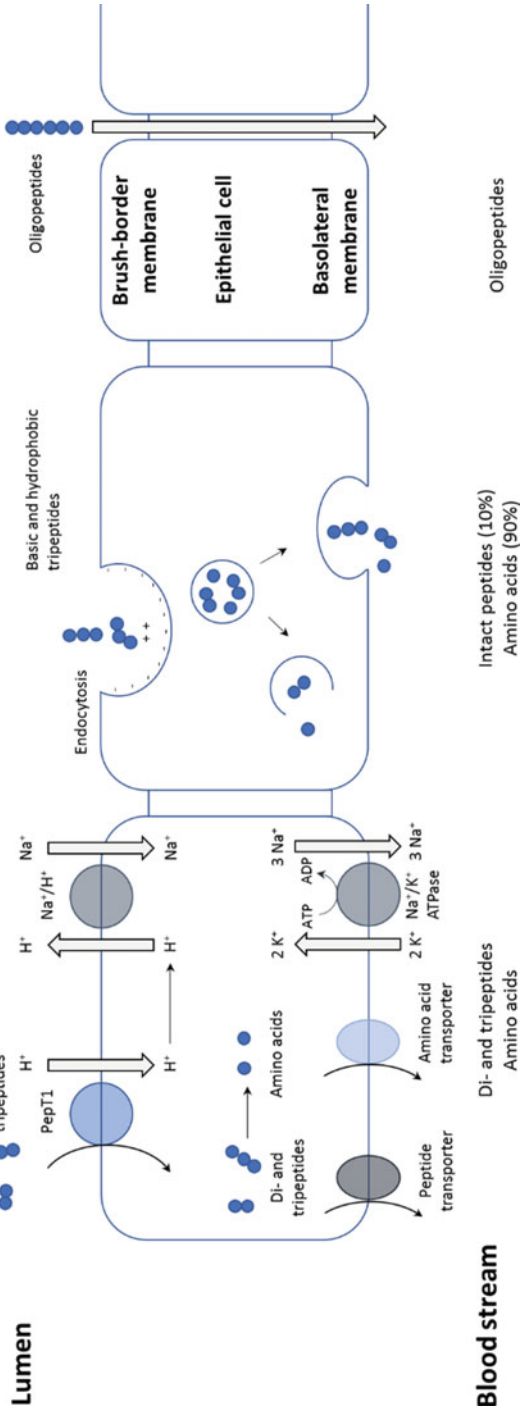


Fig. 5 Peptide transport mechanisms across intestinal cells. (Modified from Hamedy and FitzGerald [154])

depends on many factors such as peptide molecular mass and chemical stability and hydrophobicity. The transport of peptides through the gastrointestinal tract and the intestinal cell barrier is mediated via three main transport mechanisms. These mechanisms are schematically represented in Fig. 5. These consist of (A) active transport via the PepT1 carrier, which transports di- and tripeptides coupled with a proton pump, (B) endocytosis-exocytosis which transports basic and hydrophobic peptides via endocytosis vesicles, and (C) tight junction paracellular diffusion, which transports intact oligopeptides through tight junction pores [154]. However, active transport via PepT1 and passive paracellular diffusion are more efficient routes than endocytosis-exocytosis-mediated transport as peptides may be hydrolyzed after endocytosis by intracellular enzymes into amino acids before reaching the bloodstream. Furthermore, larger peptides and single amino acids have been shown to be less easily absorbed by gastrointestinal cells than short peptides (i.e., containing two to six amino acids) [162]. Transfer across the gastrointestinal membrane also depends on the amino acid sequence of the peptide [163]. Bioactive peptides isolated from fish have been reported to be resistant to the gastrointestinal digestion process and to be able to pass through intestinal membranes to reach the bloodstream. For example, *in vivo* studies in hypertensive rats have shown that the antihypertensive effects of bioactive peptides derived from fish, such as salmon, sardine, sole, tuna, and Alaska pollock, remain stable after passage through the digestion and assimilation processes. For example, Hou et al. (2016) showed that Pro-Thr-Gly-Ala-Asp-Tyr derived from tryptic hydrolysates of Alaska pollock frame could significantly enhance the humoral, cellular, and non-specific immune system in immunosuppressed mice [164]. This indicates that this peptide was resistant to digestion and was able to pass into the bloodstream. However, the stability of bioactive peptides can be enhanced via several strategies which have been developed by the pharmaceutical industry. Among these, encapsulation and structural modification of peptides at C- and/or N- terminal residues, including glycosylation and alkylation, have been shown to improve the bioavailability of peptides. Furthermore, peptides containing Thr, Glu, Phe, and His amino acids seem to be absorbed significantly faster compared to their free amino acid mixture equivalent [163]. The presence of a high percentage of Hyp and Pro amino acids also seems to improve resistance to hydrolysis by gastrointestinal enzymes [165]. Some of these approaches have been used to improve the bioavailability of fish-derived bioactive peptides. For example, the encapsulation of rainbow trout peptides in biopolymer-coated nanoliposomes was an efficient technique to maintain their antioxidant capacity [166].

7 Conclusion

The study of fish protein for the generation of bioactive peptides has increased in the last few years, and fish processing by-products as well as underutilized fish species have been identified as potential sources for bioactive peptides. However, even though several studies with regard to the extraction and hydrolysis of proteins

from fish and fish by-products, as well as the purification, characterization, and identification of bioactive peptides, have been carried out, more research is required to fully exploit and deliver their potential to consumers. While interesting studies on the use of fish processing by-products as functional food ingredients have been carried out, more research is needed in addressing the large-scale production of these products, their bioavailability, compatibility with different food matrices, long-term stability, and *in vivo* efficiency. Furthermore, it is necessary to determine the mechanisms by which peptides and hydrolysates can mediate their physiological effects. More nutrikinetic and metabolomic studies are required in order to understand the relationship between the dose administered and physiological effect. Marketing and economic studies are also required to establish consumer needs and preferences. Finally, *in vivo* validation studies are required to generate health promoting claims acceptable for international food safety agency approval.

Acknowledgments Aurélien V. Le Gouic and Pádraigín A. Harnedy were funded by the Department of Agriculture, Food and the Marine, Ireland, under grant issue 14/F/873 and 13/F/467, respectively.

References

1. Teh LCL, Sumaila UR (2013) Contribution of marine fisheries to worldwide employment. Fish Fish 14:77–88
2. FAO (2016) The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. FAO, Rome
3. Chalamaiah M, Dinesh Kumar B, Hemalatha R et al (2012) Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chem 135:3020–3038
4. Commission Delegated (20 October 2014) Regulation (EU) No 1392/2014 – establishing a discard plan for certain small pelagic fisheries in the Mediterranean Sea, Brussels
5. García-Moreno PJ, Pérez-Gálvez R, Espejo-Carpio FJ et al (2017) Functional, bioactive and antigenicity properties of blue whiting protein hydrolysates: effect of enzymatic treatment and degree of hydrolysis. J Sci Food Agric 97:299–308
6. Neves AC, Harnedy PA, O'Keeffe MB et al (2017) Bioactive peptides from Atlantic salmon (*Salmo salar*) with angiotensin converting enzyme and dipeptidyl peptidase IV inhibitory, and antioxidant activities. Food Chem 218:396–405
7. Sukkhown P, Jangchud K, Lorjaroenphon Y et al (2018) Flavored-functional protein hydrolysates from enzymatic hydrolysis of dried squid by-products: effect of drying method. Food Hydrocoll 76:103–112
8. Ettelaie R, Zengin A, Lishchuk SV (2017) Novel food grade dispersants: review of recent progress. Curr Opin Colloid Interface Sci 28:46–55
9. Lafarga T, Hayes M (2017) Bioactive protein hydrolysates in the functional food ingredient industry: overcoming current challenges. Food Rev Int 33:217–246
10. Sidhu KS (2003) Health benefits and potential risks related to consumption of fish or fish oil. Regul Toxicol Pharmacol 38:336–344
11. Ross A, Vincent A, Savolainen OI et al (2017) Dietary protein sources beyond proteins and amino acids – a comparative study of the small molecular weight components of meat and fish using metabolomics. FASEB J 31:652.613
12. Pangestuti R, Kim S-K (2017) Bioactive peptide of marine origin for the prevention and treatment of non-communicable diseases. Mar Drugs 15:67

13. Felix M, Romero A, Rustad T et al (2017) Physicochemical, microstructure and bioactive characterization of gels made from crayfish protein. Food Hydrocoll 63:429–436
14. Vidotti RM, Viegas EMM, Carneiro DJ (2003) Amino acid composition of processed fish silage using different raw materials. Anim Feed Sci Technol 105:199–204
15. Villamil O, Váquiro H, Solanilla JF (2017) Fish viscera protein hydrolysates: production, potential applications and functional and bioactive properties. Food Chem 224:160–171
16. Friedman M (1996) Nutritional value of proteins from different food sources. A review. J Agric Food Chem 44:6–29
17. Venugopal V (2009) Seafood proteins: functional properties and protein supplements. In: Venugopal V (ed) Marine products for healthcare: functional and bioactive nutraceutical compounds from the ocean. CRC Press, Boca Raton, pp 51–102
18. Bechtel PJ (1986) Muscle development and contractile proteins. In: Muscle as food. Academic, San Diego, pp 1–35
19. Lowey S, Risby D (1971) Light chains from fast and slow muscle myosins. Nature 234:81–85
20. Gordon AM, Homsher E, Regnier M (2000) Regulation of contraction in striated muscle. Physiol Rev 80:853–924
21. Lanier T, Yongsawatdigul J, Carvajal-Rondanelli P (2013) Surimi gelation chemistry. In: Park J (ed) Surimi and surimi seafood, 3rd edn. CRC Press, Boca Raton, pp 101–140
22. Kim S-K, Mendis E (2006) Bioactive compounds from marine processing byproducts – a review. Food Res Int 39:383–393
23. Pearson AM, Young RB (1989) The connective tissues: collagen, elastin, and ground substance. In: Pearson AM (ed) Muscle and meat biochemistry. Academic, San Diego, pp 338–390
24. Kristinsson HG, Lanier TC, Halldorsdottir SM et al (2013) Fish protein isolate by pH shift. In: Park J (ed) Surimi and surimi seafood, 3rd edn. CRC Press, Boca Raton, pp 169–192
25. Hayes M, Mora L, Hussey K et al (2016) Boarfish protein recovery using the pH-shift process and generation of protein hydrolysates with ACE-I and antihypertensive bioactivities in spontaneously hypertensive rats. Innovative Food Sci Emerg Technol 37:253–260
26. Park J, Graves D, Draves R et al (2013) Manufacture of surimi. In: Park J (ed) Surimi and surimi seafood, 3rd edn. CRC Press, Boca Raton, pp 55–100
27. Nguyen E, Jones O, Kim YHB et al (2017) Impact of microwave-assisted enzymatic hydrolysis on functional and antioxidant properties of rainbow trout *Oncorhynchus mykiss* by-products. Fish Sci 83:317–331
28. Auwal SM, Zarei M, Abdul-Hamid A et al (2017) Optimization of bromelain-aided production of angiotensin I-converting enzyme inhibitory hydrolysates from stone fish using response surface methodology. Mar Drugs 15:104
29. Salampessy J, Reddy N, Phillips M et al (2017) Isolation and characterization of nutraceutically potential ACE-inhibitory peptides from leatherjacket (*Meuchenia sp.*) protein hydrolysates. LWT Food Sci Technol 80:430–436
30. Klomklao S, Benjakul S (2017) Utilization of tuna processing byproducts: protein hydrolysate from skipjack tuna (*Katsuwonus pelamis*) viscera. J Food Process Preserv 41:e12970
31. Venkatesan J, Anil S, Kim S-K et al (2017) Marine fish proteins and peptides for cosmeceuticals: a review. Mar Drugs 15:143
32. Cermeño M, FitzGerald RJ, O'Brien NM (2016) *In vitro* antioxidant and immunomodulatory activity of transglutaminase-treated sodium caseinate hydrolysates. Int Dairy J 63:107–114
33. Jeewanthi RKC, Lee N-K, Paik H-D (2015) Improved functional characteristics of whey protein hydrolysates in food industry. Korean J Food Sci Anim Resour 35:350–359
34. Adler-Nissen J (1976) Enzymatic hydrolysis of proteins for increased solubility. J Agric Food Chem 24:1090–1093
35. Pacheco-Aguilar R, Mazorra-Manzano MA, Ramírez-Suárez JC (2008) Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease. Food Chem 109:782–789
36. Guérard F, Decourcelle N, Sabourin C et al (2010) Recent developments of marine ingredients for food and nutraceutical applications: a review. J Sci Halieut Aquat 2:21–27

37. Chéret R, Delbarre-Ladrat C, de Lamballerie-Anton M et al (2007) Calpain and cathepsin activities in post mortem fish and meat muscles. Food Chem 101:1474–1479
38. Busconi L, Folco EJ, Martone C et al (1984) Identification of two alkaline proteases and a trypsin inhibitor from muscle of white croaker (*Micropogon opercularis*). FEBS Lett 176:211–214
39. Li Q, Zhang L, Lu H et al (2017) Comparison of postmortem changes in ATP-related compounds, protein degradation and endogenous enzyme activity of white muscle and dark muscle from common carp (*Cyprinus carpio*) stored at 4 °C. LWT Food Sci Technol 78:317–324
40. Kleekayai T, Harnedy PA, O'Keeffe MB et al (2015) Extraction of antioxidant and ACE inhibitory peptides from Thai traditional fermented shrimp pastes. Food Chem 176:441–447
41. Wenno MR, Suprayitno E, Aulanni'am Aulanni'am H (2016) Identification and molecular interaction, mechanism and angiotensin converting enzyme inhibitory peptide from Bakasang (fermented Skipjack tuna (*Katsuwonus pelamis*)). Int J PharmTech Res 9:591–598
42. Mouritsen OG, Duelund L, Calleja G et al (2017) Flavour of fermented fish, insect, game, and pea sauces: garum revisited. Int J Gastron Food Sci 9:16–28
43. Kumar S, Nayak BB (2015) Health benefits of fermented fish. In: Prakash Tamang J (ed) Health Benefits of Fermented Foods and Beverages. CRC Press, Boca Raton, FL, 475–488
44. Skåra T, Axelsson L, Stefánsson G et al (2015) Fermented and ripened fish products in the northern European countries. J Ethnic Foods 2:18–24
45. Wu H-C, Chen H-M, Shiau C-Y (2003) Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber australasicus*). Food Res Int 36:949–957
46. Wisuthiphaet N, Kongruang S, Chamcheun C (2015) Production of fish protein hydrolysates by acid and enzymatic hydrolysis. J Med Bioeng 4:466–470
47. Beddows CG (1997) Fermented fish and fish products. In: Wood BJB (ed) Microbiology of fermented foods. Springer, Boston, pp 416–440
48. Korhonen H, Pihlanto A (2006) Bioactive peptides: production and functionality. Int Dairy J 16:945–960
49. Udenigwe CC, Aluko RE (2012) Food protein-derived bioactive peptides: production, processing, and potential health benefits. J Food Sci 77:R11–R24
50. Bouglé D, Bouhallab S (2017) Dietary bioactive peptides: human studies. Crit Rev Food Sci Nutr 57:335–343
51. Jo C, Khan FF, Khan MI et al (2017) Marine bioactive peptides: types, structures, and physiological functions. Food Rev Int 33:44–61
52. Wilkins E, Wilson L, Wickramasinghe K et al (2017) European cardiovascular disease statistics 2017. European Heart Network, Brussels
53. Zielińska E, Baraniak B, Karaś M (2017) Antioxidant and anti-inflammatory activities of hydrolysates and peptide fractions obtained by enzymatic hydrolysis of selected heat-treated edible insects. Nutrients 9:970
54. Nongonierma AB, Lalmahomed M, Paolella S et al (2017) Milk protein isolate (MPI) as a source of dipeptidyl peptidase IV (DPP-IV) inhibitory peptides. Food Chem 231:202–211
55. Vieira EF, da Silva DD, Carmo H et al (2017) Protective ability against oxidative stress of brewers' spent grain protein hydrolysates. Food Chem 228:602–609
56. Mao X, Bai L, Fan X et al (2017) Anti-proliferation peptides from protein hydrolysates of *Pyropia haitanensis*. J Appl Phycol 29:1623–1633
57. Nongonierma AB, Hennemann M, Paolella S et al (2017) Generation of wheat gluten hydrolysates with dipeptidyl peptidase IV (DPP-IV) inhibitory properties. Food Funct 8:2249–2257
58. Nongonierma AB, Paolella S, Mudgil P et al (2017) Dipeptidyl peptidase IV (DPP-IV) inhibitory properties of camel milk protein hydrolysates generated with trypsin. J Funct Foods 34:49–58

59. Mojica L, Luna-Vital DA, González de Mejía E (2017) Characterization of peptides from common bean protein isolates and their potential to inhibit markers of type-2 diabetes, hypertension and oxidative stress. J Sci Food Agric 97:2401–2410
60. Zhou D-Y, Liu Z-Y, Zhao J et al (2017) Antarctic krill (*Euphausia superba*) protein hydrolysates stimulate cholecystokinin release in STC-1 cells and its signaling mechanism. J Food Process Preserv 41:e12903
61. Flaim C, Kob M, Di Pierro AM et al (2017) Effects of a whey proteins supplementation on oxidative stress, body composition and glucose metabolism among overweight people affected by diabetes mellitus or impaired fasting glucose: a pilot study. J Nutr Biochem 50:95–102
62. Nobile V, Duclos E, Michelotti A et al (2016) Supplementation with a fish protein hydrolysate (*Micromesistius poutassou*): effects on body weight, body composition, and CCK/GLP-1 secretion. Food Nutr Res 60:29857
63. Poprac P, Jomova K, Simunkova M et al (2016) Targeting free radicals in oxidative stress-related human diseases. Trends Pharmacol Sci 38:592–607
64. Neves AC, Harnedy PA, O'Keeffe MB et al (2017) Peptide identification in a salmon gelatin hydrolysate with antihypertensive, dipeptidyl peptidase IV inhibitory and antioxidant activities. Food Res Int 100:112–120
65. Li-Chan ECY, Hunag S-L, Jao C-L et al (2012) Peptides derived from Atlantic salmon skin gelatin as dipeptidyl-peptidase IV inhibitors. J Agric Food Chem 60:973–978
66. Gu R-Z, Li C-Y, Liu W-Y et al (2011) Angiotensin I-converting enzyme inhibitory activity of low-molecular-weight peptides from Atlantic salmon (*Salmo salar L.*) skin. Food Res Int 44:1536–1540
67. Ahn C-B, Cho Y-S, Je J-Y (2015) Purification and anti-inflammatory action of tripeptide from salmon pectoral fin byproduct protein hydrolysate. Food Chem 168:151–156
68. Jung W-K, Karawita R, Heo S-J et al (2006) Recovery of a novel Ca-binding peptide from Alaska Pollack (*Theragra chalcogramma*) backbone by pepsinolytic hydrolysis. Process Biochem 41:2097–2100
69. Hou H, Fan Y, Li B et al (2012) Purification and identification of immunomodulating peptides from enzymatic hydrolysates of Alaska pollock frame. Food Chem 134:821–828
70. Guo L, Harnedy PA, O'Keeffe MB et al (2015) Fractionation and identification of Alaska pollock skin collagen-derived mineral chelating peptides. Food Chem 173:536–542
71. Nikoo M, Benjakul S, Ehsani A et al (2014) Antioxidant and cryoprotective effects of a tetrapeptide isolated from Amur sturgeon skin gelatin. J Funct Foods 7:609–620
72. Lee S-H, Qian Z-J, Kim S-K (2010) A novel angiotensin I converting enzyme inhibitory peptide from tuna frame protein hydrolysate and its antihypertensive effect in spontaneously hypertensive rats. Food Chem 118:96–102
73. Je J-Y, Qian Z-J, Byun H-G et al (2007) Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. Process Biochem 42:840–846
74. Chi C-F, Wang B, Hu F-Y et al (2015) Purification and identification of three novel antioxidant peptides from protein hydrolysate of bluefin leatherjacket (*Navodon septentrionalis*) skin. Food Res Int 73:124–129
75. Egerton S, Culloty S, Whooley J et al (2017) Characterization of protein hydrolysates from blue whiting (*Micromesistius poutassou*) and their application in beverage fortification. Food Chem 245:698–706
76. Rochelle HDL, Courois E, Cudennec B et al (2015) Fish protein hydrolysate having a satietogenic activity, nutraceutical and pharmacological compositions comprising such a hydrolysate and method for obtaining same. Compagnie des Pêches Saint Malo Santé, Museum National D'Histoire Naturelle. US Patent 14/085,350, 22 Jan 2015
77. Harnedy PA, Parthsarathy V, McLaughlin CM et al (2018) Blue whiting (*Micromesistius poutassou*) muscle protein hydrolysate with *in vitro* and *in vivo* antidiabetic properties. J Funct Foods 40:137–145

78. Song R, Wei R, Zhang B et al (2011) Antioxidant and antiproliferative activities of heated sterilized pepsin hydrolysate derived from half-fin anchovy (*Setipinna taty*). Mar Drugs 9:1142–1156
79. You L, Zhao M, Liu RH et al (2011) Antioxidant and antiproliferative activities of loach (*Misgurnus anguillicaudatus*) peptides prepared by papain digestion. J Agric Food Chem 59:7948–7953
80. Hsu K-C, Li-Chan ECY, Jao C-L (2011) Antiproliferative activity of peptides prepared from enzymatic hydrolysates of tuna dark muscle on human breast cancer cell line MCF-7. Food Chem 126:617–622
81. Huang S-L, Jao C-L, Ho K-P et al (2012) Dipeptidyl-peptidase IV inhibitory activity of peptides derived from tuna cooking juice hydrolysates. Peptides 35:114–121
82. Ko J-Y, Kang N, Lee J-H et al (2016) Angiotensin I-converting enzyme inhibitory peptides from an enzymatic hydrolysate of flounder fish (*Paralichthys olivaceus*) muscle as a potent anti-hypertensive agent. Process Biochem 51:535–541
83. Mahmoodani F, Ghassem M, Babji AS et al (2014) ACE inhibitory activity of pangasius catfish (*Pangasius sutchi*) skin and bone gelatin hydrolysate. J Food Sci Technol 51:1847–1856
84. Kim HJ, Park KH, Shin JH et al (2011) Antioxidant and ACE inhibiting activities of the rockfish *Sebastes hubbsi* skin gelatin hydrolysates produced by sequential two-step enzymatic hydrolysis. Fish Aquat Sci 14:1–10
85. Chalamaiah M, Hemalatha R, Jyothirmayi T et al (2014) Immunomodulatory effects of protein hydrolysates from rohu (*Labeo rohita*) egg (roe) in BALB/c mice. Food Res Int 62:1054–1061
86. Yang J-I, Tang J-Y, Liu Y-S et al (2016) Roe protein hydrolysates of Giant Grouper (*Epinephelus lanceolatus*) inhibit cell proliferation of oral cancer cells involving apoptosis and oxidative stress. Biomed Res Int 2016:12
87. Jang HL, Liceaga AM, Yoon KY (2017) Isolation and characteristics of anti-inflammatory peptides from enzymatic hydrolysates of sandfish (*Arctoscopus japonicus*) protein. J Aquat Food Prod Technol 26:234–244
88. Senphan T, Benjakul S (2014) Antioxidative activities of hydrolysates from seabass skin prepared using protease from hepatopancreas of Pacific white shrimp. J Funct Foods 6:147–156
89. Ngo D-H, Ryu B, Kim S-K (2014) Active peptides from skate (*Okamejei kenojei*) skin gelatin diminish angiotensin-I converting enzyme activity and intracellular free radical-mediated oxidation. Food Chem 143:246–255
90. Aissaoui N, Chobert J-M, Haertlé T et al (2017) Purification and biochemical characterization of a neutral serine protease from *Trichoderma harzianum*. Use in antibacterial peptide production from a fish by-product hydrolysate. Appl Biochem Biotechnol 182:831–845
91. Karnjanapratum S, Benjakul S, O'Callaghan YC et al (2016) Purification and identification of antioxidant peptides from gelatin hydrolysates of unicorn leatherjacket skin. Ital J Food Sci 29:158–170
92. Vilas Boas LCP, de Lima LMP, Migliolo L et al (2017) Linear antimicrobial peptides with activity against Herpes simplex virus 1 and Aichi virus. Pept Sci 108:e22871
93. Rajapakse N, Jung W-K, Mendis E et al (2005) A novel anticoagulant purified from fish protein hydrolysate inhibits factor XIIa and platelet aggregation. Life Sci 76:2607–2619
94. Wang L, Dong C, Li X et al (2017) Anticancer potential of bioactive peptides from animal sources. Oncol Rep 38:637–651
95. Ishak NH, Sarbon NM (2017) A review of protein hydrolysates and bioactive peptides deriving from wastes generated by fish processing. Food Bioprocess Technol. https://doi.org/10.1007/s11947-017-1940-1
96. Karoud W, Sila A, Krichen F et al (2017) Characterization, surface properties and biological activities of protein hydrolysates obtained from hake (*Merluccius merluccius*) heads. Waste Biomass Valorization. https://doi.org/10.1007/s12649-017-0069-9

97. Yesmine BH, Antoine B, da Silva Ortência Leocádia NG et al (2017) Identification of ACE inhibitory cryptides in Tilapia protein hydrolysate by UPLC–MS/MS coupled to database analysis. J Chromatogr B 1052:43–50
98. Gauthier SF, Vachon C, Savoie L (1986) Enzymatic conditions of an in vitro method to study protein digestion. J Food Sci 51:960–964
99. Fekete S, Veuthey J-L, Guillarme D (2012) New trends in reversed-phase liquid chromatographic separations of therapeutic peptides and proteins: theory and applications. J Pharm Biomed Anal 69:9–27
100. Lemieux L, Piot J-M, Guillochon D et al (1991) Study of the efficiency of a mobile phase used in size-exclusion HPLC for the separation of peptides from a casein hydrolysate according to their hydrodynamic volume. Chromatographia 32:499–504
101. Ghassem M, Arihara K, Mohammadi S et al (2017) Identification of two novel antioxidant peptides from edible bird's nest (*Aerodramus fuciphagus*) protein hydrolysates. Food Funct 8:2046–2052
102. WHO (2014) Global status report on noncommunicable diseases 2014. World Health Organization, Geneva
103. Brieger K, Schiavone S, Miller FJ et al (2012) Reactive oxygen species: from health to disease. Swiss Med Wkly 142:w13659
104. Machlin LJ, Bendich A (1987) Free radical tissue damage: protective role of antioxidant nutrients. FASEB J 1:441–445
105. Barbour JA, Turner N (2014) Mitochondrial stress signaling promotes cellular adaptations. Int J Cell Biol 2014:156020
106. Sae-Leaw T, Karnjanapratum S, O'Callaghan YC et al (2017) Purification and identification of antioxidant peptides from gelatin hydrolysate of seabass skin. J Food Biochem 41:e12350
107. Huang D, Ou B, Prior RL (2005) The chemistry behind antioxidant capacity assays. J Agric Food Chem 53:1841–1856
108. Hernández-Ledesma B, Dávalos A, Bartolomé B et al (2005) Preparation of antioxidant enzymatic hydrolysates from α-lactalbumin and β-lactoglobulin. Identification of active peptides by HPLC-MS/MS. J Agric Food Chem 53:588–593
109. Murase H, Nagao A, Terao J (1993) Antioxidant and emulsifying activity of N-(long-chain-acyl) histidine and N-(long-chain-acyl) carnosine. J Agric Food Chem 41:1601–1604
110. Park P-J, Jung W-K, Nam K-S et al (2001) Purification and characterization of antioxidative peptides from protein hydrolysate of lecithin-free egg yolk. J Am Oil Chem Soc 78:651–656
111. Husain Z, Schwartz RA (2013) Food allergy update: more than a peanut of a problem. Int J Dermatol 52:286–294
112. Pawankar R (2014) Allergic diseases and asthma: a global public health concern and a call to action. World Allergy Organ J 7:12–14
113. Kawai T, Akira S (2006) Innate immune recognition of viral infection. Nat Immunol 7:131–137
114. Le Y, Zhou Y, Iribarren P et al (2004) Chemokines and chemokine receptors: their manifold roles in homeostasis and disease. Cell Mol Immunol 1:95–104
115. Martin P, Leibovich SJ (2005) Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Biol 15:599–607
116. Medzhitov R (2007) Recognition of microorganisms and activation of the immune response. Nature 449:819–826
117. Andersen MH, Schrama D, Thor Straten P et al (2006) Cytotoxic T cells. J Invest Dermatol 126:32–41
118. Portnoy JM, Van Osdol T, Williams PB (2004) Evidence-based strategies for treatment of allergic rhinitis. Curr Allergy Asthma Rep 4:439–446
119. Meltzer EO (2017) Sublingual immunotherapy: a guide for primary care. J Fam Pract 66:S58–S58
120. Reyes-Díaz A, González-Córdova AF, Hernández-Mendoza A et al (2017) Immunomodulation by hydrolysates and peptides derived from milk proteins. Int J Dairy Technol. https://doi.org/10.1111/1471-0307.12421
121. Tsuruki T, Kishi K, Takahashi M et al (2003) Soymetide, an immunostimulating peptide derived from soybean β-conglycinin, is an fMLP agonist. FEBS Lett 540:206–210

122. Jaziri Mh, Migliore-Samour D, Casabianca-Pignède M-R et al (1992) Specific binding sites on human phagocytic blood cells for Gly-Leu-Phe and Val-Glu-Pro-Ile-Pro-Tyr, immunostimulating peptides from human milk proteins. Biochim Biophys Acta 1160:251–261
123. Takahashi M, Moriguchi S, Ikeno M et al (1996) Studies on the ileum-contracting mechanisms and identification as a complement C3a receptor agonist of oryzatensin, a bioactive peptide derived from rice albumin. Peptides 17:5–12
124. Cicero AFG, Fogacci F, Colletti A (2017) Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review. Br J Pharmacol 174:1378–1394
125. Chalamaiah M, Yu W, Wu J (2018) Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: a review. Food Chem 245:205–222
126. Subhan F, Kang HY, Lim Y et al (2017) Fish scale collagen peptides protect against $CoCl_2$/TNF-α-induced cytotoxicity and inflammation via inhibition of ROS, MAPK, and NF-κB pathways in HaCaT cells. Oxid Med Cell Longev. https://doi.org/10.1155/2017/9703609
127. Elango R, Laviano A (2017) Protein and amino acids: key players in modulating health and disease. Curr Opin Clin Nutr Metab Care 20:69–70
128. Li G-H, Le G-W, Shi Y-H et al (2004) Angiotensin I–converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. Nutr Res 24:469–486
129. Natesh R, Schwager SLU, Sturrock ED et al (2003) Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. Nature 421:551–554
130. Wu J, Aluko RE, Nakai S (2006) Structural requirements of angiotensin I-converting enzyme inhibitory peptides: quantitative structure−activity relationship study of di- and tripeptides. J Agric Food Chem 54:732–738
131. de Oliveira-Sales EB, Nishi EE, Boim MA et al (2010) Upregulation of AT1R and iNOS in the rostral ventrolateral medulla (RVLM) is essential for the sympathetic hyperactivity and hypertension in the 2K-1C wistar rat model. Am J Hypertens 23:708–715
132. Chatterjee S, Khunti K, Davies MJ (2017) Type 2 diabetes. Lancet 389:2239–2251
133. Eisenbarth GS (2007) Update in type 1 diabetes. J Clin Endocrinol Metab 92:2403–2407
134. IDF (2015) IDF diabetes atlas. IDF, Brussels
135. Thomas MC, Paldánius PM, Ayyagari R et al (2016) Systematic literature review of DPP-4 inhibitors in patients with type 2 diabetes mellitus and renal impairment. Diabetes Ther 7:439–454
136. Mahmood N (2016) A review of α-amylase inhibitors on weight loss and glycemic control in pathological state such as obesity and diabetes. Comp Clin Pathol 25:1253–1264
137. Zhang L, Chen Q, Li L et al (2016) Alpha-glucosidase inhibitors and hepatotoxicity in type 2 diabetes: a systematic review and meta-analysis. Sci Rep 6:32649
138. Lacroix IME, Li-Chan ECY (2016) Food-derived dipeptidyl-peptidase IV inhibitors as a potential approach for glycemic regulation – current knowledge and future research considerations. Trends Food Sci Technol 54:1–16
139. Stoimenis D, Karagiannis T, Katsoula A et al (2017) Once-weekly dipeptidyl peptidase-4 inhibitors for type 2 diabetes: a systematic review and meta-analysis. Expert Opin Pharmacother 18:843–851
140. Lovshin JA (2017) Glucagon-like peptide-1 receptor agonists: a class update for treating type 2 diabetes. Can J Diabetes 41:524–535
141. Gourgari E, Aroda VR, Wilhelm EE et al (2017) A comprehensive review of the FDA-approved labels of diabetes drugs: indications, safety, and emerging cardiovascular safety data. J Diabetes Complications 31:1719–1727
142. Nishi T, Hara H, Tomita F (2003) Soybean β-conglycinin peptone suppresses food intake and gastric emptying by increasing plasma cholecystokinin levels in rats. J Nutr 133:352–357
143. Sharara AI, Bouras EP, Misukonis MA et al (1993) Evidence for indirect dietary regulation of cholecystokinin release in rats. Am J Physiol Gastrointest Liver Physiol 265:G107–G112
144. Cudennec B, Ravallec-Plé R, Courois E et al (2008) Peptides from fish and crustacean by-products hydrolysates stimulate cholecystokinin release in STC-1 cells. Food Chem 111:970–975
145. Cudennec B, Fouchereau-Peron M, Ferry F et al (2012) *In vitro* and *in vivo* evidence for a satiating effect of fish protein hydrolysate obtained from blue whiting (*Micromesistius poutassou*) muscle. J Funct Foods 4:271–277

146. Greco E, Winquist A, Lee T et al (2017) The role of source of protein in regulation of food intake, satiety, body weight and body composition. J Nutr Health Food Eng 6:00223
147. Madani Z, Sener A, Malaisse WJ et al (2015) Sardine protein diet increases plasma glucagon-like peptide-1 levels and prevents tissue oxidative stress in rats fed a high-fructose diet. Mol Med Rep 12:7017–7026
148. Umezawa H, Aoyagi T, Ogawa K et al (1984) Diprotins A and B, inhibitors of dipeptidyl aminopeptidase IV, produced by bacteria. J Antibiot 37:422–425
149. Chinembiri T, du Plessis L, Gerber M et al (2014) Review of natural compounds for potential skin cancer treatment. Molecules 19:11679
150. Creagh EM (2014) Caspase crosstalk: integration of apoptotic and innate immune signalling pathways. Trends Immunol 35:631–640
151. Roomi MW, Shanker N, Niedzwiecki A et al (2015) Induction of apoptosis in the human prostate cancer cell line DU-145 by a novel micronutrient formulation. Open J Apoptosis 4:11
152. Ding G-F, Huang F-F, Yang Z-S et al (2011) Anticancer activity of an oligopeptide isolated from hydrolysates of Sepia ink. Chin J Nat Med 9:151–155
153. Alemán A, Pérez-Santín E, Bordenave-Juchereau S et al (2011) Squid gelatin hydrolysates with antihypertensive, anticancer and antioxidant activity. Food Res Int 44:1044–1051
154. Harnedy PA, Fitzgerald RJ (2013) Bioactive proteins and peptides from macroalgae, fish, shellfish and marine processing waste. In: Kim S-K (ed) Marine proteins and peptides. Wiley, Chichester, pp 5–39
155. Minekus M, Alminger M, Alvito P et al (2014) A standardised static in vitro digestion method suitable for food – an international consensus. Food Funct 5:1113–1124
156. Liu Y, Pischetsrieder M (2017) Identification and relative quantification of bioactive peptides sequentially released during simulated gastrointestinal digestion of commercial kefir. J Agric Food Chem 65:1865–1873
157. Vilcacundo R, Martínez-Villaluenga C, Hernández-Ledesma B (2017) Release of dipeptidyl peptidase IV, α-amylase and α-glucosidase inhibitory peptides from quinoa (*Chenopodium quinoa* Willd.) during in vitro simulated gastrointestinal digestion. J Funct Foods 35:531–539
158. Phongthai S, D'Amico S, Schoenlechner R et al (2018) Fractionation and antioxidant properties of rice bran protein hydrolysates stimulated by *in vitro* gastrointestinal digestion. Food Chem 240:156–164
159. Nieva-Echevarria B, Jacobsen C, García Moreno PJ et al (2016) Evaluation of the antioxidant activity in food model system of fish peptides released during simulated gastrointestinal digestion. Paper presented at the 14th Euro Fed Lipid Congress, Ghent, 18–21 Sept 2016
160. Sanchón J, Fernández-Tomé S, Miralles B et al (2018) Protein degradation and peptide release from milk proteins in human jejunum. Comparison with *in vitro* gastrointestinal simulation. Food Chem 239:486–494
161. Toopcham T, Mes JJ, Wichers HJ et al (2017) Bioavailability of angiotensin I-converting enzyme (ACE) inhibitory peptides derived from *Virgibacillus halodenitrificans* SK1-3-7 proteinases hydrolyzed tilapia muscle proteins. Food Chem 220:190–197
162. Grimble GK, Keohane PP, Higgins BE et al (1986) Effect of peptide chain length on amino acid and nitrogen absorption from two lactalbumin hydrolysates in the normal human jejunum. Clin Sci 71:65–69
163. Keohane PP, Grimble GK, Brown B et al (1985) Influence of protein composition and hydrolysis method on intestinal absorption of protein in man. Gut 26:907–913
164. Hou H, Fan Y, Wang S et al (2016) Immunomodulatory activity of Alaska pollock hydrolysates obtained by glutamic acid biosensor – artificial neural network and the identification of its active central fragment. J Funct Foods 24:37–47
165. FitzGerald RJ, Meisel H (2000) Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. Br J Nutr 84:33–37
166. Ramezanzade L, Hosseini SF, Nikkhah M (2017) Biopolymer-coated nanoliposomes as carriers of rainbow trout skin-derived antioxidant peptides. Food Chem 234:220–229

Edible Insects as Source of Proteins

15

Ewelina Zielińska, Monika Karaś, Anna Jakubczyk, Damian Zieliński, and Barbara Baraniak

Contents

E. Zielińska (✉) · M. Karaś · A. Jakubczyk · B. Baraniak
Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Lublin, Poland
e-mail: ewelina.ziel@tlen.pl; monika.karas@up.lublin.pl; anna.jakubczyk@up.lublin.pl; barbara.baraniak@up.lublin.pl

D. Zieliński
Department of Ethology and Animal Welfare, University of Life Sciences in Lublin, Lublin, Poland
e-mail: damian.zielinski@up.lublin.pl

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_67

Abstract

The potential of insects as a source of protein for future food and feed is the object of numerous studies. The nutritional value of edible insects is well established, and other aspects of consumption thereof are investigated. In this chapter, we aim to summarize the main features of insects as food. We briefly describe the history of the usage of insects as food for humans and refer to the current acceptance of insects by Europeans based on conducted surveys. We characterize the most common insect species with the biggest potential to be used as food and feed in the EU according to EFSA. We describe the nutritional value of insects and the possibility of application thereof in the food and feed industry, keeping in mind the safety of consumption. In addition, the ecological aspect of insect breeding is discussed. A review of the growing edible insect market in Europe and the USA is also provided. Moreover, we analyze the current legal status of insect intake in Europe. We aim to make this chapter a current conclusion about the consumption of insects.

Keywords

Entomophagy · Edible insects · Protein · Environment · Nutritive value · Functional properties · Insect products · Bioactive peptides · Preferences of consumers

1 Introduction

The practice of eating insects is known as entomophagy. The term "entomophagy" itself is relatively new in English and some other European languages. The Google Ngram currently dates the first published mention of "entomophagy" to 1871, in a volume entitled "Sixth annual report on the noxious, beneficial and other insects of the state of Missouri" by Charles V. Riley. In French, the term "entomophagie" was found in data from 1810 [1]. Today, we can find "entomophagy" in Oxford Dictionaries online, which defines the term as "the practice of eating insects, especially by people." The greater interest in entomophagy is reflected in the increase in the number of publications. As reported by Evans et al. [1], the number of publications

in the Web of Science containing "entomophagy" amounted to 16 in 2001–2010 and as many as 49 in 2011–2015. In turn, 50 articles were published between 2016 and September 2017.

The rising interest in edible insects is reflected in the emergence of companies dealing with the production and processing of insects as well as the expanding scientific literature. The resulting publications present the use of insects in feeding farm animals, accompanying animals, and humans [2], whereas, research grants are focused on the use of insects in the food industry in various forms [3]. The consumption of insects as a whole can be difficult for Western consumers; therefore, the easiest way for processing insects is grinding them to flour [4]. It is also possible to isolate individual components of insects such as protein [5], fat [6], and chitin [7].

Eating insects by humans is not a new concept; it occurs globally [8] but is still rare in Europe [9]. Why not eat insects? Is it worth it? The answer is simple – definitely yes. Entomophagy has several advantages. First of all insects are a good source of protein, essential fats, and antioxidant peptides [10–13]. Many insects are rich in microelements such as iron, calcium, and zinc and in vitamins [14, 15]. Secondly, insect breeding is environmentally friendly. Insects emit significantly fewer greenhouse gases (GHGs) and ammonia than most livestock [16]. Moreover, insects require less space, feed, and water for breeding than livestock [17]. Economic factors are also important. Insect rearing can be low-tech or very sophisticated, depending on the level of investment [18]. For these three main reasons, insects have been highlighted as an important food source in response to the growing concerns about the future of world food security.

In this chapter, we describe the state of the knowledge of various aspects of insect consumption and their potential to be used in the food industry. We will also discuss the historical and cultural background of insect consumption, and we will focus on the biology of popular species.

2 History and Cultural Acceptance of Entomophagy

Despite the lack of tradition and the reluctance of some consumers, edible insects are increasingly becoming part of the diet of the people of not only Africa, Asia, and Latin America but also the USA, Australia, and Europe [18]. In many countries, they are considered not only as food providing nutrients but also as a delicacy. Although for many Europeans, insects are still associated with something unpleasant, it should be noted that in countries such as Thailand, Madagascar, and Mexico, they could only be consumed by the royal elites and the rich [19].

2.1 History and Tradition of Eating Insects by Humans

Habits, geographic regions, cultures, and religious beliefs have the main influence on food and diet practices. It is known that insects were consumed in ancient times, not only by animals but also by our primate ancestors [20]. Already in the fourth century

BCE, cicadas (nymphs, in particular) were described by Aristotle as a delicacy in Ancient Greece [21]. The first mention of insects as food ingredients can be found in the Old Testament (Leviticus 11.20–23), where we can read: "All winged insects moving on four feet you will regard as detestable for eating. Of all these winged insects you may eat only the following: those with the sort of legs above their feet which enable them to leap over the ground. These are the ones you may eat: the various kinds of migratory locust, the various kinds of solham locust, hargol locust and hagab locust. But all other winged insects on four feet you will regard as detestable for eating" [22]. Noteworthy is the fact that only some insect species were allowed to be eaten. Moreover, the New Testament (Matthew, 3, 4) mentions locusts as the food of John the Baptist [22]. Entomophagy was described in the early centuries AD in Greek literature by many philosophers; however, the approach to insect consumption was not unequivocal because many Greeks in the first century AD believed that cicadas should not be eaten because these insects were a swallow food and themselves were scary and musical [23]. In turn, in the first century AD, Roman historian Pliny the Elder (author of the encyclopedia – *Historia Naturalis*) mentioned that cicadas were eaten in the East [23] and the Cossus, i.e., an insect living on oak trees, was a meal valued by the Romans [23, 24]. In the early third century AD, in one of his works (*The Deipnosophistae* of Athenaeus), which is named the first cookbook, Athenaeus of Naucratis described grasshoppers and cicadas that were eaten as an incentive to appetite [25]. As shown by the abovementioned sources, the tradition of eating insects dates back several thousand years, and the mention of insect food can be found not only in the literature of the Christian religion but also in the Jewish and Islamic culture. As far as Christianity is concerned, we find already mentioned data on insect eating in the Bible, whereas species of kosher locusts were largely accepted in the Jewish culture in ancient times. In the following years, the tradition of consuming insects among the Jewish diaspora due to the lack of knowledge of the various types of insects known in the Torah as "winged swarming things" significantly decreased. Probably, also the influence of the Western culture was the reason for the disappearance of the tradition of eating insects by Jews [26]. In the Islamic tradition, we can also find several references to eating insects. El-Mallakh and El-Mallakh [27] find insects in the text of the Koran (e.g., gnat, locust, lice, bee, ant, and spider) that have played an important role in historical events or religious instructions. Only locusts, despite the association with plagues, could be designed to eat (Sahih Muslim, 21.4801). Moreover, insects were seen as low life forms (with the exception of bees) that had a negative influence on the daily life of humans. In the early literature of the Far East, especially in the Chinese literature, we can find information about insects as food. The *Compendium of Materia Medica*, i.e., a Chinese herbology and medicine book written by Li Shizhen during the Ming dynasty [28], provides information about the consumption of insect tea prepared from feces of moths, e.g., *Aglossa dimidiatus*, *Hydrillodes morosa*, and *Nodaria niphona*, which exhibited potential activity in treatment of otitis media [29]. The same source of interesting data about insects provides information that *Megachile* spp. China were used as medicine for cancer, *Endoclita sinensis* (grape tree borer) was used to enhance health, and larvae and adults of

Apriona germari japonica (mulberry longhorn, mulberry borer) were consumed [30] or used for treatment of cardiovascular diseases and brain fever [28]. As can be seen, insects are an important part of human nutrition history. They were very important sources of nutritional compounds not only for Africans, Asians, Americans, Latin Americans, and Indians in the west of North America. Some species were more important such as grasshoppers, caterpillars, beetle grubs and (sometimes) adults, winged termites (some of which are very large in the tropics), bee, wasp, and ant brood (larvae and pupae) as well as winged ants, cicadas, and a variety of aquatic insects. It should be noted that these insects were eaten not only during the periods of famine as the ultimate food, but they were also elements of the normal diet. Due to the tradition of nutrition, taste, and nutritional compounds among some people such as the Yukpa people of Colombia and Venezuela or the Pedi of South Africa, traditional insect dishes are more preferred than meat dishes [31]. It should be emphasized that insect consumption in many countries is not a new trend in nutrition and is part of the culture and tradition of the East and tropical countries. Moreover, the history of entomophagy is very old – from ants and larvae of beetles eaten in the tribes of Africa and Australia to maintain a balanced diet to fried locusts and beetles, which are popular also today in Thailand. It is estimated that insects are part of a diet of about two billion people worldwide, and 1900 species have been documented in the scientific literature as edible species. Similar to meat eaters who do not eat all kinds of meat, insect consumers do not eat all insect species. The most populous groups of insects include locusts, crickets, cicadas, beetles, wasps, caterpillars, leaf and plant hoppers, termites, scale insects, true bugs, dragonflies, flies, ants, grasshoppers, and bees [18]. In cultures where insects are regarded as food components, there are special local standards for the preparation thereof to make them suitable for consumption [32].

2.2 Do Europeans Already Eat Insects?

Although it may seem that Europeans do not eat insects, this is not exactly true. Even though many people claim that they would never eat insects, they actually consume them every day. Insects are everywhere. In spite of farmers' efforts, insects are found in cereals, spices, vegetables, and fruits, and despite the enforced limits on the specific insect content, they reach finished products such as flour, frozen food, chocolate, ketchup, coffee, fruit juice, and many other foods. Calculations indicate that each of us unknowingly consumes about 1 lb (500 g) of insects per year [33].

Furthermore, sometimes we eat insects consciously. The cochineal scale insect (*Dactylopius coccus*) produces a red dye called carmine (E120) when it is crushed. This dye is widely used in the food industry, e.g., in beverages, sauces, yogurt, and baked goods. In turn, cheese from sheep milk casu marzu (literally "rotten cheese") is a Sardinian delicacy. It is left to ripen for so long that it starts to rot. The rotting process attracts cheese flies that then lay their eggs on the cheese. The fly larvae eat their way into the cheese, digesting fats along the way and making the hard cheese soft and runny on the inside. The cheese is eaten with larvae, which are about 8 mm

long. However, the most popular and totally accepted product, although processed through an insect, is honey. People may not realize anymore that it comes from an insect and it is the vomit of some kind of larva [33]. Nevertheless, since honey is delicious, perhaps a chance should be given to other insects.

2.3 Preferences of Consumers in Western Countries

Although there are historical data on the use of insects for nutrition and insects are considered delicacies in many parts of the world, in most Western countries, eating insects is regarded as a disgusting practice arousing distaste and associated with primitive behavior [34]. In these cultures, insects are completely rejected as food ingredients, because they are unclean, unhealthy, and thus involved in the risk associated with food contamination and consuming insects [35]. The current growing scientists and consumers' interest in insects as food should be noted. It seems that there are three main barriers for the sector of edible insects: consumer acceptance, technology, and regulation. Hoverer, a study conducted in Switzerland has indicated nine main factors that have an influence on the willingness to consume insects. These are convenience orientation, discernibility of insects in food, food neophobia, expected food healthiness, perceived health benefits of meat, food technology neophobia, need for familiarity, and binary variables: gender and prior consumption. It is interesting that food neophobia does not play the most important role in the willingness to consume insects [36].

The current European Union policy on the food sector is cautious about new food sources. Classification of products as a "new food" and new food technologies as well as legislation of edible insects in human diet is still under consideration [37]. Nevertheless, according to Lensvelt and Steenbekkers [38] and Caparros Megido et al. [39], crickets and mealworms are most widely accepted as edible insects by people from Western countries. Factors that have an influence on this trend include increased interest in non-European cultures, developing ecology, environmental concern, and increased consumer awareness of technological issues in food production. In some countries, ten species of reared insects are mainly used as animal feed; however, some of these species have recently been sold as food in China, Thailand, the Netherlands, South Africa, Belgium, France, and the USA [40]. Commercial insect consumption in Western countries is currently limited to experimental restaurants where insects are served as a delicacy and something different or original [41] or to products that contain insecticides such as protein isolated as a nutrient ingredient for athletes. There are many studies on consumer preferences, relationships between human nutritional habits and entomophagy, or factors that have the strongest influence on consumer acceptance of insects as food. The results are not clear and depend on the countries and the awareness of participants in the experiments.

In recent years, the consumption of animal-based proteins, especially meat, aimed to maintain a well-balanced diet has been identified as one of the most important topics both for consumers and for food producers. It is known that the intensity of animal production generates climate change, has a strong influence on the environment, and is

expensive [42]. For this reason, studies mainly focus on determination of consumers' readiness to replace meat with other products in the diet [43]. Alternative nutritional approaches are vegetarian, macrobiotic, or anthroposophical diets, in which meat is either not eaten at all or very rarely and the protein is replaced by a vegetable healthy protein. Last but not least, insects are considered a potential source of protein [44]. The insects are not associated as food but as something nasty and food contamination. However, results of a study conducted in Belgium have shown that more than 65% of respondents disagree with the idea of including insects as food compounds [45]. Another study provided data from a representative survey of Dutch consumers on their habits in preparation of meat meals, meat substitution, and reduction of this ingredient in the diet, showing that the meal form, cooking skills, product familiarity, and preferences for plant-based foods had an influence on the consumer preferences. The most important factor that has an influence on acceptance of edible insects by consumers is their invisibility and the way of preparation of the meal [39, 46]. The results of the study with Dutch consumers, in which pictures of food with whole insects and with protein isolated from insects but used as an ingredient of the product, e.g., pizza, indicated that the photos with visible insects were rated much more negatively than those showing protein derived from insects and processed in the pizza. It seems that the pizza flavor has a positive effect on accepting insects as food, as chocolate-coated locusts were rated less negatively than fully visible insects [42]. The aim of another experiment was to determine how the product preparation, individual traits (e.g., food neophobia), and familiarity influence the acceptance of insects as food by consumers. The Dutch respondents rated eight pictures with four dishes (familiar foods). There was beef stew, curry, and brownie and spice cake in two variants: the mealworms were ground and not visible in the picture, and the insects were visible in the dishes. The results indicated that adding mealworms to familiar meals did not guarantee high product acceptability. Moreover, the products with edible insects were ranked lower, even if they were visually identical with products used as a control [47]. It would be worth exploring whether insects would be more readily accepted as a snack food than a part of the main meal. Since consumers have little experience with insects as food, it is difficult to conclude that the taste of dishes is based only on the presentation of the meal [48]. Hence, insects presented in various forms in a dish can cause different sensations and perceptions of taste, which affect the readiness of consumers to eat insects. In turn, a study conducted in Italy indicated that students engaged in a so-called bug banquet with cookies made with "insect flour" willingly tried the product and claimed that they would try other insects in the future. The main role in the decision to try a cookie made with cricket flour was played by curiosity, but negative opinions of family members and friends and the disgust factor prevented the consumers from eating the insects. In general, respondents consider insects as a rich source of protein and other nutrients, which may influence the desire to introduce them into the diet. This largely depends on the market availability and regulatory framework, food category (e.g., bakery product with insect flour), marketing strategies, methods of preparation, and culinary trends [49].

Another study provided some data about the sector of edible insects in the Netherlands, the progress of the sector, and limitations in the legitimacy process. Interviews were held with experts and stakeholders, including, e.g., industry experts,

researchers, government officials, and farmers in the emerging sector, about the strengths, weaknesses, opportunities, and threats of the edible insect industry. It should be noted that almost all respondents rated high the use of edible insects for economic and social purposes. Edible insects can also constitute a new business model in the Netherlands, be an alternative to soy and fishmeal, and provide a solution to global protein deficiency. However, the development of this sector should not be rushed and not limited to the feed market [37].

A study assessing the perception of entomophagy among the Belgian population was carried out by Caparros Megido et al. [39]. The results indicated slight neophobia, but consumers agreed to test insect preparations. Two species of edible insects were tested (house crickets and mealworms); they were prepared as a snack with known flavors and crispy texture. The results have suggested that people will be eating, preparing, and cooking insects in the near future. It has been shown that edible insects that are prepared as snacks with common flavors such as chocolate, chili, caramel, or curry arise curiosity and are more likely to be eaten than raw insects. It should be noted that, even in 2012, insects were not accepted as an alternative or replacement of meat in Belgium [43].

Determination of the acceptance of edible insects as food and an alternative source of protein was the aim of a study conducted by Gere et al. [50] in Hungary. The results showed that less than 11% of respondents did not regard insects, algae, soy, and whey as an alternative source of protein, but half of them knew algae and whey as this kind of protein source, and soy had the highest familiarity scores. Moreover, almost 60% of the respondents have heard about edible insects but do not know them. Interestingly, those who intended to reduce their meat intake in the coming year suggested insects as a replacement for fresh meat, but the general scores of food neophobia were significantly higher than the scores of food technology neophobia. The results confirm findings reported by other authors, i.e., in the case of European consumers, food neophobia is a barrier for the consumption of insects. Table 1 summarizes the acceptance of insects as alternative proteins among Western consumers.

We can conclude that edible insects may be introduced to the diet of the Western consumers in the future; yet, although the product design is important, it is insufficient to popularize insect consumption. Moreover, people's knowledge about the environment and positive aspects of edible insects as bioactive components of food should be increased. In the future, edible insects may become such a delicacy for European consumers as seafood, which in some European countries is a source of disgust.

3 Examples of Popular Insect Species for Human Consumption

The word insect derives from the Latin word "insectum," meaning "with a notched or divided body," literally "cut into sections." In the Online Etymological Dictionary [55], we can also find: "The Latin word is Pliny's loan-translation of Greek entomon

Table 1 Acceptance and methods of preparation of insects as alternative proteins among Western consumers

Country, year of study, and sample	Method of preparation	Main research question/intervention	Provided information
Hungary, 2016, 400 adult meat consumers [50]	No info	Have you ever heard about this alternative source of protein, and would you be prepared to eat insects as a meat substitute?	89% of the respondents know about the alternative protein sources. 60% of the respondents have heard about insect consumption and know what this means. Insects can be an alternative source of protein for people who want to reduce meat consumption, but neophobia is a barrier
Italy, 2015, 109 people (53% female and 47% male), aged between 18 and 25 years old [49]	Cookie with cricket flour	Would you like to taste an edible insect? The reasons for the decision	Almost all respondents tasted the cookie and are willing to try other insects in the future. The barriers include negative opinions of family members and friends and the disgust factor. Market availability (regulatory framework), food category (e.g., bakery product with insect flour) marketing strategies, gastronomy (preparation), culinary trends, and education have an influence on insect consumption in the future
German- and French-speaking parts of Switzerland, 2015, 379 cases [36]	No info	Predictors that are currently used to explain the willingness to consume insects	There are nine significant variables reliably predicting the willingness to consume insects: convenience orientation, discernibility of insects in food, expected food healthiness, need for

(*continued*)

Table 1 (continued)

Country, year of study, and sample	Method of preparation	Main research question/intervention	Provided information
			familiarity, perceived health benefits of meat, food technology neophobia, food neophobia, and binary variables: gender and prior consumption. Food neophobia was not found to be the key predictor of the willingness to consume insects but shares its rank with various predictors. Further, the analysis revealed one significant two-way interaction effect
Belgium, 2014, students interested in insect food, 79, 44% males, 56% females [39]	Burger with a mixture of beef, lentils, and mealworm	Overall liking of the hybrid burger in comparison with a beef burger	Females liked the beef burger better than the burger containing mealworms. Males perceived no significant difference between the beef burger and the mealworm burger. The major factors influencing the results were knowledge of entomophagy, previous experience with insect tasting, and gender
Denmark and Italy, no info, 136 students from Denmark (61 females), and 128 from Italy (71 females) [51]	Insects	Impact of individual and social benefits of eating insects on acceptance of insect-based food products. Effect of communication, also comparing messages based on individual versus societal benefits of insect	Communication has an influence on the intention and behavior and depends on the nation, gender, and knowledge of entomophagy Negative associations with insects have a strong

(*continued*)

Table 1 (continued)

Country, year of study, and sample	Method of preparation	Main research question/intervention	Provided information
		consumption on the possibility to foster people's willingness to eat insect-based food	influence on avoidance of consumption of insect-based food
Netherlands, no info; 100 participants were all occasional or regular consumers of beef, whereas a minority claimed to have tasted the novel ingredients before [52]	Burger with "100% ground beef," "75% ground beef and 25% ground lamb brain," "75% ground beef and 25% ground frog meat," and "75% ground beef and 25% ground mealworms." For brevity, these labels will be mentioned as "beef burger" and "novel burgers"	Consumer acceptance of new food	Unwillingness to eat new food related more with food appropriateness than the actual taste of meals. Consumers accept new ingredients in known foods with known flavors
Germany, 2014, 502 adults [46]	Insects as meat substitute, silkworm (deep-fried), crickets (deep-fried), silkworm drink, cricket cookies, cricket cookies choco chip	A study of consumers' willingness to eat different insect-based processed and unprocessed food	The results indicated that the willingness to eat was significantly higher for processed food items such as drinks and cookies than for unprocessed food items. The age of the respondents has no influence on the willingness to eat insects
Netherlands, no info, 976 adults from 18 to 94 years [47]	Eight mealworm product images with differences: mealworm visibility (visible/invisible), flavor (savory/sweet) carrier, and origin carrier (Western/ Asian)	How the product preparation, familiarity, and individual beliefs influence the consumer acceptance of insect-based food	The respondents accepted edible insects when these were added to familiar foods, and it depended on the perceived appropriateness of mealworms as food and the perceived appropriateness of the product combination
Canada, no info, 134 students from junior high, high school, and university [53]	Spring rolls filled with carrots, celery, bean sprouts, sauce, and whole cooked	A study of the first reaction to insects as a food idea	The disgust was reported, and "bug banquets" were found to be the most

(*continued*)

Table 1 (continued)

Country, year of study, and sample	Method of preparation	Main research question/intervention	Provided information
	crickets and mealworms, roasted crickets and roasted seasoned mealworms, and roasted mealworms garnishing rice crispy squares		effective way to change attitudes toward insects as food
Switzerland, 2015, 104, 55% men [54]	Tortilla chips: made with a traditional corn flour recipe and cricket flour as an additional ingredient	Could processed foods like cookies or chips made with a small amount of cricket flour be used to increase the general acceptance of insects as a food source?	The results have shown that the positive experience with a processed insect product can increase people's willingness to eat unprocessed insects. The products with edible insects as an ingredient can taste good, and processed insect food may be a first step to accept insects as food by western consumers

'insect,' which was Aristotle's term for this class of life, in reference to their 'notched' bodies. First in English in 1601 in Holland's translation of Pliny. In zoology, in reference to a class of animals, 1753."

Insects have a three-part body (head, thorax, and abdomen) with a chitinous exoskeleton, three pairs of jointed legs, compound eyes, and two antennae. They are among the most diverse groups of animals on the planet: there are more than one million described species, which is more than half of all known living organisms. Until recently, the total number of species is estimated at 6–10 million [56], but the current estimates are up to 30 million [57]. Among the millions of insect species living on earth, 2111 are considered as edible (Fig. 1) [58]. These facts show that insects are an important source of protein in our diet. Members of Lepidoptera, Hemiptera, Coleoptera, Diptera, Orthoptera, and Hymenoptera are the most commonly consumed insects. The popularity of edible species varies among regions or cultures [59]. In Europe, the consumption of insects is marginal, but the interest in insects is still growing. Therefore, the Scientific Committee of the European Food Safety Authority issued a scientific opinion "Risk profile related to production and consumption of insects as food and feed" [60]. The report contains a list of insect species that were found to have the biggest potential to be used as food and feed in the EU:

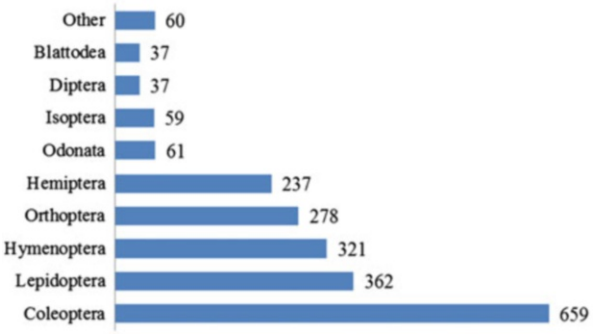


Fig. 1 Number of recorded edible insect species per group in the world [58]

Musca domestica: Common housefly
Hermetia illucens: Black soldier fly
Tenebrio molitor: Mealworm
Zophobas atratus: Giant mealworm
Alphitobius diaperinus: Lesser mealworm
Galleria mellonella: Greater wax moth
Achroia grisella: Lesser wax moth
Bombyx mori: Silkworm
Acheta domesticus: House cricket
Gryllodes sigillatus: Banded cricket
Locusta migratoria migratorioides: African migratory locust
Schistocerca americana: American grasshopper

At the same time, EFSA notes that this list does not need to be considered definitive or exhaustive. This selection is undoubtedly supported by the large number of scientific studies of these species.

3.1 Diptera

Flies (order Diptera) are a very popular insect order consumed by humans [58]. *Musca domestica* and *Hermetia illucens* are representatives of this order. They are probably characterized by the largest reproductive capacity, shortest life cycles, and rapid growth rates, which makes them one of the most attractive species of insects for human consumption [61]. The black soldier fly (*Hermetia illucens*) can be used commercially to solve a number of environmental problems associated with manure and other organic waste, such as reducing manure mass, moisture content, and offensive odors. At the same time, they provide high-value feedstuff for cattle, pig, poultry, and fish [62]. *H. illucens* has become an object of interest to researchers who work on the possibility of using this insect in animal feed [63–65]. It can also be used

in solving another problem: the increasing amount of food waste, which can cause environmental pollution if not managed properly [66]. The black soldier fly exhibits rapid food intake ranging from 25 to 500 mg of fresh matter/larva/day feeding on a wide range of decaying organic materials, such as rotting fruits and vegetables, fish offal, and animal manure and human excreta [67].

3.2 Coleoptera

Insects of this order are most commonly consumed in the world with 659 documented species being consumed (Fig. 1). Of the several edible groups within this order, the most interesting is probably the family Tenebrionidae. The *Tenebrio molitor*, *Zophobas atratus*, and *Alphitobius diaperinus* species listed by EFSA belong to this family. Tenebrionids have a bad reputation as pests of meal, flour, and other stored and packaged cereal foods, but, despite this, the yellow mealworm, *Tenebrio molitor*, has been reared by zoos, aquaria, and commercial dealers as food for animals since at least the eighteenth century [59]. Moreover, the mealworm (*T. molitor*) is the subject of many studies. Its properties and nutritional value are well known [4, 5, 14, 68–70]. The insect can be easily reared on many types of vegetables and grains in a small space. There are even special devices for breeding insects at home. A Livin Farms hive is a device that facilitates breeding of mealworms for the needs of your household. The system is divided into multiple vertically stacked chambers beginning with pupae, placed into the topmost pupation component, where they hatch into meal beetles (*T. molitor*). After the adult beetles mate, they lay eggs that fall through into the egg layer where the mealworms are hatched. Every week, the mealworms will be lowered into the next compartment until they reach the sixth drawer for the weekly harvest. The mealworms can survive on oats and vegetable scraps, while in-built smart systems help maintain ideal microclimates for growth and remove foul odors. The weekly full harvest process should take approximately 8–9 weeks for setup [71].

3.3 Lepidoptera

Caterpillars are the second most consumed insect order by humans, with 362 documented species being consumed (Fig. 1). *Galleria mellonella*, *Achroia grisella*, and *Bombyx mori* are representatives of the EFSA list. They are eaten in the larval form, and the largest size (up to about 60 mm) is reached by the silkworm *Bombyx mori*. They are very high in fat but also in microelements such as iron and zinc [68]. They are less popular for consumption in Europe, but they are massively reared for the animal feed industry. Moreover, these larvae are the subject of research on bioactive substances derived from their organism. Studies on the antihypertensive properties of peptides from *B. mori* are being conducted [72, 73]. *B. mori* and *G. mellonella* have been found to produce antimicrobial peptides [74–77].

3.4 Orthoptera

Insects of this order such as grasshoppers, locusts, and crickets are known to all insect-eating cultures. Two hundred seventy-eight species of crickets, grasshoppers, and katydids are recorded as being consumed by humans (Fig. 1). Acrididae, Gryllidae, and Tettigoniidae are the most commonly consumed insect families [61].

The house cricket *Acheta domesticus* is most readily available to Western consumers. Studies have shown that, when kept at temperatures of 30 °C or higher and fed diets equal in quality to those used in bringing conventional livestock to market condition, this cricket exhibits food conversion efficiency about twice as high as that of broiler chickens and pigs. In addition, female crickets have much higher fecundity than other animals. Each cricket lays 1200–1500 eggs over a period of 3–4 weeks [59]. Another cricket *Gryllodes sigillatus* is also well known in Europe, for example, as food for exotic pets. The tropical house cricket is probably native to Southwestern Asia but has been spread by commerce to tropical regions worldwide [78]. *Locusta migratoria migratorioides* and *Schistocerca americana* are members of the Acrididae family. Despite the extensive practice of farming insects, these species are more difficult in commercial breeding than the crickets mentioned above [18]. There is a prototype device for breeding crickets or grasshoppers at home. The Lepsis is a vessel that can be used to grow insects for food. The product consists of four individual units that are each designed to breed, grow, harvest, and kill grasshoppers, and they combine to form a decorative kitchen product [79].

4 Nutritional Value of Edible Insects

The nutrient compositions of many edible insects were compiled and published in numerous studies [80–85]. The nutritional values of insects are expressed as the content of proteins (amino acid profile), fat, fibers, dietary energy, minerals, and vitamins. The nutritional values of edible insects depend on many factors, e.g., the origin of the insect, stage of metamorphosis, diet, and environmental factors (temperature, humidity) [86]. Therefore, it is very difficult to generalize about the nutritional value of the 2000 edible insect species.

4.1 Energy Content

The energy content in most edible insects is considerable due to the high levels of protein and fat. The energy value depends on the stage of insects, i.e., larvae or pupae are usually richer in energy than adults. Ramos-Elorduy et al. [87] analyzed 78 species of insects and estimated their energy value in the range from 293 to 762 kcal/100 g. On the basis of 113 literature reports, Rumpold and Schlüter [70] have found that 79.65% of insects are characterized by energy content above 400 kcal/100 g and 40.94% above 500 kcal/100 g. The mean energy content in edible insects is in the range of 409.78–508.89 kcal/100 g (based on dry matter), with maximum energy

Table 2 Nutritional content of selected insect species compared with common protein commodities

Name (*Latin name*)	Protein content (% in dry mass)	Fat (%)	Fiber (%)	Energy content (KCAL/100G)
Banded cricket (*Gryllodes sigillatus*)	70.00 [14]	18.23 [14]	3.65 [14]	452.00 [14]
Black soldier fly (*Hermetia illucens*)	17.50–36.00 [81]	14.00 [80]	6.70 [89]	199.00 [80]
	17.50 [80]	32.60 [90]		
	47.00 [89]			
Common housefly (*Musca domestica*)	9.30 [89]	11.90 [89]	–	552.40 [70]
	63.10–63.99 [70]	15.50–24.31 [70]		
Giant mealworm (*Zophobas atratus*)	41.50 [90]	36.20 [90]	–	–
(*Zophobas morio*)	19.85–51.60 [81]	40.80–42.04 [70]	1.54–2.50 [81]	575.53 [70]
	43.13–46.79 [70]		9.26–13.0 [70]	
	46.80–55.16 [91]			
Greater wax moth (*Galleria mellonella*)	15.39 [81]	51.40–60.00 [70]	8.92–19.52 [70]	650.13 [70]
	33.98–41.25 [70]			
House cricket (*Acheta domesticus*)	7.50–23.70 [80]	2.41–6.71 [80]	1.18–4.03 [81]	135.10–170.90 [80]
	15.60–73.60 [81]	9.80–24.00 [70]	6.20–22.08 [70]	414.41–455.19 [70]
	55.00–70.75 [70]	14.40–22.10 [89]	9.60–10.20 [89]	
	66.60–67.20 [89]			
Lesser mealworm (*Alphitobius diaperinus*)	48.60–60.00 [81]	8.50 [3]	4.40–9.10 [81]	–
	61.70 [90]	24.30 [90]		
		29.00 [92]		
Locust (*Schistocerca americana*) (*Schistocerca gregaria*)	61.10 [70]	17.00 [70]	10.00 [70]	427.00 [70]
	76.00 [14]	12.97 [14]	2.53 [14]	432.00 [14]
Migratory locust (*Locusta migratoria*)	62.20 [91]	12.61	23.61	364.70
	13.00–28.00 f.m [18]			

(*continued*)

Table 2 (continued)

Name (*Latin name*)	Protein content (% in dry mass)	Fat (%)	Fiber (%)	Energy content (KCAL/100G)
Mealworm (*Tenebrio molitor*)	11.42–30.38 [80]	5.40–19.94 [83]	1.39–21.00 [81]	130.00–482.00 [80]
	13.68–24.59 [83]	6.42–22.98 [80]	2.10 [83]	160.00–283.00 [83]
	14.00–25.00 f.m. [18]	14.88–43.08 [70]	5.00–20.22 [70]	379.61–577.44 [70]
	20.00–68.60 [81]	25.00 [90]		
	47.00–65.29 [70]			
Silkworm (*Bombyx mori*)	10.00–17.00 f.m. [18]	5.62–14.78 [80]	2–6.39 [70]	120.52–135.48 [80]
	10.14–25.66 [80]			
	17.90–22.89 [81]			
	48.70–69.84 [70]			
	53.80–64.70 [89]	8.09–37.10 [89]	6.40 [89]	389.60–555.00 [70]
Commonly consumed protein source				
Egg, whole, dried	12.56 [93]	9.51 [93]	–	592.00 [93]
Beef, grass-fed, ground, raw	19.42 [93]	12.73 [93]	–	198.00 [93]
Chicken ground, raw	17.44 [93]	8.10 [93]	–	143.00 [93]
Soybeans, mature seeds, raw (*Glycine max*)	28.70–50.10 [94]	19.94 [93]	9.30 [104] – 11.90 [94]	403.00–446.00 [93, 94]
Broad beans, mature seeds, raw (*Vicia faba*)	22.00–38.20 [94]	1.53 [93]	25.00 [93]	320.00–341.00 [93, 94]
Lentils/raw (*Lens culinaris*)	24.63 [93]	1.06 [93]	10.70 [93]	352.00 [93]
Wheat, durum (*Triticum durum*)	13.68 [93]	2.47 [93]	10.70 [93]	339.00 [93]

contents as high as 762.00–776.85 kcal/100 g in moth *Phassus triangularis* [70, 88]. Among the species of edible insects listed in Table 2, the maximum energy contents was found in the greater wax moth (650.13 kcal/100 g) [10], and the minimum value was estimated in the silkworm (*B. mori*) 128 ± 7.48 kcal/100 g [80]. Evidently, the energy content in most edible insects is high, even in comparison to meat.

4.2 Protein and Essential Amino Acid Profile

In recent years the protein content is a leading trend in the development of food products. Protein is a very important basic macronutrient for humans, as it plays many functions in the body and, above all, is a source of essential amino acids and energy. The growing desire for unconventional non-animal-based protein sources has led to an interest in pulses and insect-based protein. As a rich protein source, edible insects are alternatives to meats within international dietary guidelines. The total protein content of insect species is presented in Table 2.

The protein content of most edible insects has been shown in many studies to be within the range of 13–77% [81, 82] or 9.3–80% [89, 95]. The protein level in insects depends on many factors, e.g., their metamorphosis stages, feeding mixtures used, varying water content, methods of preparation and processing applied before consumption of insects (e.g., drying, boiling, or frying), and primarily methods used from protein determination (Dumas technique or Kjeldahl method) [89, 90, 95]. Many studies have reported analysis of the nutritional value of edible insects; however, these data are not always comparable due to the variations between insects and because of the varying methodologies employed to analyze the compounds. The Kjeldahl method is widely used for determination of the crude protein content in food [18]. Therefore, many scientists use this method in their studies devoted to insects. This procedure evaluates the total concentration of nitrogen (N), which is converted to protein by multiplying it by the nitrogen-to-protein conversion factor of 6.25. Because insects contain many N-rich compounds that are not digested by humans (chitin and proteins tightly embedded in its matrix), the Kjeldahl method can overestimate the content of digestible protein. In view of the above, Jonas-Levi and Martinez [81] proposed evaluation of digestible nitrogen by quantifying N in the cuticle and subtracting it from the total nitrogen content and calculation of a new N-conversion factor, which should be similar for all insect species and their development stages.

The highest percentage of protein (80%) was found in the adult stage of the gypsy moth (*Porthetria dispar*) and (78.8%) in the German cockroach (*Blattella germanica*) [88]. Among insects recommended by EFSA, the cricket (*Acheta domesticus*) was characterized by the highest protein content (73.60%) [81].

Comparison of the conventional protein source in human nutrition with insect protein demonstrates that the content of proteins in many insects is comparable to that in beef or chicken meat. As shown in Table 2, some insects are comparable to eggs, meat, and plants. Moreover, insects are richer in protein than cereals, e.g., wheat 13.68%, pseudocereals: buckwheat 13.1% and amaranth 13.5% [96] and legume seeds including dried peas (*Pisum sativum*) 14.2–36.1%, chickpeas (*Cicer arietinum*)19.1–31.2%, beans (*Phaseolus vulgaris*) 15.2–36.0%, and soybeans (*Glycine max*) 28.7–50.1% [94]. Finke et al. [97] investigated insects as a source of protein used for feeding rats. They observed that the amino acid composition in cricket protein (*Acheta domesticus* and *Anabrus simplex*) was equal or better than that in soy protein. By contrast, silkworm protein showed a significant lower quality than casein.

A major consideration with respect to the inclusion of insects in food products relates to the quality of their dietary protein. Protein quality depends on the kind of amino acids present in their sequence (essential or nonessential) and protein digestibility. However, the quality of insect proteins in comparison to other animal and plant proteins has to be assessed through the amino acid requirements in humans. The most important aspect and characteristic of protein from a nutritional standpoint is its amino acid composition. Phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, and lysine are classified as essential amino acids, and histidine is a semi-essential amino acid.

Cereal proteins that are major components in human diets and animal feed worldwide are rich in sulfur amino acids, but they are poor in lysine, tryptophan, and threonine (e.g., maize) [98–100]. Similar to cereals, legumes (soybean, pea, chickpea, lentil, beans) contain high amounts of essential amino acids such as arginine, leucine, and lysine but are poor in sulfur-containing amino acids (particularly methionine) and tryptophan [31].

The amino acid spectra in edible insect have been shown in many studies [70, 80, 89, 101–103]. Analysis of many edible insects has revealed that their essential amino acid content is 10–30%, covering 35–50% of all types of amino acids, which is close to the amino acid model proposed by the World Health Organization and FAO [89]. In some insect species, essential amino acids are very well represented [104], while in others they are limited. It was reported in literature that insect proteins were low in the Met + Cys [103] and high in Lys, Trp and Thr [31], or Phe [105]. As demonstrated by Williams et al. [89], Trp is a limited amino acid in ants, bee (*Apis mellifera*), and silkworm (*B. mori*), Ile in caterpillar, and Lys in termites. The essential amino acids (EAA) of selected insects compared to plant and animal protein sources are shown in Table 3. The giant mealworm (*Z. atratus*) protein is low in Met, silkworm (*B. mori*) in Met and Phe, and common housefly (*M. domestica*) in Ile.

Generally, proteins derived from animal foods (meats, milk, eggs) are complete, but protein malnutrition continues to be a problem in many countries around the world. Insects are a promising alternative source for nutritional and functional proteins. Insects are characterized by high protein quality with beneficial potential for human nutrition. The great number of investigations on the health benefits of edible insects associated with consumption thereof increase interest in developing innovative technologies to expand the use of insects in food or feed products. Apart from their nutritional properties, proteins from edible insects possess functional properties that play an important role in food formulation and processing [18].

4.3 Lipids and Essential Fatty Acid Profile

Fats are one of the three main macronutrients, along with carbohydrates and protein. Dietary consumption of fatty acids has an impact on human health. Some insects are rich in fat, in a wide range from 4.56% to 60% (Table 2). The fat content is higher in

Table 3 Essential amino acid (EAA) content of selected insect species compared with common protein commodities [70, 93]

EAA [mg/g protein]		Egg, whole, raw, fresh	Wheat, durum	Soybeans mature seeds. Raw	Silkworm (*Bombyx mori*)	House cricket (*Acheta domesticus*) (adults)	Mealworm (*Tenebrio molitor*)	Giant mealworm (*Zophobas morio*)	Common housefly (*Musca domestica*) (larvae)	Summary of the adult EAA requirements FAO/WHO/UNU (2017) [mg/g protein]
Essential amino acids	**His**	24.60	23.54	30.06	25.8–29.5	22.7–23.4	28.7–37.9	30.5	30.9	15
	Ile	53.42	38.96	54.01	32.3–33	36.4–45.9	43.5–50.3	47.2	**22.8**	30
	Leu	86.46	68.27	90.68	48.9–52.7	66.7–100	82.2–106.4	97	45.3	59
	Lys	72.61	**22.15**	74.16	47.3–50	51.1–53.7	44.3–64.9	52.3	81.6	45
	Met	30.25	16.15	**14.99**	**12.5–14**	14.6–19.6	12.7–19.5	**10.7**	36.6	16
	Cys	21.66	20.91	17.95	8.6–9.1	8.33–9.8	6.8–10.9	7.6	6.6	6
	Phe	54.14	49.78	58.15	**28.4–29**	31.7–30.2	26.2–43.7	34.5	55.8	30
	Thr	44.27	26.75	48.40	28.4–31.2	6.3–7.6	34.2–41.8	39.6	35.5	23
	Trp	13.30	12.87	16.20	6.8–7.5	31.1–36.1	8.0–11.0	9.1	49.5	6
	Val	68.31	43.42	55.60	39.8–40.9	48.4–52.2	58.8–69.0	52.3	45.6	39
	Total EAA	469.03	322.807	460.21	278.80–296.90	317.33–378.50	345.4–455.4	380.8	410.2	269

the larval stage than in the adult stage [82]. Larvae of the greater wax moth (~60%) and mealworm (~43%) exhibit the highest amounts of fat [10, 90, 98, 100].

Lipids are a source of not only energy but also essential fatty acids (FA). Many studies have found that replacing saturated fatty acids (SFA) with monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in the diet reduces the risk of cardiovascular diseases. The evaluation of fat quality is a complex issue, since SFAs elevate the LDL cholesterol concentration in serum, while MUFAs and PUFAs (in particular, those from the n-6 family) have been shown to decrease LDL cholesterol concentrations. Therefore, it is recommended that SFA should be replaced with PUFA (n-3 and n-6) in the diet, and the total intake of SFA should not exceed 10% of energy [106]. The fatty acids of many insects contain more PUFA and are comparable to those of poultry and fish in their degree of unsaturation [70, 104]. In contrast, beef and pork contain very low levels of PUFA but high content of MUFA [107]. The highest MUFA proportion was found in beetle larvae, while PUFA levels were determined in adult crickets [102]. The ratio of fatty acids recommended for human nutrition is SFA/MUFA/PUFA 1.25:1.5:1. The ratio found in *Zophobas morio* by Kourimska et al. [105] was 1.9:1.4:1. The determined MUFA/PUFA ratio meets the requirements for human consumption (1.4:1), but the amount of SFA is significantly higher. Similar results were obtained by Zielińska et al. [14] (1.34:1.4:1.1) for *Schistocerca gregaria*. In turn, Tzompa-Sosa et al. [11] obtained a lower ratio (1.4:1.6:1) in the case of this species. The amount of SFA in the mealworm (*T. molitor*) was significantly lower. This is confirmed by the results obtained by Zielińska et al. (2015) (1:1.7:1.2). Tzompa-Sosa et al. [11] obtained a significant content of MUFA (1.1:2.3:1), whereas Bednářová et al. [91] reported a greater amount of PUFA (0.7:0.8:1). A greater amount of PUFA was determined by Zielińska et al. [14] in the case of *Gryllodes sigillatus* (1:1:1), but the amount of SFA and MUFA was considerably lower than recommended. The quality and quantity of fatty acids in foods are very important from the nutritional point of view. The fatty acid composition in edible insects has been summarized in many studies [14, 68, 70]. The differences between reported values of fat contents in many studies may have been associated with the variety of insects and with changing the insects' feed composition [70, 95, 108]. The main MUFA detected in edible insects are palmitoleic acid and oleic acid. The silkworm larva is a potential source of such fatty acids as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are important to support a healthy cardiovascular system [109]. The highest content of essential α-linolenic acid and oleic acid was determined in *T. molitor* larvae and *A. domesticus* [11, 102, 110]. Therefore, the mealworm could be the most suitable insect of all analyzed species for human consumption. PUFA that can be found in the fatty acid spectra of edible insects are represented by linoleic, linolenic, arachidonic, and eicosapentaenoic acids [70, 92, 103].

4.4 Fiber Content

The fiber content of edible insects was investigated in many studies and referred to as crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF)

[111–113]. According to Rumpold and Schlüter [70], the average fiber content in edible insects ranges from 5.06% (termites) to 13.56% (true bugs). The average fiber content in the species of edible insects listed in Table 2 ranges from 2% (silkworm) to 22.08% (house cricket). The differences in the fiber content likely result from the presence of different compounds that are bound to chitin. The exoskeleton of edible insects contains a significant amount of different compounds, including chitin and substances that are bound to chitin (e.g., protein, lipids, and other compounds) [111, 114]. Moreover, Finke [111] suggests that insects with harder cuticles do not necessarily contain more chitin than softer-bodied insects, but rather they contain higher levels of cross-linking proteins that are essential for sclerotization. Chitin is considered as an indigestible fiber by humans, because chitinase found in human gastric juices is inactive [115]. However, it was found that this enzyme is active in the organisms of inhabitants of tropical countries where the consumption of insects has a long-term tradition [18, 89, 105]. Removal of chitin improves the digestibility of insect protein [111].

Chitin has been associated with defense against parasitic infections and some allergic conditions; it acts as an anticoagulant and protects against certain pathogens in the blood and skin. The positive activity of chitin involves significant reduction of serum cholesterol, functions as a hemostatic agent for tissue repair, enhancement of burn and wound healing, enhancement of pollutant removal from waste-water effluent, improvement of the washability and antistatic nature of textiles, inhibition of growth of pathogenic soil fungi and nematodes, and an increase in wheat, barley, oat, and pea yields by as much as 20% [116–118].

4.5 Microelements

Many edible insects are a good source of macro- and micronutrients that are necessary for a healthy organism [40, 119]. As shown in many studies, edible insects are a rich source of phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), calcium (Ca), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and selenium (Se). Therefore, edible insects can supply necessary nutritive elements for human body functions. Very important is the supply of Fe and Zn in the diet, given the worldwide deficiencies in these minerals among humans. There are nutritional studies on the use of insects (caterpillar cereal) in prevention of diseases associated with iron deficiency diseases such as anemia and stunting [120, 121]. Most edible insects have equal or higher iron contents than beef [104]. An excellent source of iron are mopane caterpillar (*Gonimbrasia belina*) (31–77 mg/100 g) and locusts (8–20 mg/100 g DM), while the iron content of the beef is 6 mg/100 g [122]. Zinc content in beef is on average 12.5 mg/100 g, while house cricket (*A. domesticus*), for example, contains 29.69 mg/100 g, chapulin (*Sphenarium histrio*) 78 mg/100 g, and house fly pupa (*M. domestica*) 85.8 mg/100 [70, 89]. Generally, edible insects are low in sodium (except caterpillar *Usta terpsichore*) and calcium (except *M. domestica* and *A. domesticus*) [70]. The composition of macro- and micronutrients largely depends on the food consumed by the insect. The macro- and microelements can be contained in the

consumed food present in the gastrointestinal tract or in components incorporated in the insect's body [89, 113, 123]. The levels of these nutrients vary considerably between species. Rumpold and Schlüter [70] concluded that edible insects generally lack sufficient amounts of Ca and K. In contrast, Gosh et al. [102] have shown that the calcium content in all studied insects was much higher than that in conventional foods of animal origin except chicken eggs. Edible insects have the potential to provide specific micronutrients such as Cu, Fe, Mg, Mn, P, Se, and Zn, and, in addition, they can be utilized in low-sodium diets. Nowak et al. [83] compared a high number of insect species and found that the mineral content in insects exhibited extreme variability within and between species. According to Oonincx and Dierenfeld [124], the high variability in microelements is a result of a small sample size, species-specific metabolism, varying accuracy of sampling and/or analytical techniques, and contamination.

4.6 Vitamins

Edible insects are also a source of vitamins, which are essential for normal growth and development of a living organism by stimulating metabolic processes and enhancing immune system functions. Due to the limited data about vitamins in insects, it cannot be indicated which insects are a good source of vitamins. Nevertheless, Bukkens [104] has shown that the level of vitamin B_1 in a whole range of insects is 0.1–4 mg/100 g and B_2 – 0.11–8.9 mg/100 g; in comparison, whole-meal bread provides 0.16 mg and 0.19 mg/100 g of these vitamins, respectively.

Rumpold and Schlüter [70] have demonstrated that edible insects contain a whole range of water-soluble vitamins, B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), B_6 (pyridoxine), B_7 (biotin), B_9 (folate), and C (ascorbic acid), and fat-soluble vitamins, A (retinol) and E (tocopherols). By comparison of the vitamin content in insects with the vitamin requirements in human nutrition of adults, Rumpold and Schlüter [70] noted that edible insects are generally rich in riboflavin, pantothenic acid, and biotin. In turn, Nowak et al. [83] collected 56 literature reports about vitamin content in edible insects and found that mealworm can be a good source of vitamin B_6 (pyridoxine), B_2 (riboflavin), B_3 (niacin), B_9 (folate), and B_{12} (cobalamin). Caterpillars are especially rich in B_1, B_2, and B_6 [82, 125].

Wakayama et al. [126] noted that many insect species were characterized by low levels of vitamin B_{12} (cyanocobalamin). It is present only in animal-origin food and is well represented in mealworm larvae (*T. molitor*) (0.47 μg/100 g) and house crickets (*A. domesticus*) (5.4 μg/100 g in adults and 8.7 μg/100 g in nymphs). According to van Huis [122], Williams et al. [89], and Rumpold and Schlüter [70], insects are generally a good source of some vitamins B but not A, C, B_3, and B_1. The content of vitamin A (as retinol or β-carotene) was less than 2 μg/100 g and less than 100 μg/100 g in yellow mealworm larvae, superworms, and house crickets [68, 104, 127]. Compared to commercially bred insects, those captured in the wild contain higher levels of carotenoids, such as astaxanthin, α- and β-carotene, lutein, or

zeaxanthin; this is associated with their diet, which is a richer source of these compounds. The high level of carotenoids in insects may be a promising source of vitamin A in human diet [122].

There are insufficient data on the content of vitamin E in insects. According to Bukkens [104], palm weevil larvae (*Rhynchophorus ferrugineus*) had 35 mg and 9 mg/100 g of α-tocopherol and β+γ tocopherol, respectively (the recommended daily intake – 15 mg). A relatively high level of vitamin E, 9.65 mg/100 g, was determined by Tong et al. [128] in freeze-dried silkworm powder (*B. mori*).

In conclusion, since the literature provides only a few data on insect vitamin content, more research is needed to identify edible insects as a source of vitamins. Moreover, Payene et al. [80] observed that the contents of minerals and vitamins varied greatly between insect species; this was suggested to be due to soil contamination of samples and variation in the diet of the insects.

Although significant variation was found in the data, many edible insects provide satisfactory amounts of energy and protein; they are also rich in monounsaturated and/or polyunsaturated fatty acids and amino acids, micronutrients (copper, iron, magnesium, manganese, phosphorous, selenium, and zinc), vitamins B_{2-12}, carotenoids, and bioactive substances, which are essential for humans.

4.7 Bioactive Substances

Insects and bioactive substances extracted from them have been used in unconventional medicine by human cultures all over the world. Insects constitute an almost inexhaustible source of compounds for scientific research. A wide range of edible insects is a source of many bioactive components (polyphenols, enzymes, peptides/proteins) [116, 129, 130].

Nongonierma and FitzGerald [131] focused on studies published between 2005 and 2017 and analyzed the literature on the generation of bioactive peptides (BAPs) from edible insect proteins following enzymatic hydrolysis. Different enzyme preparations (Alcalase™ 2.4 L, Flavourzyme™, Protamex™, papain, trypsin, and pepsin) were used during hydrolysis of insect proteins to obtain bioactive peptides. The authors suggested that silkworm (*B. mori*) is currently the most widely studied species. Generally, the peptides obtained by enzymatic hydrolysis of edible insect proteins showed angiotensin-converting enzyme (ACE) inhibitory activity and, hence, antioxidant and antidiabetic properties. Moreover, selected species of edible insects studied by Zielińska et al. [12, 13] have high antiradical activity and an ability to chelate iron ions. They can inhibit lipoxygenase and cyclooxygenase-2 activity after the digestion and absorption process. Moreover, the heat treatment process positively affects the antioxidant properties of peptides derived from these species.

A majority of studies were focused on insect protein-derived peptides with ACE inhibitory activity. Vercruysse et al. [132–134] were one of the first authors presenting on ACE inhibitory activity of protein hydrolyzates from insects (*B. mori*, *B. terrestris*, *S. gregaria*, and *S. littoralis*). Dai et al. [135] investigated an angiotensin I-converting enzyme (ACE) inhibitor peptide (Tyr-Ala-Asn) derived from *T. molitor*

larva protein hydrolyzate. Wang et al. [136–138] examined antihypertensive properties of a novel peptide (Ala-Ser-Leu) obtained from silkworm pupa protein on spontaneously hypertensive rats and the effect of these peptides on the fermentation and quality of yogurt. The peptides, Ala-Pro-Pro-Pro-Lys-Lys, Val-Glu-Ile-Ser, Lys-His-Val, and Gly-Asn-Pro-Trp-Met, were identified in *B. mori* protein hydrolyzed by Wang et al. [138], Li et al. [139], Jia [140], and Tao [141], respectively. In their study, Wu et al. [142] investigated a silkworm larvae protein isolate (SLPI) as a source of bioactive peptides. The SLPI hydrolyzate exhibited strong angiotensin I-converting enzyme (ACE) inhibitory activity and antioxidant activity. The second large group of peptides comprises antioxidant peptides obtained by proteolysis of insect proteins with the use of different enzymes [12, 13, 132]. There are only few investigations evaluating the α-glucosidase inhibitory activity of insect protein hydrolyzates. However, Zhang et al. [143] used a quantitative structure-activity relationship (QSAR) approach for prediction of α-glucosidase inhibitory activity of peptides from *B. mori* proteins. Moreover, alkaloids, flavonoids, anthraquinones, tannins, phlobatannins, steroids, triterpenoids, and cyanogen glycosides were detected in hydrophilic and lipophilic extracts from *Encosternum delegorguei* (Hemiptera: Tessaratomidae) hydrophilic and lipophilic extracts [130]. These extracts were characterized by high levels of flavonoids and free radical scavenging activity, but the potential trade-off from the elevated levels of cyanogen glycosides after processing needs further investigation.

Peptides obtained from insects (Fig. 2) also have antibacterial and antifungal properties. A few antifungal compounds have been found in insects, for example, termicin from termites, drosomycin from *Drosophila melanogaster*, heliomicin from the tobacco budworm (*Heliothis virescens*), and the gallerimycin peptide from greater wax moth (*G. mellonella*) larvae [144]. The extract from housefly larvae possesses broad antibacterial activity against both Gram-negative and Gram-positive bacteria [145]. *T. molitor* produces antimicrobial and antifungal tenecin 4 [146].

In turn, Chinese black ants contain compounds with anti-inflammatory, immunosuppressive, and renoprotective activities [147]. Protein fractions extracted and purified from housefly larvae have antiviral and antitumor activities [148]. Elpidina and Goptar [149] reported that proteinases from *T. molitor* presumably have potential for oral administration to treat celiac disease. This insect contains a wide spectrum of digestive proteinases operating in the midgut with a sharp pH gradient from acid to alkaline values. Two digestive peptidases from the acidic AM, i.e., cysteine cathepsin L and post-proline cleaving peptidase, readily hydrolyze bonds formed by the major amino acids of cereal gluten – proline and glutamine.

In summary, potentially bioactive peptides derived from insect proteins and phenolic compounds have been identified (Fig. 3). Many of these peptides had interesting activities (mainly antihypertensive) even in small animals. The bioactive potency of edible insect protein hydrolyzates/peptides has been shown by Nongonierma and FitzGerald [131] to be similar or higher than that of other dietary proteins (plants and animals).

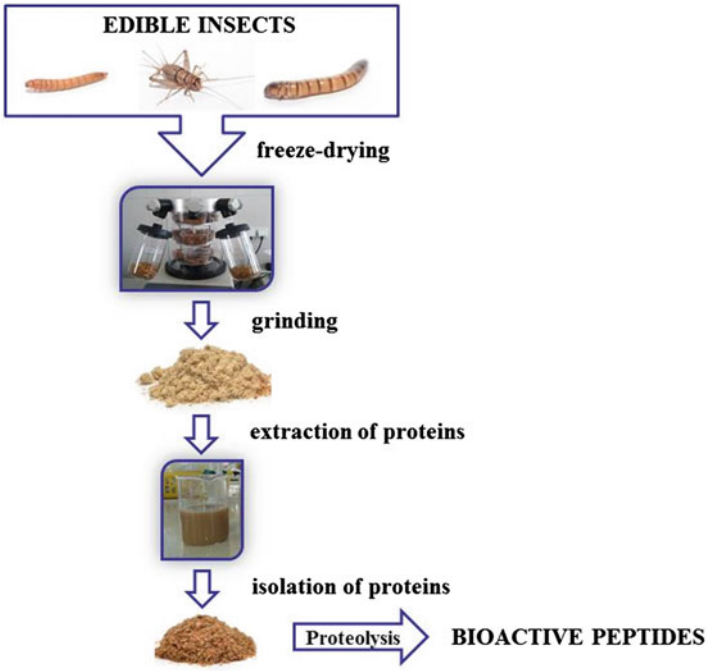


Fig. 2 Method for obtaining the bioactive peptides from edible insect proteins

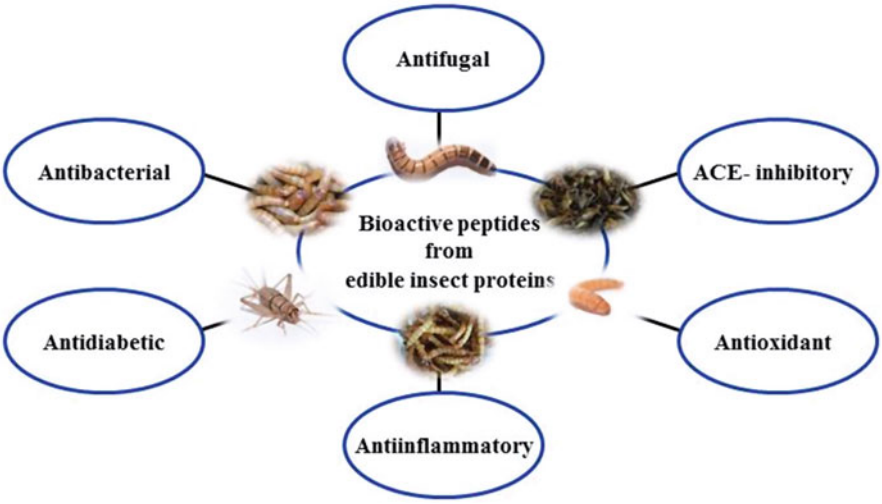


Fig. 3 Edible insects as a source of bioactive peptides

Fig. 4 Insect flour and extracted insect protein (flour and extracted protein from cricket *G. sigillatus* and flour and extracted protein from mealworm *T. molitor*, respectively)

5 Processing Edible Insects for Food

In tropical countries, whole insects are often consumed after a suitable thermal process. However, Western consumers may be reluctant to accept this form. Some consumers are not convinced to eat insects because of the bad associations with their appearance. It is easier to accept products where the insect additive is not visible [41, 45]. In typical western societies, consumers find insects more appealing when used as an ingredient of foods with familiar flavors and textures [150]. There are many possibilities of using insects in an invisible form, for example, grinding insects to so-called insect flour or extraction of the main insect ingredients such as protein, fat, and chitin (Fig. 4).

5.1 Insect Flour

Cricket flour is the most popular insect product in Europe because of its versatility [151]. The addition of the flour to traditionally eaten foods is a great way to introduce insects into our diet without altering our habits. The powder form seems to be ideal for use in the food industry as it can be used for all kinds of products without changing their textural or functional properties; moreover, this additive can improve the products [152, 153]. Furthermore, the process of flour production is not complicated and, hence, cost-effective.

The easiest way to make flour from insects is to dry them first because of their high moisture content [68]. Drying is important because it reduces not only moisture but also water activity in the product, which is important in microbiological terms. Insects can be dried in industrial driers or lyophilized, which is probably the best possible method for preserving the chemical and other properties of food (Fig. 5). Reduced water content in the finished product guarantees longer storage time, but this is not the only advantage of producing insect flour. Milling the whole insects retains all their components in the flour, i.e., micronutrients or chitin in addition to proteins.

Fig. 5 The process of freeze-drying edible insects

5.2 Extracted Insect Protein

Supplementation of food products (e.g., meat products) with legume protein is a known practice. Extraction of insect proteins to increase the protein content in a food product could be a useful way of increasing acceptability among wary consumers. In this case, a small step method is a good idea. The gradual introduction of insect food proteins will allow consumers to get acquainted with the new product that at first sight is not different from what is known to them. However, supplementation of food products with insect ingredients (e.g., proteins) requires extensive research of their properties. A good understanding of the properties of proteins will allow specific application in food production according to the potential shown. Proper use of extracted proteins will make it possible to create the desired properties of food without altering the taste or smell of products that are well known to consumers.

Extracted proteins may also be part of the diet of individuals with increased protein requirements such as sportsmen. Importantly, insect protein contains a set of essential amino acids and high content of BCAA (branched-chain amino acids) [83]. Protein isolates can be an ingredient of nutrition supplements or protein shakes – a process already being carried out [154, 155].

5.3 Extracted Fat

Fat can be extracted from insects during flour production. This treatment prevents unsaturated fatty acids from exposure to undesirable oxidation processes; however, fat is not an unnecessary by-product. Oils are essential to the food industry for many uses, and oils from insects are valuable due to the composition of fatty acids. Many species of insects are high in unsaturated fatty acids including omega-3 fatty acids with a desirable omega-6 to omega-3 fatty acid (n6/n3) ratio [14, 156]. Oils from different species, and species fed on different diets, can have different fatty acids profiles. By changing insects' diet, oils with the desired composition of fatty acids can be received [11]. Oils obtained from

insects can be widely used in the food industry: from production of salad dressings or supplements of traditionally consumed dishes to attempts at replacement of fats used conventionally in food technology.

5.4 Chitin

Chitin seems to be a by-product of flour production, but, in fact, it is a high-value product. Primarily, chitin acts as a dietary fiber but this is not the only function. Chitin and chitosan (produced commercially by deacetylation of chitin) are natural products that are compatible with plant and animal tissues, biologically functional, biodegradable, nontoxic, and eco-friendly [7, 157]. Due to these properties, chitin and its derivatives have many application areas including wastewater treatment, pharmacy, medicine, cosmetics, weight loss, edible biofilm production, and reduction of low-density lipoprotein (LDL) cholesterol levels in the blood. The vast majority of chitin currently used for these applications comes from unsustainable harvesting of shrimp which, like much of the ocean's other resources, is constantly being overharvested [151]. Moreover, chitin has shown antioxidant, antimicrobial, and antitumor potential [7, 158, 159]. The surface morphology, acetylation degree, and molecular weight are the three main criteria determining the industrial use of chitin and its derivatives [7, 157].

5.5 Functional Properties of Insect Protein

Supplementation of food products with insect flour or extracted ingredients requires extensive knowledge of their properties. In the case of proteins, these properties include, among others, solubility, thermal stability, and techno-functional properties such as water and oil holding capacity, gelling, foaming, and emulsifying capacity. These properties determine the strict application of insect flour or protein isolates in food products. Furthermore, enzymatic modification of proteins is a useful mechanism to improve the functionality compared with the native unhydrolyzed proteins [152].

Good solubility of proteins is important in many uses, mainly for formation of emulsions, foams, and gels in designed food products. Zhao et al. [160] observed that the lowest protein solubility for proteins from *T. molitor* was reached around the isoelectric point and increased with either increasing or decreasing pH. Good solubility of insect protein in a wide pH range ensures varied use thereof in food industry – as a food additive in acid and alkaline food. Yi et al. [3] investigated the techno-functional properties of proteins from five insect species: *Tenebrio molitor* (larvae), *Zophobas morio* (larvae), *Alphitobius diaperinus* (larvae), *Acheta domesticus* (adult), and *Blaptica dubia* (adult). It was found that the investigated insect proteins had the ability to form gels depending on their concentration and on pH and could potentially be used as gelling agents or texturizers in food. Omotoso [153] studied the functional properties of *Cirina forda* (Lepidoptera: Saturniidae) – one of the most widely eaten insects in Southern Nigeria. The water holding capacity

of dried ground insect was 300%, whereas the water holding capacity values for silkworm (*Bombyx mori*) larvae and pupae were 175% and 115%, respectively. Acid-extracted protein fractions from five different insect species, including cricket (*Acheta domesticus*) protein or mealworm (*Tenebrio molitor*) protein, were shown to have poor or no foam capacity, over a range of pH [3]. In turn, crickets (*G. sigillatus*) heated at 50 °C for 30 min were found to have a FC of 100% with a FS of 90% after 1 h [152].

Hall et al. [152] demonstrated that controlled enzymatic hydrolysis of whole crickets successfully yielded protein hydrolyzates with improved protein functionality. Improved solubility of hydrolyzates makes them suitable for acidic food systems, such as nutritional beverages and sports drinks. The authors also observed better emulsifying and foaming properties for cricket protein hydrolyzates than the unhydrolyzed cricket protein.

These are only a few examples of the functional properties of insects. Due to the biodiversity of insects, these properties can vary considerably; therefore, it is important to investigate the properties of insect proteins before using them in food products.

6 Why Should We Look for New Sources of Nutrients?

It is estimated that around 7.6 billion people live in the world, and the urban population has been constantly increasing by approximately 1.84% per year between 2015 and 2020. To maintain proper health and functioning of the organism, it is necessary to have access to safe food rich in nutrients [161]. It is estimated that the human population in 2050 will amount to nine billion, and the demand for meat will increase by 76%. Therefore, the issues of food quantity and safety as well as the quality of food, feed, and fuel and environmental protection have become a priority for policy makers [162–164]. In order to achieve sustainable development and stability of food markets, the impact of all human activities on the environment should be reduced. Therefore, it is necessary to change and improve the technology of food production and the approach of consumers and food producers in a stepwise manner. The International Human Dimensions Programme identified food, water, and energy as the most important targets of quality change [165]. It should be noted that these main activities are mutually independent, since food production is dependent on freshwater resources and energy production [166]. Given the impact of animal production on the environment, e.g., soil erosion, deforestation, greenhouse gas emission, and water pollution, increasing production does not seem to be a good solution for the protein amount requirements in the future. Moreover, the prices of soybean and fishmeal that are used in feeding due to the demand are still increasing [17]. Therefore, we are looking for alternative sources of protein and nutrients. As edible insects are a diverse food and feed market, they can become an alternative source of protein, and the main reasons why the insects can be an alternative for lamb, beef, poultry, and pork are the economic, environmental,

and nutritional aspects. Nevertheless, the European Novel Food Regulation prohibits the production and processing of edible insects on a commercial scale for humans [164].

7 Environmental Impact of Entomophagy

Conversion of plant protein to animal protein is naturally inefficient (6 kg of vegetable protein is needed to obtain 1 kg of meat protein) and responsible for the negative influence on the environmental condition [167]. Furthermore, only 15% of protein and energy are applied as human food, and 85% are wasted. In 2000, 942 and 617 million tons of grains were used for food and feed, respectively [168]. It is worth noting that this is more than 500 tons of wasted food production and turned into polluting emissions by animal metabolism. The actual conversion efficiency of protein depends on the animal type and breed and the conditions of farming, such as climate and feed. Poultry and pigs are more productive species of animals than cows if they are grass-fed. Moreover, their compound stomachs are not adapted to feeding with maize. Furthermore, the use of water, amount of feeding stuff, and influence on the environment (water pollution or gas emission) depend on the type of animal protein production. For example, to produce 1 kg of beef, pork, and chicken meat, 15,400 l, 6000 l, and 3400 l of water, respectively, need to be used [169]. It should be noted that generally 1 kg of animal protein requires about 100 times more water than 1 kg of grain protein [167]. It should be noted that generally 1 kg of animal protein requires about 100 times more water than 1 kg of grain protein [167]. Since water is the main part of feed and occupies a particular place in food production, the low energy conversion efficiency, especially in the case of cattle, is obvious. In general, insects are characterized by a lower feed conversion rate (FCR), which is defined as the amount of feed required to grow 1 kg of conventional livestock animals, which corresponds to high feed efficiency. To produce 1 kg of beef, lamb, pork, and chicken meat, 7.7, 6.3, 3.6, and 2.2 kg of feed are required [170]. In comparison, 1.7 kg of feed is needed to obtain 1 kg of cricket meat [171]. Further, 2.2 kg of feed are used production for 1 kg of mealworm [172], 2.7 kg of feed for the Argentinean cockroach, 1.8 kg of feed for the black soldier fly, and 2.3 kg of feed for the house cricket [173]. This factor depends on the mode of feeding.

It should be noted that production of edible insects has a lower negative influence on the environment than breeding conventional livestock animals. Production of greenhouse gases (GHS), i.e., carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), is said to be an important cause of climate change. The latter two gases are considered as the key global warming factors [16]. According to the life cycle analysis (LCA) which takes into account all animal production, the contribution of the animal sector to global greenhouse gas emissions is 9% for CO_2 (on-farm energy, animal product processing expenditures, feed and animal transport, fertilizer production for feed crops, and land-use changes), 35–40% for CH_4 (enteric fermentation in ruminants and farm animal manure), and 65% for N_2O (farm manure and

urine) [174]. A relevant indicator of the environmental impact is emission of ammonia (NH_3) by animal livestock related to manure and urine production, which causes nitrification and acidification of soil [175]. The GHS production by five species of insects, the first three of which are considered edible: *Tenebrio molitor*, *Acheta domesticus*, *Locusta migratoria*, *Pachnoda marginata*, and *Blaptica dubia*, and comparison with the GSH production by livestock animals were the objects of an experimental study [16]. The comparison with GHS production by animal livestock is not easy since the differences in the size of the animals determine differences in the metabolic rate and, hence, CO_2 production. However, the CO_2 eq. (g/kg mass gain) production in the case of the aforementioned insect species was estimated at 7.58, 1.57, 17.72, 121.86, and 37.54, respectively. This factor is in the range of 79.59–1130 for pigs and 2850 CO_2 eq. (g/kg mass gain) for beef cattle [16]. Moreover, in the case of *Anabrus simplex* (Orthoptera, Tettigoniidae), a production rate of 37 g CO_2/kg BM/day was reported, while 40 g CO_2/kg BM/day were noted for the locust *Schistocerca americana* (Orthoptera, Acrididae) [176] and 94 g of CO_2/kg BM/day for adult *Tribolium castaneum* (Coleoptera, Tenebrionidae) [177]. It should be noted that CO_2 production is influenced by the species, temperature, feeding status, stage of development, and activity level [16, 178, 179]. As far as the CH_4 production is concerned, *Acheta domesticus* and *Locusta migratoria* did not produce this compound, and 4.96 CH_4 (g/kg mass gain) were produced by *Pachnoda marginata*, 0.16 CH_4 (g/kg mass gain) by *Tenebrio molitor*, and 1.46 CH_4 (g/kg mass gain) by *Blaptica dubia*. In comparison to pigs, which produced 1.92 CH_4 (g/kg mass gain) and beef cattle, 114 CH_4 (g/kg mass gain) [16], these are low values, and this suggests that edible insects have a lower negative influence on the environment than livestock animals. Similarly, lower values of NH_3 emission were recorded in the case of edible insects. There are many reports about NH_3 emission by livestock animals. For example, pigs emit 4.8–75 mg/kg BM/day [180–182], cattle 14–170 mg/kg BM/day [180, 183], and poultry 72–436 mg/kg BM/day [180, 184]. The emission of NH_3 in the case of insects was different, i.e., from 1.57 to 4.29 mg/kg BM/day for B. *dubia* and from 2.46 to 8.01 mg/kg BM/day for *A. domesticus* [16]. Furthermore, edible insects need less space than livestock animals; they are able to live in high densities (kg biomass per m^2) and exhibit low vulnerability to disease (high resistance). Besides, crickets can eat a wide range of organic and waste biological material, and the farm takes up little space (2000 insects/m^2) [185]. It has been reported that around three quarters of the world's agricultural crop is used for livestock production either directly or indirectly [186].

A high impact of entomophagy on the environment is exerted by the potential decrease in application of pesticides, as collecting edible insects from crops or wild plants can prevent pests and thus decrease the demand for insecticides. Insects can also be used to compost manure that is produced in huge amounts by animal livestock, especially by poultry, pigs, and beef cattle. This can be a way to prevent soil acidification [187]. Moreover, organic material is the diet of edible insects, and thus waste of food can be reduced [8]. The production of edible insects has great potential not only in terms of supply of protein and nutrient for humans but also as an economic aspect in the food sector.

8 Safety Aspect of Edible Insects as Food

One of the aspects of the lack of acceptance of edible insects by Western consumers is the safety of insect consumption. Although insects are a good source of protein, nutrients, and minerals, it should be kept in mind that not all insect are safe to eat.

Like other food ingredients derived from plants or animals, insects can cause allergies (Table 4). Three allergens, arginine kinase (AK), hemocyanin (HC), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), have been identified in the muscle of *Macrobrachium rosenbergii*. Moreover, a novel specific allergen

Table 4 Main allergens identified in animals

Allergen substance	Origin
Arginine kinase (AK)	Black tiger prawn (*Penaeus monodon*) [191]
	Pacific white shrimp (*Litopenaeus vannamei*) [192]
	Giant freshwater prawn (*Macrobrachium rosenbergii*) [193]
	Banana shrimp (*Penaeus merguiensis*) [194]
	Moth (*Plodia interpunctella*) [195]
	American cockroach (*Periplaneta americana*) [196]
	German cockroach (*Blattella germanica*) [197]
	Silkworm (*Bombyx mori*) [190]
	Locust (*Locusta migratoria manilensis*) [198]
	Myxobacteria (*Myxococcus xanthus*) [199]
Tropomyosin (TM)	Indian white prawn (*Penaeus indicus*) [200]
	Spiny lobster (*Panulirus stimpsoni*) [201]
	Brown shrimp (*Penaeus aztecus*) [202]
	Pacific white shrimp (*Litopenaeus vannamei*) [203]
	Black tiger prawn (*Penaeus monodon*) [204]
	Carpet clam (*Paphia textile*) [205]
	Lobster (*Panulirus stimpsoni*) [201]
	German cockroach (*Blattella germanica*) [206]
	American cockroach (*Periplaneta Americana*) [207]
	Silverfish (*Lepisma saccharina*) [208]
Glyceraldehyde 3-phosphate dehydrogenase	Banana shrimp (*Penaeus merguiensis*) [194]
	Giant freshwater prawn (*Macrobrachium rosenbergii*) [188]
	Sardine (*Sardinops sagax*) [209]
	Cricket (*Gryllus bimaculatus*) [188]
Hemocyanin	Giant freshwater prawn (*Macrobrachium rosenbergii*) [188, 210] Lanchester's freshwater prawn (*Macrobrachium lanchesteri*) [188]
	Cricket (*Gryllus bimaculatus*) [188]
Hexamerin1B	Cricket (*Gryllus bimaculatus*) [188]
	Firebrat (*Thermobia domestica*) [211]

hexamerin1B (HEX1B) was detected in *Gryllus bimaculatus* [188]. Allergenicity may depend on the stage of development. Pupae of the African silkworm (*Anaphe venata*) have thiaminase in their composition, which can cause thiamine deficiency [189], and arginine kinase has been identified in *B. mori* (silkworm) larvae [190]. Certain insects can produce toxins as a defense mechanism. Inset specimens collected in the wild may contain pesticides or other chemicals from the environment.

Insects also can directly or indirectly cause diseases. Food poisoning due to the content of aflatoxins and cases of botulism and parasitic diseases were noted after consumption of edible insects [212]. Edible insects can also be a source of zoonotic agents such as bacteria, fungi, and parasites, or vectors [213]. *Escherichia coli*, *Klebsiella aerogenes*, and *Staphylococcus* were identified in freshly collected palm grubs (*Rhynchophorus phoenicis*) from Nigeria [214]. Following the recent scientific findings, the European Food Safety Authority (EFSA) indicates that the possible microbiologic threat from insects accepted for consumption is comparable to other threats associated with animal protein. The biological and chemical hazard of insect-based food and feed products mainly depends on insects' diet as well as production and processing methods. Moreover, experts have demonstrated that the insect species used as a food ingredient is not the main risk, but the diet, handling, and storage of farmed insects can pose some threat. Insects cannot be considered as possible biological vectors or amplifiers of mammalian prions because they cannot replicate in the insect body, provided that no ruminant or human substrates are used as feed. Moreover, there are no data about accumulation of heavy metals by edible insects; therefore, this aspect is largely unknown. Utilization of postconsumer food wastes and organic sidestreams to feed insects, which is nowadays not allowed in the European Union, should be extensively studied in the future [60, 215].

The risk for health and safety of insects as food can be prevented by consuming insects from farms where insects are fed without pollution. It should be noted that storage conditions, preparation methods, and proper heat treatment decrease the risk for human health and the harmful dose-dependent effects of the compounds. Scientific findings about the benefits and risk factors related to consumption of edible insects are a valuable source of information for the EU Commission to consider the use of insects as feed and food in a uniform fashion within the EU and beyond.

9 European Union Policy on Insects as Food

It is known that insects are not traditional food in Europe. The law governing insects as food and feed is the European Novel Food Regulation (ENFR), which was first established in 1997 (EC 258/97), revised in 2011 (EC 1169/2011), and repealed and replaced in 2015 (EC 2015/2283). Within the European Union, insects have a status of "novel foods," which means "any food that was not used for human consumption to a significant degree within the Union before 15 May 1997 irrespective of the dates of accession of Member States to the Union" and which requires that a risk assessment be performed prior to marketing according to Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel

foods [216]. It shall apply from 1 January 2018. In this regulation, we can find short information about insects: "The scope of this Regulation should, in principle, remain the same as the scope of Regulation (EC) No 258/97. However, on the basis of scientific and technological developments that have occurred since 1997, it is appropriate to review, clarify and update the categories of food which constitute novel foods. Those categories should cover whole insects and their parts."

According to Regulation (EC) No 258/97, it was not totally clear that insects were novel food. Some national food agencies (Belgium, the Netherlands, and the UK) did not recognize that the original Novel Food Act from 1997 clearly specified this issue. Therefore, they accepted companies dealing with insects as food. In Belgium, FASFC (Federal Agency for the Safety of the Food Chain) has established a list of insects that are accepted as food if they are produced in the EU, if the whole insects are used, and if requirements for food safety have been respected [217].

Considering the standards of insect production for food, the European Council requested the European Food Safety Authority (EFSA) for a scientific opinion to assess the microbiological, chemical, and environmental risks arising from the production and consumption of insects as food and feed. In October 2015, EFSA issued a scientific opinion "Risk profile related to production and consumption of insects as food and feed" [60]. A list of 12 insect species that were reported to have the biggest potential to be used as food and feed in the EU was established, thereby indicating that the list should serve as guidance in the overall assessment and should not be considered definitive or exhaustive. In conclusion, EFSA recommended to initiate research on the few issues mentioned such as bacteria, viruses, allergens, prions, chemicals, processing, and environment [60].

10 Insect Business

The increase in the interest in edible insects can be seen on the basis of the number of emerging companies in Europe and the USA. These companies deal with the mass breeding of insects, optimization of this production, processing of insects, and production of feed and food products, most often snacks containing insects. These changes may revolutionize the food market but for some still remain incomprehensible and surprising. In choosing food as in many other areas of life, we often follow stereotypes. The reluctance to consume insects is most often attributed to negative associations, i.e., the mental barrier. Improving the rank of insects can help the catering industry, for example, by placing them in recipes and introducing them to restaurant menus. Emerging start-ups and blogs devoted to insect consumption are an important part of the process of overcoming barriers and stereotypes.

10.1 Insect Farming

Insect farming has the same characteristics as other animal production systems: insects need access to water and feed (substrate) to supply energy and nutrients for

growth and excrete intestinal content (frass). Little information is available about mass production of insects for feed and food. In European insect farms, insects are kept in a closed environment, in boxes/cages, where the atmosphere, substrate, water, etc., can be controlled. For assurance of economically profitable and sustainable insect production, an appropriate level of mechanization, cheap insect feed production, building design, and temperature-controlled rooms have to be adapted.

One of the main elements of insect production is feeding. Most insect species farmed for food or feed around the world are omnivores, but the productivity of farmed insects can be improved by providing a correctly balanced diet [90]. An integrated rearing system with mechanization, information, and automation can be applied competitively and effectively for large-scale insect farming. Smaller farms are not mechanized at all or only to a small extent. Climatic conditions such as temperature or humidity and rearing area are dependent on the species of insects. The advantage of insect farming is the use of a small area. To minimize the space needed to farm a maximum quantity of insects, rearing boxes can be held in multilevel shelves, which are filled with as many rearing boxes as possible to minimize the space usage per kilogram of insect produced [218].

Conventional mealworm production is based on trays to hold larvae during their development and adults, and the most common tray size used is a standard 65 L × 50 W × 15 H cm. Such a tray has sufficient depth to prevent larvae or adults from escaping. Crickets are grown in large 36–60 cm deep boxes filled with different materials, most commonly cardboard, such as egg cartons or packing dividers for shipping to increase surface space. Rearing boxes can be made of different materials, but smooth surfaces are preferred because these make it difficult for crickets to climb the walls [218].

Moreover, traceability of edible insects, as with all agricultural products, from farm to table is essential to maintain safety and trace any hazards or disease outbreaks to their source. Each new production batch of insects harvested must be properly catalogued in a way that can be traced to the raw material used to feed and water that batch [218].

In Europe there are several professional insect farms intended for consumption by animals and humans. “Micronutris” from France produces cricket *G. sigillatus* and mealworm *T. molitor*, of which they produce appetizers, pralines, biscuits, and pastas [219]. In turn, “Proti-Farm” (Netherlands) delivers high-quality insect ingredients of the buffalo (*Alphitobius diaperinus*) for the food and personal care and pharmaceutical industries under the EntoPure brand. Proti-Farm products are HACCP certified, and the factories are certified with FSCC22000, ISO14000, and ISO26000 [220]. Insects farmed for human consumption in the Netherlands (Bugs Organic Food) can be obtained from Ruig and sons at Bugs Originals [221]. A very popular insect in the USA is the house cricket *A. domesticus* produced by “Cowboy Cricket Farms.” They sell three cricket products: chocolate chirp cookie, cricket powder, and cricket flour [222]. “AgriProtein,” operating worldwide, produces two products (a natural protein meal and oil) which can be used as a growth facilitator in agricultural feed preparations. Some of the by-products of their larvae can also be used and sold as a soil. And they also sell the dried larvae directly as by the pet food industry. Protix has

developed a broad range of insect-derived products such as insect protein meal, purified insect oils and lipids, chitin and chitin derivatives, and insect fertilizer pellets [223]. In turn, "Open Bug Farm," a project created in the USA, establishes a new branch of agriculture involved in insect breeding. This is an innovation platform to stimulate interaction between farmers, researchers, and hobbyists who want to change the world with edible insects [224].

10.2 Insect-Based Companies

It is said that the consumption of insects in Europe is new; however, analysis of the number of entrepreneurs dealing with this topic reveals that the issue is highly absorbing. The best example is the list of entrepreneurs around the world that was created for the first edition of World Edible Insect Day (the 23rd of October 2015) by Bugburger. It includes companies, organizations, and individuals who are working on an edible-insect product, planning to deliver one, or just advocating the benefits of eating insects [225]. At present, this list contains 180 items from Europe and the USA, but it is probably incomplete. The list comprises such categories as insect products, insect restaurants, insects as animal feed, online stores, professional insect farmers, and research projects.

Sweets are a very popular category of insect products. The species mostly used are crickets and mealworms. Examples of popular bars are "Kriket" from Belgium, "SWARM Protein" from Germany, "Jimini's" from France, "Crickstart" from the USA, "Jungle Bar" from Iceland, and "Yumpa Bar" and "Sens" from the UK. Attempts are made to create protein shakes and nutrition supplements from insects. In turn, Hotlix, who started their business in 1970, are the pioneers of "insect candy." They sell classic lollipops with worms, dried grasshoppers, etc. They have relied on the scary/yuck effect of insects. Newly established companies have a very serious approach to insect snacks [225]. The assortment is enhanced with pralines, muesli, cookies, and crispy insects [226]. However, insect flour is the most popular product because it is relatively easy to produce, and it may be the basis for many products such as cookies ("Crickelle" the cricket crackers) [227], bread, and pasta ("Aldento") [228]. Some European shops offer burgers and meatballs made from mealworms, for example, supermarket chain "Coop" in Switzerland [229]. Most of the products are sold online in shops such as "Delibugs" (Netherlands) [230], "Insectes Comestibles" (France) [231], and "Minifood" (Belgium) [232].

Insect products are not only designed for humans but also for animals. "Wilder Harrier" offers dog food with added cricket protein [233]; "Conscientious Cat" proposed insect-based cat food [234]. Several companies see the potential in feeding livestock, fish, pigs, and poultry with insects instead of other protein sources: "Protix" (Netherlands), "Ynsect" (France), "Entomo Farm" (France), "Diptera" (Italy), or "HiProMine" (Poland). It is not possible to mention all the companies involved in this business due to their large number, but research projects should be highlighted as well. The main research center in Europe that conducts extensive

research on insects is the Wageningen University [235]. On their web page, there is a list of all edible insects in the world [58].

European and global industry organizations have also been organized. The International Platform of Insects for Food and Feed (IPIFF) "is the voice of the insect sector towards the European Union. IPIFF is an EU non-profit organization which represents the interests of the insect production sector towards EU policy makers, European stakeholders, and citizens." They promote insects as a source of animal proteins for both human consumption and animal feed. Members include Ynsect (France), Protix (Netherlands), Hermetia (Germany), Koppert (Netherlands), Proti-Farm (Netherlands), HiProMine (Poland), Micronutris (France), Jimini's (France), Entomo Farm (France), nextProtein, Andromeda, MealFood Europe, and NextAlim with 26 associated members [236]. In turn, "the ASEAN Food and Feed Insects Association – AFFIA – is a voluntary group of companies, institutions, and natural persons which promotes and supports activities that relate to the use of insects as food and as feed" [237]. Moreover, a journal devoted to edible insects was established in 2015. "Journal of Insects as Food and Feed" is an online journal issued four times a year by Wageningen Academic Publishers. The journal aims to cover the whole chain of insect collecting or rearing to marketing edible insect products [238].

11 Insects as a Source of Nutrients for Animals

The increased production of livestock, poultry, and fish is forced by the continuously growing human population and the decrease in areas available for agricultural production [17]. The increasing intensity of farming animals requires higher amounts of feed with high-value nutrients to cover their nutritional requirements [31, 63, 85, 239]. The nutritive needs of monogastric species include a high quality and quantity of protein in the diet, provided proteins should have an adequate amino acid profile, high digestibility, good palatability, and no anti-nutritional factors [240]. Nowadays, the most useful protein sources in livestock feeding are fishmeal and soy meal [241]. The European Union currently imports 40 million tons of crop proteins each year (mainly soya). The profitability of the EU livestock and aquaculture sectors is at risk, taking into account the fluctuations in soybean meal prices as well as the prices of fishmeal, which have risen fourfold over the last decade [242, 243]. These economic issues underscore the strategic importance of developing an alternative protein source involving new protein sources in animal feed [244]. Insects might be a sustainable solution answering the urgent need for an alternative source of feed compounds: fishmeal, fish oils, and soymeal [185, 245].

The farming of insect intended for animal feed has been the subject of studies for years [246–248]; however, insects have not yet become a replacement for traditional forage based on plant material [168] Both scientists and feed industrial sectors have begun to reconsider the use of insects as feedstuff for farming animals [249] given the economic, environmental, and nutritional aspects of entomophagy [17, 66, 85, 250–252]. Moreover, insects can be reared on organic waste, and they exhibit

favorable feed conversion efficiency [85, 253]. Therefore, it seems justified to consider inclusion of insect proteins in commercial feed manufacturing. It is important to elaborate intensive farming systems with selected insect species that have a short life cycle, can be easily reared, are highly nutritious, and provide high concentrations of proteins [239]. The use of insects as a diet compound is justified by the fact that in nature, most insectivores (e.g., fish and birds) prey on a variety of arthropod species [85]. The natural behavior of chicks is to pick up and eat insects from the ground, which indicates that poultry are evolutionarily adapted to be insectivores [254].

Among the millions of insect species living on earth, over 2000 are considered as edible for humans, and only several species have the potential to be a new source of nutrients for farmed animals [58, 255]. Currently, only few species of insects are massively reared for food and feed, i.e., yellow mealworm (*T. molitor*), domestic cricket (*A. domestica*), black soldier fly (*H. illucens*), the domestic house fly (*M. domestica*), locusts (*L. migratoria*, *S. gregaria*, *Oxya* sp.), and silkworms (*B. mori*) [185, 253, 256]. However, as suggested by van Huis et al. [256], when insects are considered as feed for poultry, aquaculture, and pigs, they have to be reared on a mass scale with stable quality. This can be achieved only by automated rearing facilities with complete optimization and synchronization of the production processes adjusted to the insect species that will be reared.

Regulation of European Commission EC 999/2001 and consecutive EU regulations on processed animal protein (PAP) led to categorization of insects as ingredients that cannot be present in the food chain or in animal feed [257]. Afterward, Regulation EC 56/2013 opened a discussion on the use of PAP obtained from non-ruminants in feed for nonruminants, which might be considered under strict conditions. In turn, as specified by Regulation EC 1069/2009, invertebrates are classified as "fit but not intended for human food chains" (category 3 materials). The use of processed animal proteins (including insects) was reauthorized for the aquaculture sector in June 2013 and is expected to be included in poultry and pig feeding in the near future [258]. Several feed companies have committed themselves to include insects and insect compounds in the forage for livestock as soon as the EU legislation allows doing so [259]. Moreover, a study conducted by Verbeke et al. [45] shows that farmers, agriculture sector stakeholders, and citizens express a favorable attitude toward the use of insects in animal feed, especially for fish and poultry. In contrast to Europe, poultry in West Africa is already fed with termites collected in the wild [260].

12 Promising Opportunities of the Use of Insects

As mentioned above, the list of positive results of the consumption of insects is long. Nevertheless, besides being food and feed, insects have other uses. Firstly, they can be used in food-waste recycling – insects have favorable feed conversion efficiency; waste sidestreams or other ingredients that cannot be used for feeding traditional livestock species can be utilized as a source of feed [173, 257, 261–264]. The most promising in this respect is the black soldier fly (*Hermetia illucens*). The use of

Hermetia for waste reduction has been extensively studied since the 1970s [265]. The life cycle of this species is linked with organic waste matter. Its larval stage can be reared on a wide range of decaying materials such as rotting fruits and vegetables, distiller grains, coffee bean pulp, fish offal, and especially on animal manure and human excreta [17, 67, 266, 267]. This species has a capability of rapid food processing – it is estimated that its food intake ranges from 25 to 500 mg of fresh matter per larva per day [67]. Moreover, the study carried out by Barroso et al. [268] showed that *Hermetia* meal (to be used as a livestock forage compound) could be easily modified by dietary manipulation of larval feed.

Secondly, insects may be used for producing oils. Oil extracted from black soldier fly larvae is a by-product of insect meal production, which makes them more useful than the source of proteins. *Hermetia*-derived oil has a good fatty acid profile, which can be optimal for feed purposes and for producing high-quality biodiesel [269]. Moreover, Zeng et al. [269] claims that the oil extracted from black soldier fly larvae meets most of the requirements set by the international standard (EN 14214) for biodiesel.

Thirdly, every biomaterial left with waste from insect farming can be used as a fertilizer. Such a biofertilizer contains insect frass with addition of any matter that falls on the floor of the rearing cage, e.g., wings, body parts, leftovers, dust, or shredded egg carton. The use of cricket fertilizer was assumed to substitute the application of mineral fertilizers on key crops in Thailand (e.g., rice paddies) [270].

Fourthly, insects have a mechanism of induced detoxification of plant material with toxic compounds. This allows herbivorous insects to catabolize secondary plant compounds before they are absorbed [271, 272]. However, a study by van Broekhoven et al. [90] showed that longtime exposure of *Z. atratus* to diet with high content of potato steam peelings caused high mortality of larvae. It should be taken into account that chronic toxicity may result in reduced growth and lower feed conversion efficiency [273, 274].

Finally, insects are able to process polystyrene. A study conducted by Yang et al. [275] confirmed that mealworms (*T. molitor*) are the very first species of insects that is capable to degrade and mineralize petroleum-based plastic polystyrene. Moreover, Yang et al. [276] reported that the gut of the mealworm should be considered as an efficient bioreactor, where the gut microbiota (especially *Exiguobacterium* sp. strain YT2) plays the key role in Styrofoam degradation.

13 Conclusions

Insects are a good source of many nutrients, although the contents of most nutrients vary widely depending on a few factors such as the insect species [10], gender [277], stage of development [68], or diet [127]. In general, most species appear to be good sources of proteins with essential amino acids, fatty acids (omega-3), most minerals, and most vitamins B [10, 14, 113]. Moreover, insects are a source of biologically active substances such as antimicrobial and antifungal peptides [75, 77, 116] and angiotensin-converting enzyme (ACE) inhibitory peptides [73], and antioxidant peptides [12, 13]. In

addition to the high nutritional value and bioactive ingredients, insects are environmentally friendly. Low greenhouse gas emissions and low drinking water and biomass consumption are just some of the factors in favor of the insect breeding [16, 172]. Furthermore, insects can be helpful in the management of by-products of the food industry [173, 187], and their feces can be used as a fertilizer [270].

Despite the many positive sides of introducing insects to our diet, there are a number of issues that must be met to make this happen. First of all, consumers need to be convinced to eat insects. They should want to do it voluntarily, being certain of their own beliefs. It is important to raise awareness about the dangers associated with the constantly growing population which is related to, among other things, food security and environmental pollution. The food industry can help by proposing products containing insects in the form acceptable to consumers. Dishes with invisible insects, e.g., only protein isolates, are a good way to expand our menu. Research on the functional properties of the insects or components isolated from their organisms [3, 152, 153] is a good start to gain detailed knowledge of insects as food ingredients. There is a need for further analysis, given the multitude of edible insect species. A better understanding of each aspect of insect consumption brings us closer to accepting them on a par with other sources of protein. At the same time, mass insect breeding systems should be developed by automating the production and optimizing the breeding parameters. In addition, veterinary control should be applied. Currently, insects cost more than they should because in Europe they are still sold as novelties. The way insects are bred certainly has an impact on their high price. Improving the farming system will probably be a factor in lowering production costs. The combination of all the above factors will help the edible insects to gain a better reputation among unconvinced consumers. However, in order to exploit the potential of insects fully, they should become an increasing proportion of our diet partially replacing other sources of protein such as pork, beef, and poultry.

For many years, the agricultural sector has been moving in the same direction – maximization of plant and animal production. The use of insects as food and feed will undoubtedly be a revolution in this sector and will change the direction of further development of agriculture.

Entomophagy becomes an object of interest of an increasing number of researchers, and its promotion through the media is still increasing. Although EU legislation is cautious about the consumption of insects, the number of companies dealing with edible insects has exponentially grown over the past few years. Start-ups are successful on the food market and their popularity exceeds expectations. This proves that perhaps insects will soon be acceptable and produced as feed and food on a large scale.

References

1. Evans J, Alemu MH, Flore R et al (2015) 'Entomophagy': an evolving terminology in need of review. J Insects Food Feed 1(4):293–305
2. Verbeke W, Spranghers T, De Clercq P, De Smet S, Sas B, Eeckhout M (2015) Insects in animal feed: acceptance and its determinants among farmers, agriculture sector stakeholders and citizens. Anim Feed Sci Technol 204:72–87

3. Yi L, Lakemond CM, Sagis LM, Eisner-Schadler V, van Huis A, van Boekel MA (2013) Extraction and characterisation of protein fractions from five insect species. Food Chem 141(4):3341–3348
4. Bußler S, Rumpold BA, Fröhling A, Jander E, Rawel HM, Schlüter OK (2016) Cold atmospheric pressure plasma processing of insect flour from *Tenebrio molitor*: impact on microbial load and quality attributes in comparison to dry heat treatment. Innov Food Sci Emerg 36:277–286
5. Kim HW, Setyabrata D, Lee YJ, Jones OG, Kim YHB (2016) Pre-treated mealworm larvae and silkworm pupae as a novel protein ingredient in emulsion sausages. Innov Food Sci Emerg 38:116–123
6. Raksakantong P, Meeso N, Kubola J, Siriamornpun S (2010) Fatty acids and proximate composition of eight Thai edible terricolous insects. Food Res Int 43(1):350–355
7. Kaya M, Erdogan S, Mol A, Baran T (2015) Comparison of chitin structures isolated from seven Orthoptera species. Int J Biol Macromol 72:797–805
8. Ramos-Elorduy J (2009) Anthropo-entomophagy: cultures, evolution and sustainability. Entomol Res 39:271–288
9. Shelomi M (2015) Why we still don't eat insects: assessing entomophagy promotion through a diffusion of innovations framework. Trends Food Sci Technol 45(2):311–318
10. Ramos-Elorduy J, Moreno JMP, Camacho VHM (2012) Could grasshoppers be a nutritive meal? Food Nutr Sci 3:164–175
11. Tzompa-Sosa DA, Yi L, van Valenberg HJ, van Boekel MA, Lakemond CM (2014) Insect lipid profile: aqueous versus organic solvent-based extraction methods. Food Res Int 62:1087–1094
12. Zielińska E, Baraniak B, Karaś M (2017) Antioxidant and anti-inflammatory activities of hydrolysates and peptide fractions obtained by enzymatic hydrolysis of selected heat-treated edible insects. Forum Nutr 9(9):970
13. Zielińska E, Karaś M, Jakubczyk A (2017) Antioxidant activity of predigested protein obtained from a range of farmed edible insects. Int J Food Sci Technol 52:306–312
14. Zielińska E, Baraniak B, Karaś M, Rybczyńska K, Jakubczyk A (2015) Selected species of edible insects as a source of nutrient composition. Food Res Int 77:460–466
15. Finke MD (2012) Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol 21:269–285
16. Oonincx DG, van Itterbeeck J, Heetkamp MJ, van den Brand H, van Loon JJ, van Huis A (2010) An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. PLoS One 5(12):e14445
17. van Huis A (2013) Potential of insects as food and feed in assuring food security. Annu Rev Entomol 58:563–583
18. van Huis A, van Itterbeeck J, Klunder H et al (2013) Edible insects: future prospects for food and feed security. FAO, Rome
19. DeFoliart G (1999) Insects as food: why the Western attitude is important. Annu Rev Entomol 44:21–50
20. van Huis A (2003) Insects as food in sub-Saharan Africa. Insect Scis Appl 23:63–85
21. Thompson DW (1907) The history of animals – Aristotle. John Bell, London
22. Anonymous (2001) The holy bible. English standard version. Crossway Bibles, Wheaton
23. Bodenheimer FS (1951) Insects as human food; a chapter of the ecology of man. Dr. W. Junk Publishers, Hague
24. Holt V (1885) Why not eat insects? Pryor Publications, Whitstable
25. Gulick CB (1927) Athenaeus: the Deipnosophists, vol 1. Loeb Classical Library, Harvard University Press, UK
26. Amar Z (2003) The eating of locusts in Jewish tradition after the Talmudic period. Torah U Madda J 11:186–202
27. El-Mallakh OS, El-Mallakh RS (1994) Insects of the Qur'an (Koran). Am Entomol 40:82–84
28. Shizhen L (1596) The compendium of Materia Medica. Shunyo-Do Shoten, Tokyo

29. Seabrooksa L, Hu L (2017) Insects: an underrepresented resource for the discovery of biologically active natural products. Acta Pharm Sin B 7(4):409–426
30. Hu P, Zha LS (2009) Records of edible insects from China. Agr Sci Tech 10:114–118
31. DeFoliart G (1992) Insects as human food: gene DeFoliart discusses some nutritional and economic aspects. Crop Prot 11(95):395–399
32. Menzel P, D'Aluisio F (1998) Man eating bugs: the art and science of eating insects. Random House, New York
33. van Huis A, van Gurp H, Dicke M (2014) The insect cookbook: food for a sustainable planet. Columbia University Press, New York
34. Fessler DMT, Navarette CD (2003) Meat is good to taboo: dietary proscriptions as a product of the interaction of psychological mechanisms and social processes. J Cogn Cult 3(1):1–40
35. Looy H, Dunkel FV, Wood JR (2014) How then shall we eat? Insect-eating attitudes and sustainable foodways. Agr Human Val 31(1):131–141
36. Schlup Y, Brunner T (2017) Prospects for insects as food in Switzerland: a tobit regression. Food Qual Pref 64:37–46
37. Marberg A, van Kranenburg H, Korzilius H (2017) The big bug: the legitimation of the edible insect sector in the Netherlands. Food Policy 71:111–123
38. Lensvelt E, Steenbekkers L (2014) Exploring consumer acceptance of entomophagy: a survey and experiment in Australia and the Netherlands. Ecol Food Nutr 53(5):543–561
39. Caparros Megido R, Sablon L, Geuens M et al (2014) Edible insects acceptance by Belgian consumers: promising attitude for entomophagy development. J Sens Stud 29(1):14–20
40. Halloran A, Muenke C, Vantomme P, van Huis A (2014) Insects in the human food chain: global status and opportunities. Food Chain 4(2):103–119
41. Verkerk MC, Tramper J, van Trijp JCM, Martens DE (2007) Insect cells for human food. Biotechnol Adv 25(2):198–202
42. Schösler H, de Boer J, Boersema JJ (2012) Can we cut out the meat of the dish? Constructing consumer-oriented pathways towards meat substitution. Appetite 58(1):39–47
43. Vanhonacker F, van Loo EJ, Gellynck X, Verbeke W (2013) Flemish consumer attitudes towards more sustainable food choices. Appetite 62:7–16
44. Vogel G (2010) For more protein, filet of cricket. Science 327:811
45. Verbeke W (2015) Profiling consumers who are ready to adopt insects as a meat substitute in a western society. Food Qual Pref 39:147–155
46. Hartmann C, Shi J, Giusto A, Siegrist M (2015) The psychology of eating insects: a cross-cultural comparison between Germany and China. Food Qual Pref 44:148–156
47. Tan HSG, van den Berg E, Stieger M (2016) The influence of product preparation, familiarity and individual traits on the consumer acceptance of insects as food. Food Qual Pref 52:222–231
48. Shan H, Tan G, Fischer ARH et al (2015) Insects as food: exploring cultural exposure and individual experience as determinants of acceptance. Food Qual Pref 42:78–89
49. Sogari G, Menozzi D, Mora C (2017) Exploring young foodies' knowledge and attitude regarding entomophagy: a qualitative study in Italy. Int J Gastr Food Sci 7:16–19
50. Gere A, Székely G, Kovács S et al (2017) Readiness to adopt insects in Hungary: a case study. Food Qual Pref 59:81–86
51. Verneau F, La Barbera F, Kolle S et al (2016) The effect of communication and implicit associations on consuming insects: an experiment in Denmark and Italy. Appetite 106:30–36
52. Tan HSG, Tibboel CJ, Stieger M (2017) Why do unusual novel foods like insects lack sensory appeal? Investigating the underlying sensory perceptions. Food Qual Pref 60:48–58
53. Looy H, Wood JR (2006) Attitudes toward invertebrates: are educational "bug banquets" effective? J Environ Educ 37(2):37–48
54. Hartmann C, Siegrist M (2016) Becoming an insectivore: results of an experiment. Food Qual Pref 51:118e122
55. Online Etymological Dictionary. Available at http://www.etymonline.com/word/insect. Accessed 1 Oct 2017

56. Delong DM (1960) Man in a world of insects. Ohio J Sci 60(4):193–206
57. Dossey AT (2010) Insects and their chemical weaponry: new potential for drug discovery. Nat Prod Rep 27:1737–1757
58. Jongenema Y (2017) List of edible insects of the world. Wageningen University, Wageningen. http://www.wur.nl/en/Expertise-Services/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm. Accessed Oct 2015
59. DeFoliart GR (2003) Food, insects as. In: Resh VH, Cardi RT (eds) Encyclopedia of insects. Academic, Cambridge, UK
60. EFSA Scientific Committee (2015) Scientific opinion on a risk profile related to production and consumption of insects as food and feed. EFSA J 13(10):4257
61. Shockley M, Dossey AT (2014) Insects for human consumption. In: Morales-Ramos JA, Rojas MG, Shapiro-Ilan DI (eds) Mass production of beneficial organisms. Academic, Cambridge, UK
62. Newton L, Sheppard C, Watson DW, Burtle G (2005) Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. North Carolina State University, North Carolina
63. Józefiak D, Józefiak A, Kierończyk B, Rawski M, Świątkiewicz S, Długosz J, Engberg RM (2016) Insects–a natural nutrient source for poultry–a review. Ann Anim Sci 16(2):297–313
64. Magalhães R, Sánchez-López A, Leal RS, Martínez-Llorens S, Oliva-Teles A, Peres H (2017) Black soldier fly (*Hermetia illucens*) pre-pupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). Aquaculture 476:79–85
65. De Marco M, Martínez S, Hernandez F et al (2015) Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. Anim Feed Sci Technol 209:211–218
66. Salomone R, Saija G, Mondello G, Giannetto A, Fasulo S, Savastano D (2017) Environmental impact of food waste bioconversion by insects: application of life cycle assessment to process using *Hermetia illucens*. J Clean Prod 140:890–905
67. Diener S, Zurbrügg C, Tockner K (2009) Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Manag Res 27(6):603–610
68. Finke MD (2002) Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol 21:269–285
69. Li L, Xie B, Dong C, Hu D et al (2015) Rearing *Tenebrio molitor* L. (Coleptera: Tenebrionidae) in the "Lunar Palace 1" during a 105-day multi-crew closed integrative BLSS experiment. Life Sci Space Res 7:9–14
70. Rumpold BA, Schlüter OK (2013) Nutritional composition and safety aspects of edible insects. Mol Nutr Food Res 57(5):802–823
71. https://inhabitat.com/livin-farms-makes-growing-sustainable-and-healthy-protein-as-easy-as-compost/livin-farms-edible-insects-2/. Accessed 10 Oct 2017
72. Jia J, Wu Q, Yan H, Gui Z (2015) Purification and molecular docking study of a novel angiotensin-I converting enzyme (ACE) inhibitory peptide from alcalase hydrolysate of ultrasonic-pretreated silkworm pupa (*Bombyx mori*) protein. Process Biochem 50(5):876–883
73. Wang W, Shen S, Chen Q et al (2008) Hydrolyzates of silkworm pupae (*Bombyx mori*) protein is a new source of angiotensin I-converting enzyme inhibitory peptides (ACEIP). Curr Pharm Biotechnol 9(4):307–314
74. Bulet P, Hetru C, Dimarcq JL, Hoffmann D (1999) Antimicrobial peptides in insects; structure and function. Dev Comp Immunol 23(4):329–344
75. Cytryńska M, Mak P, Zdybicka-Barabas A, Suder P, Jakubowicz T (2007) Purification and characterization of eight peptides from *Galleria mellonella* immune hemolymph. Peptides 28(3):533–546
76. Ponnuvel KM, Koundinya PR, Sinha RK, Kamble CK (2007) Immune mechanism in *Bombyx mori* L. against microbial pathogens. Indian Silk 46:9–11

77. Mak P, Zdybicka-Barabas A, Cytryńska M (2010) A different repertoire of *Galleria mellonella* antimicrobial peptides in larvae challenged with bacteria and fungi. Dev Comp Immunol 34(10):1129–1136
78. http://entnemdept.ufl.edu/creatures/misc/crickets/gsigilla.html. Accessed 1 Oct 2017
79. https://inhabitat.com/mansour-ourasanah-designs-a-vessel-for-farming-edible-insects-at-home/. Accessed 10 Oct 2017
80. Payne CL, Scarborough P, Rayner M, Nonaka K (2016) A systematic review of nutrient composition data available for twelve commercially available edible insects, and comparison with reference values. Trends Food Sci Technol 47:69–77
81. Jonas-Levi A, Martinez JJI (2017) The high level of protein content reported in insects for food and feed is overestimated. J Food Compost Anal 62:184–188
82. Xiaoming C, Ying F, Hong Z et al (2010) Review of the nuritive value of edible insects. In: Durst PB, Johnson DV, Leslie RL, Shono K (eds) Forest insects as food: humans bite back, proceedings of a workshop on Asia-Pacific resources and their potential for development. FAO, Bangkok
83. Nowak V, Persijn D, Rittenschober D, Charrondiere UR (2016) Review of food composition data for edible insects. Food Chem 193:39–46
84. Rothman JM, Raubenheimer D, Bryer MA, Takahashi M, Gilbert CC (2014) Nutritional contributions of insects to primate diets: implications for primate evolution. J Hum Evol 71:59–69
85. Anankware PJ, Fening KO, Osekre E, Obeng-Ofori D (2015) Insects as food and feed: a review. Int J Agric Res Rev 3(1):143–151
86. Finke MD, Oonincx DGAB (2014) Insects as food for insectivores. In: Morales-Ramos J, Rojas G, Shapiro-Ilan DI (eds) Mass production of beneficial organisms: invertebrates and entomopathogens. Elsevier, New York
87. Ramos-Elorduy J, Moreno JMP, Prado EE et al (1997) Nutritional value of edible insects from the state of Oaxaca, Mexico. J Food Compost Anal 10:142–157
88. Ramos-Elorduy J, Pino MJM, Correa SC (1998) Edible insects of the state of Mexico and determination of their nutritive values. An Inst Biol Univ Nac Auton Mex Ser Zool 69:65–104
89. Williams JP, Williams JR, Kirabo A et al (2016) Nutrient content and health benefits of insects. In: Dossey AT, Morales-Ramos JA, Guadalupe Rojas M (eds) Insects as sustainable food ingredients: production, processing and food applications. Academic, Cambridge, UK
90. van Broekhoven S, Oonincx DGAB, van Huis A, van Loon JJ (2015) Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. J Insect Physiol 73:1–10
91. Bednářová M, Borkovcová M, Mlček J, Rop O, Zeman L (2013) Edible insects-species suitable for entomophagy under condition of Czech Republic. Acta Univ Agric Silvic Mendel Brun 61(3):587–593
92. Adámková A, Kouřimská L, Borkovcová M et al (2016) Nutritional values of edible Coleoptera (*Tenebrio molitor*, *Zophobas morio* and *Alphitobius diaperinus*) reared in the Czech Republic. Potravinarstvo 10(1):663–671
93. USDA National Nutrient Database. http://www.nal.usda.gov/fnic/foodcomp/search/. Accessed 5 Oct 2017
94. Ofuya ZM, Akhidue V (2005) The role of pulses in human nutrition: a review. J Appl Sci Environ Manag 9(3):99–104
95. Adámková A, Mlček J, Kouřimská L et al (2017) Nutritional potential of selected insect species reared on the island of sumatra. Int J Environ Res Public Health 14(5):521
96. Mota C, Santos M, Mauro R et al (2016) Protein content and amino acids profile of pseudocereals. Food Chem 193:55–61
97. Finke MD, Defoliart G, Benevenga NJ (1989) Use of a four parameter logistic model to evaluate the quality of the protein from three insect species when fed to rats. J Nutr 119:864–871

98. Day L (2013) Proteins from land plants–potential resources for human nutrition and food security. Trends Food Sci Tech 32(1):25–42
99. Jansen GR (1968) Amino-acid supple mentation and the world food problem. In evaluation of novel protein products. In: Bender AE, Kihlberg R, Löfkvist B, Munck L (eds) Evaluation of novel protein products. Pergamon Press, Stockholm
100. Kamau EH, Serrem CA, Wamunga FW (2017) Rat bioassay for evaluation of protein quality of soy-fortified complementary foods. J Food Res 6(6):35
101. Bednářová M, Borkovcová M, Komprda T (2014) Purine derivate content and amino acid profile in larval stages of three edible insects. J Sci Food Agr 94(1):71–76
102. Ghosh S, Lee SM, Jung C, Meyer-Rochow VB (2017) Nutritional composition of five commercial edible insects in South Korea. J Asia Pac Entomol 20(2):686–694
103. Mba ARF, Kansci G, Viau M, Hafnaoui N, Meynier A, Demmano G, Genot C (2017) Lipid and amino acid profiles support the potential of *Rhynchophorus phoenicis* larvae for human nutrition. J Food Compos Anal 60:64–73
104. Bukkens GF (2005) Insects in the human diet: nutritional aspects. In: Paoletti MG (ed) Ecological implications of minilivestock: potential of insects, rodents, frogs and snails. Taylor & Francis, Oxford
105. Kouřimská L, Adámková A (2016) Nutritional and sensory quality of edible insects. NFS J 4:22–26
106. http://who.int/nutrition/topics/FFA_interim_recommendations/en/. Accessed 27 Sept 2017
107. De Foliart GR (1991) Insect fatty acids: similar to those or poultry and fish in their degree of unsaturation but higher in the polyunsaturates. Food Insects Newsl 4:1–4
108. Komprda T, Zorníková G, Rozíková V, Borkovcová M, Przywarová A (2013) The effect of dietary Salvia hispanica seed on the content of n-3 long-chain polyunsaturated fatty acids in tissues of selected animal species, including edible insects. J Food Compos Anal 32(1):36–43
109. Paul D, Dey S (2014) Essential amino acids, lipid profile and fat-soluble vitamins of the edible silkworm *Bombyx mori* (Lepidoptera: Bombycidae). Int J Trop Insect Sci 34:239–247
110. Ravzanaadii N, Kim SH, Choi WH et al (2012) Nutritional value of mealworm, *Tenebrio molitor* as food source. Int J Indust Entomol 25(1):93–98
111. Finke MD (2007) Estimate of chitin in raw whole insects. Zoo Biol 26(2):105–115
112. Lease HM, Wolf BO (2010) Exoskeletal chitin scales isometrically with body size in terrestrial insects. J Morphol 271(6):759–768
113. Finke MD (2015) Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. Zoo Biol 34(6):554–564
114. Kramer KJ, Hopkins TL, Schaefer J (1995) Applications of solids NMR to the analysis of insect sclerotized structures. Insect Biochem Mol Biol 25(10):1067–1080
115. Paoletti MG, Norberto L, Damini R, Musumeci S (2007) Human gastric juice contains chitinase that can degrade chitin. Ann Nutr Metab 51:244–251
116. Mlcek J, Borkovcova M, Rop O, Bednarova M (2014) Biologically active substances of edible insects and their use in agriculture, veterinary and human medicine. J Cent Eur Agric 15(4):225–237
117. Chen X, Feng Y, Chen Z (2009) Common edible insects and their utilization in China. Entomol Res 39:299–303
118. Goodman WG (1989) Chitin: a magic bullet? Food Insects Newsl 3:6–7
119. Belluco S, Losasso C, Maggioletti M (2013) Edible insects in a food safety and nutritional perspective: a critical review. Compr Rev Food Sci Food Saf 12:296–313
120. Bauserman M, Lokangaka A, Gado J (2015) A cluster-randomized trial determining the efficacy of caterpillar cereal as a locally available and sustainable complementary food to prevent stunting and anaemia. Public Health Nutr 18:1785–1792
121. Christensen DL, Orech FO, Mungai MN et al (2006) Entomophagy among the Luo of Kenya: a potential mineral source? Int J Food Sci Nutr 57:198–203
122. van Huis A (2017) New sources of animal proteins: edible insects. In: Purslow PP (ed) New aspects of meat quality: from genes to ethics. Woodhead Publishing, Cambridge, UK

123. La'Toya VL, Toddes BD, Wyre NR (2017) Effects of various diets on the calcium and phosphorus composition of mealworms (*Tenebrio molitor* larvae) and superworms (*Zophobas morio* larvae). Am J Vet Res 78(2):178–185
124. Oonincx DGAB, Dierenfeld ES (2012) An investigation into the chemical composition of alternative invertebrate prey. Zoo Biol 31(1):40–54
125. Ramos-Elorduy J (2005) Insects: a hopeful food source. In: Paoletti MG (ed) Ecological implications of minilivestock: potential of insects, rodents, frogs and snails. Taylor & Francis, Oxford
126. Wakayama EJ, Dillwith JW, Howard RW et al (1984) Vitamin B12 levels in selected insects. Insect Biochem 14(2):175–179
127. Oonincx DGAB, van der Poel AFB (2011) Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*). Zoo Biol 30(1):9–16
128. Tong L, Yu X, Liu H (2011) Insect food for astronauts: gas exchange in silkworms fed on mulberry and lettuce and the nutritional value of these insects for human consumption during deep space flights. Bull Entomol Res 101:613–622
129. Ratcliffe N, Azambuja P, Mello CB (2014) Recent advances in developing insect natural products as potential modern day medicines. J Evid Based Complement Altern Med 2014:904958
130. Musundire R, Zvidzai JC, Chidewe C (2014) Bio-active compounds composition in edible stinkbugs consumed in south-eastern districts of Zimbabwe. Int J Biol 6(3):36–45
131. Nongonierma AB, FitzGerald RJ (2017) Unlocking the biological potential of proteins from edible insects through enzymatic hydrolysis: a review. Innov Food Sci Emerg Technol 43:239–252
132. Vercruysse L, Smagghe G, Beckers T, van Camp J (2009) Antioxidative and ACE inhibitory activities in enzymatic hydrolysates of the cotton leafworm, *Spodopter littoralis*. Food Chem 114:38–43
133. Vercruysse L, Smagghe G, Herregods G, van Camp J (2005) ACE inhibitory activity in enzymatic hydrolysates of insect protein. J Agr Food Chem 53:5207–5211
134. Vercruysse L, Smagghe G, Matsui T, van Camp J (2008) Purification and identification of an angiotensin I converting enzyme (ACE) inhibitory peptide from the gastrointestinal hydrolysate of the cotton leafworm, *Spodoptera littoralis*. Process Biochem 43:900–904
135. Dai C, Ma H, Luo L, Yin X (2013) Angiotensin I-converting enzyme (ACE) inhibitor peptide derived from *Tenebrio molitor* (L.) larva protein hydrolysate. Eur Food Res Tech 236:681–689
136. Wang W, Wang N, Zhang Y (2014) Antihypertensive properties on spontaneously hypertensive rats of peptide hydrolysates from silkworm pupae protein. Food Nutr Sci 5:1202–1211
137. Wang W, Wang N, Liu C et al (2017) Effect of silkworm pupae peptide on the fermentation and quality of yogurt. J Food Proc Preserv 41:e12893
138. Wang W, Wang N, Zhou Y et al (2011) Isolation of a novel peptide from silkworm pupae protein components and interaction characteristics to angiotensin I-converting enzyme. Eur Food Res Technol 232:29–38
139. Li X, Li Y, Huang X et al (2014) Identification and characterization of a novel angiotensin I-converting enzyme inhibitory peptide (ACEIP) from silkworm pupa. Food Sci Biotech 23:1017–1023
140. Jia J, Wu Q, Yan H, Gui Z (2015) Purification and molecular docking study of a novel angiotensin-I converting enzyme (ACE) inhibitory peptide from alcalase hydrolysate of ultrasonic-pretreated silkworm pupa (*Bombyx mori*) protein. Process Biochem 50:876–883
141. Tao M, Wang C, Liao D et al (2017) Purification, modification and inhibition mechanism of angiotensin I-converting enzyme inhibitory peptide from silkworm pupa (Bombyx mori) protein hydrolysate. Process Biochem 54:172–179
142. Wu QY, Jia JQ, Tan GX, Xu JL, Gui ZZ (2011) Physicochemical properties of silkworm larvae protein isolate and gastrointestinal hydrolysate bioactivities. Afr J Biotech 10(32):6145–6153
143. Zhang Y, Wang N, Wang W, Wang J, Zhu Z, Li X (2016) Molecular mechanisms of novel peptides from silkworm pupae that inhibit α-glucosidase. Peptides 76:45–50

144. Faruck MO, Yusof F, Chowdhury S (2016) An overview of antifungal peptides derived from insect. Peptides 80:80–88
145. Hou L, Shi Y, Zhai P et al (2007) Antibacterial activity and in vitro anti-tumor activity of the extract of the larvae of the housefly (*Musca domestica*). J Ethnopharmacol 111(2):227–231
146. Chae J, Kurokawa K, So Y et al (2011) Purification and characterization of tenecin 4, a new anti-Gram-negative bacterial peptide, from the beetle *Tenebrio molitor*. Dev Comp Immunol 36:540–546
147. Tang JJ, Fang P, Xia HL et al (2015) Constituents from the edible Chinese black ants (*Polyrhachis dives*) showing protective effect on rat mesangial cells and anti-inflammatory activity. Food Res Int 67:163–168
148. Wang Y, Zhao Y, Lei C, Zhu F (2012) Antiviral and antitumor activities of the protein fractions from the larvae of the housefly, *Musca domestica*. Afr J Biotechnol 11(39):9468–9474
149. Elpidina EN, Goptar IA (2007) Digestive peptidases in *Tenebrio molitor* and possibility of use to treat celiac disease. Entomol Res 37:139–147
150. Tan HSG, Fischer AR, van Trijp HC, Stieger M (2016) Tasty but nasty? Exploring the role of sensory-liking and food appropriateness in the willingness to eat unusual novel foods like insects. Food Qual Pref 48:293–302
151. Dossey AT, Tatum JT, McGill WL (2016) Modern insect-based food industry: current status, insect processing technology, and recommendations moving forward. In: Dossey AT, Morales-Ramos JA, Rojas MG (eds) Insects as sustainable food ingredients: production, processing and food applications, 1st edn. Academic, Cambridge, UK
152. Hall FG, Jones OG, O'Haire ME, Liceaga AM (2017) Functional properties of tropical banded cricket (*Gryllodes sigillatus*) protein hydrolysates. Food Chem 224:414–422
153. Omotoso OT (2006) Nutritional quality, functional properties and anti-nutrient compositions of the larva of *Cirina forda* (Westwood) (Lepidoptera: Saturniidae). J Zhejiang Univ Sci B 7(1):51–55
154. http://criknutrition.com/. Accessed 18 Oct 2017
155. http://bugmuscle.com/. Accessed 18 Oct 2017
156. Paul A, Frederich M, Megido RC, Alabi T, Malik P, Uyttenbroeck R, Francis F, Blecker C, Haubruge E, Lognay G, Danthine S (2017) Insect fatty acids: a comparison of lipids from three orthopterans and *Tenebrio molitor* L. larvae. J Asia Pac Entomol 20(2):337–340
157. Dutta PK, Dutta J, Tripathi VS (2004) Chitin and chitosan: chemistry, properties and applications. J Sci Ind Res 63:20–31
158. Muzzarelli RA (2011) Biomedical exploitation of chitin and chitosan via mechano-chemical disassembly, electrospinning, dissolution in imidazolium ionic liquids, and supercritical drying. Mar Drugs 9(9):1510–1533
159. Park BK, Kim MM (2010) Applications of chitin and its derivatives in biological medicine. Int J Mol Sci 11(12):5152–5164
160. Zhao X, Vázquez-Gutiérrez JL, Johansson DP, Landberg R, Langton M (2016) Yellow mealworm protein for food purposes-extraction and functional properties. PLoS One 11(2):e0147791
161. WHO. Food safety Fact sheet Reviewed October 2017. http://www.who.int/mediacentre/factsheets/fs399/en/. Accessed 25 Sept 2017
162. Aiking H (2011) Future protein supply. Trends Food Sci Technol 22:112–120
163. Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050: the 2012 revision. ESA Working paper. FAO, Rome
164. Marberg A, van Kranenburg H, Korzilius H (2017) The big bug: the legitimation of the edible insect sector in the Netherlands. Food Pol 71:111–123
165. Vellinga P, Herb N (1999) Industrial transformation science plan. IHDP, Bonn
166. Aiking H, De Boer J, Vereijken JM (2006) Sustainable protein production and consumption: pigs or peas? Springer, Dordrecht
167. Pimentel D, Pimentel M (2003) Sustainability of meat-based and plant-based diets and the environment. Am J Clin Nutr 78:660S–663S
168. Msangi S, Rosegrant M (2009) World agriculture in a dynamically- changing environment: IFPRI's long-term outlook for food and agriculture under additional demand and constraints. FAO, Rome. http://www.fao.org/wsfs/forum2050/wsfs-background-documents/wsfs-expert-papers/en/. Accessed 23 Sept 2017

169. Mekonnen MM, Hoekstra AY (2010) The green, blue and grey water footprint of farm animals and animal products. UNESCO-IHE, Delft
170. van Huis A (2010) Opinion: bugs can solve food crisis. The Scientist. http://www.the-scientistcom/?articlesview/articleNo/29292/title/Opinion-Bugs-can-solve-food-crisis/. Accessed 24 Sept 2017
171. Collavo A, Glew RH, Huang YS, Chuang LT, Bosse R, Paoletti MG (2005) House cricket small-scale farming. In: Paoletti MG (ed) Ecological implications of minilivestock: potential of insects, rodents, frogs and snails. Taylor & Francis, Oxford
172. Oonincx DGAB, de Boer IJM (2012) Environmental impact of the production of mealworms as a protein source for humans – a life cycle assessment. PLoS One 7:e51145
173. Oonincx DGAB, van Broekhoven S, van Huis A, van Loon JJA (2015) Feed conversion, survival and development, and composition of four insect species on diets composed of food by- products. PLoS One 10(12):e0144601
174. Steinfeld H, Gerber P, Wassenaar T et al (2006) Livestock's long shadow; environmental issues and options. FAO, Rome
175. Aarnink AJA, Keen A, Metz JHM, Speelman L, Verstegen MWA (1995) Ammonia emission patterns during the growing periods of pigs housed on partially slatted floors. J Agr Econ Res 62:105–116
176. Greenlee KJ, Harrison JF (2004) Development of respiratory function in the American locust *Schistocerca americana* I. Across-instar effects. J Exp Biol 207:497–508
177. Emekci M, Navarro S, Donahaye E, Rindner M, Azrieli A (2002) Respiration of *Tribolium castaneum* (Herbst) at reduced oxygen concentrations. J Stored Prod Res 38:413–425
178. Gouveia SM, Simpson SJ, Raubenheimer D, Zanotto FP (2000) Patterns of respiration in *Locusta migratoria* nymphs when feeding. Physiol Entomol 25:88–93
179. Emekci M, Navarro S, Donahaye E, Rindner M, Azrieli A (2004) Respiration of *Rhyzopertha dominica* (F.) at reduced oxygen concentrations. J Stored Prod Res 40:27–38
180. Koerkamp PW, Metz JHM, Uenk GH et al (1998) Concentrations and emissions of ammonia in livestock buildings in northern Europe. J Agr Econ Res 70:79–95
181. Nicks B, Laitat M, Vandenheede M et al (2003) Emissions of ammonia, nitrous oxide, methane, carbon dioxide and water vapor in the raising of weaned pigs on straw-based and sawdust-based deep litters. Anim Res 52:299–308
182. Cabaraux JF, Philippe FX, Laitat M et al (2009) Gaseous emissions from weaned pigs raised on different floor systems. Agric Ecosyst Environ 130:86–92
183. Harper LA, Flesch TK, Powell JM et al (2009) Ammonia emissions from dairy production in Wisconsin. J Dairy Sci 92:2326–2337
184. Demmers TGM, Burgess LR, Short JL et al (1999) Ammonia emissions from two mechanically ventilated UK livestock buildings. Atmos Environ 33:217–227
185. Makkar HPS, Tran G, Heuzé V, Ankers P (2014) State-of-the-art on use of insects as animal feed. Anim Feed Sci Technol 197:1–33
186. Foley JA, Ramankutty N, Brauman KA et al (2011) Solutions for a cultivated planet. Nature 478:337–342
187. Pastor B, Velasquez Y, Gobbi P, Rojo S (2015) Conversion of organic wastes into fly larval biomass: bottlenecks and challenges. J Insects Food Feed 1:179–193
188. Srinroch C, Srisomsap C, Chokchaichamnankit D et al (2015) Identification of novel allergen in edible insect, *Gryllus bimaculatus* and its cross-reactivity with *Macrobrachium* spp. allergens. Food Chem 184:160–166
189. Nishimune T, Watanabe Y, Okazaki H, Akai H (2000) Thiamin is decomposed due to Anaphe spp. entomophagy in seasonal ataxia patients in Nigeria. J Nutr 130:1625–1628
190. Liu Z, Xia L, Wu Y et al (2009) Identification and characterization of an arginine kinase as a major allergen from silkworm (*Bombyx mori*) larvae. Int Arch Allergy Immunol 150:8–14
191. Yu CJ, Lin YF, Chiang BL, Chow LP (2003) Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. J Immunol 170:445–453
192. García-Orozco KD, Aispuro-Hernández E, Yepiz-Plascencia G et al (2007) Molecular characterization of arginine kinase, an allergen from the shrimp *Litopenaeus vannamei*. Int Arch Allergy Immunol 144:23–28

193. Yadzir ZHM, Misnan R, Abdullah N et al (2012) Identification of the major allergen of *Macrobrachium rosenbergii* (giant freshwater prawn). Asian Pac J Trop Biomed 2(1):50–54
194. Khanaruksombat S, Srisomsap C, Chokchaichamnankit D et al (2014) Identification of novel allergen from muscle and various organs in banana shrimp (*Fenneropenaeus merguiensis*). Ann Allergy Asthma Immunol 113:301–306
195. Binder M, Mahler V, Hayek B et al (2001) Molecular and immunological characterization of arginine kinase from the Indianmeal moth, *Plodia interpunctella*, a novel cross-reactive invertebrate panallergen. J Immunol 167:5470–5477
196. Sookrung N, Chaicumpa W, Tungtrongchitr A et al (2006) *Periplaneta americana* arginine kinase as a major cockroach allergen among Thai patients with major cockroach allergies. Environ Health Perspect 114:875–880
197. Chuang JG, Su SN, Chiang BL et al (2010) Proteome mining for novel IgE-binding proteins from the German cockroach (*Blattella germanica*) and allergen profiling of patients. Proteomics 10:3854–3867
198. Li M, Wang XY, Bai JG (2006) Purification and characterization of arginine kinase from locust. Protein Pept Lett 13(4):405–410
199. Bragg J, Rajkovic A, Anderson C et al (2012) Identification and characterization of a putative arginine kinase homolog from *Myxococcus xanthus* required for fruiting body formation and cell differentiation. J Bacteriol 194(10):2668–2676
200. Shanti KN, Martin BM, Nagpal S et al (1993) Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. J Immunol 151:5354–5363
201. Leung PS, Chen YC, Mykles DL et al (1998) Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen. Mol Mar Biol Biotechnol 7:12–20
202. Daul CB, Slattery M, Reese G, Lehrer SB (1994) Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. Int Arch Allergy Immunol 105:49–55
203. Liu GM, Huang YY, Cai QF et al (2011) Comparative study of in vitro digestibility of major allergen, tropomyosin and other proteins between Grass prawn (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*). J Sci Food Agric 91:163–170
204. Rahman AMA, Kamath S, Lopata L et al (2010) Analysis of the allergenic proteins in black tiger prawn (*Penaeus monodon*) and characterization of the major allergen tropomyosin using mass spectrometry. Rapid Commun Mass Spectrom 24:2462–2470
205. Yadzir ZHM, Misnan R, Bakhtiar F et al (2015) Tropomyosin and actin identified as major allergens of the carpet clam (*Paphia textile*) and the effect of cooking on their allergenicity. Biomed Res Int 2015:254152
206. Jeong KY, Lee J, Lee IY et al (2003) Allergenicity of recombinant Bla g 7, German cockroach tropomyosin. Allergy 58(10):1059–1063
207. Asturias JA, Gómez-Bayón N, Arilla MC et al (1999) Molecular characterization of American cockroach tropomyosin (*Periplaneta americana* allergen 7), a cross-reactive allergen. J Immunol 162(7):4342–4348
208. Barletta B, Di Felice G, Pini C (2007) Biochemical and molecular biological aspects of silverfish allergens. Protein Pept Lett 14(10):970–974
209. van der Ventel ML, Nieuwenhuizen NE, Kirstein F et al (2011) Differential responses to natural and recombinant allergens in a murine model of fish allergy. Mol Immunol 48:637–646
210. Piboonpocanun S, Jirapongsananuruk O, Tipayanon T et al (2011) Identification of hemocyanin as a novel non cross-reactive allergen from the giant freshwater shrimp *Macrobrachium rosenbergii*. Mol Nutr Food Res 55:1492–1498
211. Pick C, Hagner-Holler S, Burmester T (2008) Molecular characterization of hemocyanin and hexamerin from the firebrat *Thermobia domestica* (Zygentoma). Insect Biochem Mol Biol 38:977–983
212. Schabel HG (2010) Forest insects as food: a global review. In: Durst PB, Johnson DV, Leslie RN, Shono K (eds) Forest insects as food: humans bite back. FAO, Bangkok

213. van der Spiegel M (2016) Safety of foods based on insects. In: Prakash V, Martin-Belloso O, Keener L et al (eds) Regulating safety of traditional and ethnic foods. Academic, Whaltam
214. Opara MN, Sanyigha FT, Ogbuewu IP, Okoli IC (2012) Studies on the production trend and quality characteristics of palm grubs in the tropical rainforest zone of Nigeria. Int J Agr Tech 8:851–860
215. Finke MD, Rojo S, Roos N et al (2015) The European food safety authority scientific opinion on a risk profile related to production and consumption of insects as food and feed. J Insects Food Feed 1(4):245–247
216. Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001
217. www.bugburger.se. Accessed 5 Oct 2017
218. Cortes Ortiz JA, Ruiz AT, Morales-Ramos JA et al (2016) Insect mass production technologies. In: Dossey AT, Morales-Ramos JA, Rojas MG (eds) Insects as sustainable food ingredients: production, processing and food applications, 1st edn. Academic, Cambridge, UK
219. https://www.micronutris.com/en/insectes-aperitifs. Accessed 5 Oct 2017
220. https://protifarm.com/products/. Accessed 5 Oct 2017
221. http://www.insects4food.org
222. https://cowboycrickets.com/. Accessed 5 Oct 2017
223. https://4ento.com/2015/03/12/top-10-insect-feed-companies/. Accessed 5 Oct 2017
224. http://www.openbugfarm.com/. Accessed 5 Oct 2017
225. http://www.bugburger.se/foretag/the-eating-insects-startups-here-is-the-list-of-entopreneurs-around-the-world/. Accessed 5 Oct 2017
226. https://www.donbugito.com/. Accessed 5 Oct 2017
227. https://www.crickefood.com/. Accessed 5 Oct 2017
228. http://www.goffardsisters.com/. Accessed 5 Oct 2017
229. https://www.coopathome.ch/en/Meat-%26-Fish/Insects/Essento-Insect-Burgers/p/5934433. Accessed 5 Oct 2017
230. https://www.delibugs.nl/. Accessed 5 Oct 2017
231. http://www.insectescomestibles.fr/. Accessed 5 Oct 2017
232. http://www.minifood.be/. Accessed 5 Oct 2017
233. https://www.wilderharrier.com/. Accessed 5 Oct 2017
234. https://www.conscientious.cat/. Accessed 5 Oct 2017
235. http://www.wur.nl/en/Expertise-Services/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects.htm. Accessed 5 Oct 2017
236. www.ipiff.org. Accessed 5 Oct 2017
237. www.affia.org. Accessed 5 Oct 2017
238. http://www.wageningenacademic.com/journals/jiff/general-information. Accessed 5 Oct 2017
239. Hossain SM, Blair R (2007) Chitin utilisation by broilers and its effect on body composition and blood metabolites. Brit Poultry Sci 48:33–38
240. Barrows FT, Bellis D, Krogdahl A et al (2008) Report of plant products in aquafeeds strategic planning workshop: an integrated interdisciplinary roadmap for increasing utilization of plant feedstuffs in diets for carnivorous fish. Rev Fish Sci 16:449–455
241. Sánchez-Muros MJ, Barroso FG, Manzano-Agugliaro F (2014) Insect meal as renewable source of food for animal feeding: a review. J Clean Prod 65:16–27
242. FEFAC (2012) Statistics 2012. European Feed Manufacturers' Federation, Brussels. http://www.fefac.eu/files/47239.pdf. Accessed 29 Oct 2017
243. Spring P (2013) The challenge of cost effective poultry and animal nutrition: optimizing existing and applying novel concepts. Lohmann Inf 48(1):38–46
244. EUFETEC (2013) Vision & SRIA document 2030 feed for food producing animals. EUFETEC (European Feed Technology Center), Brussels

245. Henry M, Gasco L, Piccolo G, Fountoulaki E (2015) Review on the use of insects in the diet of farmed fish: past and future. Anim Feed Sci Technol 203:1–22
246. Bondari K, Sheppard DC (1987) Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aure*us (Steindachner). Aquacult Fish Manag 18:209–220
247. Hem S, Toure S, Sagbla C, Legendre M (2008) Bioconversion of palm kernel meal for aquaculture: experiences from the forest region (Republic of Guinea). Afr J Biotechnol 7:1192–1198
248. Newton L, Sheppard C, Watson DW et al (2005) Using the black soldier fly, *Hermetia illucens*, as a value added tool for the management of swine manure. Animal and Poultry Waste Management Center. North Carolina State University, Raleigh
249. Stamer A (2015) Insect proteins – a new source for animal feed. EMBO Rep 16(6):676–680
250. Raubenheimer D, Rothman JM (2012) Nutritional ecology of entomophagy in humans and other primates. Annu Rev Entomol 58:141–160
251. de Marco M, Martínez S, Hernandez F et al (2015) Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. Anim Feed Sci Technol 209:211e218
252. Ramaswamy SB (2015) Setting the table for a hotter, flatter, more crowded earth: insects on the menu? J Insects Food Feed 1(3):171–178
253. Veldkamp T, Bosch G (2015) Insects: a protein-rich feed ingredient in pig and poultry diets. Anim Front 5(2):45–50
254. Bovera F, Loponte R, Marono S et al (2015) Use of *Tenebrio molitor* larvae meal as protein source in broiler diet: effect on growth performance, nutrient digestibility, and carcass and meat traits. J Anim Sci 94:639–647
255. Premalatha M, Abbasi T, Abbasi T, Abbasi SA (2011) Energy-efficient food production to reduce global warming and ecodegradation: the use of edible insects. Renew Sust Energ Rev 15(9):4357–4360
256. van Huis A, Dicke M, van Loon JJA (2015) Insects to feed the world. J Insects Food Feed 1(1):3–5
257. Veldkamp T, van Duinkerken G, van Huis A et al (2012) Insects as a sustainable feed ingredientin pig and poultry diets – a feasibility study. Wageningen UR Livestock Research, Wageningen
258. Smith R, Pryor R (2013) Mapping exercise report with regard to current legislation & regulation: Europe and Africa & China (PROteINSECT Deliverable 5.1). Minerva HCC, Andover
259. AllAboutFeed (2014) Why are insects not allowed in animal feed? White Paper. Reed Business Media, Doetinchem. http://www.allaboutfeed.net/Global/Whitepapers/Whitepaper_Insect_meal.pdf. Accessed 29 Oct 2017
260. Kenis M, Hien K (2014) Prospects and constraints for the use of insects as human food and animal feed in West Africa. Book of Abstracts of conference on insects to feed the world, The Netherlands, 14–17 May 2014
261. Čičková H, Newton GL, Lacy RC, Kozánek M (2015) The use of fly larvae for organic waste treatment. Waste Manag 35:68–80
262. Collavo A, Glew RH, Huang YS et al (2005) House cricket small-scale farming. In: Paoletti MG (ed) Ecological implications of Minilivestock: potential of insects, rodents, frogs and snails. Science Publishers, Enfield
263. Lundy ME, Parrella MP (2015) Crickets are not a free lunch: protein capture from scalable organic side-streams via high-density populations of *Acheta domesticus*. PLoS One 10:e0118785
264. Smetana S, Mathys A, Knoch A, Heinz V (2015) Meat alternatives: life cycle assessment of most known meat substitutes. Int J Life Cycle Assess 20:1254–1267

265. Newton GL, Booram CV, Barker RW, Hale OM (1977) Dried *Hermetia illucens* larvae meal as a supplement for swine. J Anim Sci 44:395–399
266. Myers HM, Tomberlin JK, Lambert BD, Kattes D (2008) Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. Environ Entomol 37:11–15
267. Banks IJ, Gibson WT, Cameron MM (2014) Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Tropical Med Int Health 19(1):14–22
268. Barroso FG, Sánchez-Muros MJ, Segura M et al (2017) Insects as food: enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. J Food Comp Anal 62:8–13
269. Zheng L, Hou Y, Li W et al (2012) Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. Energy 47:225–229
270. Halloran A, Hanboonsong Y, Roos N, Bruun S (2017) Life cycle assessment of cricket farming in north-eastern Thailand. J Clean Prod 156:83–94
271. Glendinning JI (2002) How do herbivorous insects cope with noxious secondary plant compounds in their diet? Entomol Exp App 104:15–25
272. Yu SJ, Hsu EL (1993) Induction of detoxification enzymes in phytophagous insects: roles of insecticide synergists, larval age, and species. Arch Insect Biochem 24:21–32
273. Chaubey MK (2008) Fumigant toxicity of essential oils from some common spices against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). J Oleo Sci 57:171–179
274. Wheeler D, Isman MB (2001) Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*. Entomol Exp Appl 98:9–16
275. Yang Y, Yang J, Wu WM et al (2015) Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 1. Chemical and physical characterization and isotopic tests. Environ Sci Technol 49(20):12080–12086
276. Yang Y, Yang J, Wu WM et al (2015) Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 2. Role of gut microorganisms. Environ Sci Technol 49(20):12087–12093
277. Sonmez E, Gulel A (2008) Effects of different temperatures on the total carbohydrate, lipid and protein amounts of the bean beetle, *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). Pak J Biol Sci 11(14):1803

Plant Proteases in Food Processing

16

Manzoor Ahmad Shah and Shabir Ahmad Mir

Contents

Abstract

Proteases are enzymes that hydrolyze protein molecules into peptides and amino acids. Proteases are the most commercially important enzymes because of their multiple applications in food and other industries. In recent decades, interest in plant proteases has been increased rapidly. The number of industrially employed enzymes of plant origin is still small but growing fast. Plants are an important source of proteases as plants require proteases throughout their life cycle. These are present in all kinds of plant tissues and, thus, can be extracted from their natural sources or can be prepared using in vitro techniques. Plant proteases can

M. A. Shah (✉)
Department of Food Science and Technology, Pondicherry University, Puducherry, India
e-mail: mashahft@gmail.com

S. A. Mir
Department of Food Technology, Islamic University of Science and Technology, Awantipora, Jammu and Kashmir, India
e-mail: shabirahmir@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_68

be extracted from natural sources by aqueous maceration of various plant organs. The crude extract thus obtained may be further purified to obtain a pure enzyme. Production of plant proteases by in vitro techniques leads to higher enzyme yields and minimizes the extraction procedures used in extraction from natural sources; these techniques reduce the effects of climate and seasonal changes and also the heterogeneity of enzymes produced from different parts of plant. Plant proteases have the ability to coagulate milk proteins and thus have been utilized as milk clotting enzymes in cheesemaking for centuries. These proteases are used as crude or in purified form; they are a substitute to the calf rennet. They are used for making different varieties of cheese in Mediterranean, West African, and southern European countries. Proteases extracted from different plant sources have been widely used in meat tenderization, in bioactive peptide production from both the plant and animal sources, and in flour/dough modification in baking industry.

Keywords
Plant protease · Papain · Bromelain · Ficin · Actinidin · Zingibain · Cardosins · Cheese · Milk clotting enzyme · Meat tenderization · Bioactive peptide · Dough modification

1 Introduction

Proteases are enzymes that hydrolyze protein molecules into peptides and amino acids. These form a very diverse and complex group of enzymes. The specificity of these enzymes is governed by the type of the amino acid and other functional groups close to the bond being hydrolyzed [1]. Proteases can be classified into many ways. Based on the site of action, they can be grouped into exoproteases and endoproteases. Exoproteases can cleave N- or C-terminal peptide bonds, while endopeptidases cleave internal peptide bonds [2]. Exoproteases can be further divided into aminopeptidases and carboxypeptidases based on the ability to cleave the N-terminal and the C-terminal peptide bond. Endoproteases are classified on the basis of their catalytic mechanism, i.e., enzyme active site. The MEROPS database classifies them into seven families: aspartic, cysteine, glutamic, metallo, asparagine, serine, and threonine. But only five types of endoproteases have been reported in plants [3].

Proteases are the most commercially important enzymes because of their multiple applications in various industries and particularly in food, pharmaceutical, detergent, and leather industries. In recent decades, interest in plant natural products has grown rapidly. The number of industrially employed enzymes of plant origin is still small but growing fast [4]. Plants are an important source of proteases as plants require proteases throughout their life cycle [5]. The most widely used plant proteases are papain, bromelain, ficin, actinidin, zingibain, and cardosins. Plant proteases are being used in dairy processing, meat tenderization, bioactive peptide production, and baking industry.

2 Sources

Plants are an important source of proteases as plant physiology and development require a diverse number of proteases [6]. Proteases are required by plants throughout their life cycle [5]. Proteases obtained from plant sources are very attractive because they can be used over a broad temperature and pH range [7]. Proteases have been isolated and purified from various plant sources (Table 1).

Cardosins and cyprosins are obtained from *Cynara cardunculus* flowers [8–24], cynarase is from *Cynara scolymus* flowers [25–30], and cardosin like protease is prepared from *Cynara humilis* [10].

Actinidin, bromelain, caprifig coagulant, cucumisin, dubiumin, ficin, hieronymain, lettucine, onopordosin, oryzasin, papain, pomiferin, procirsin, salpichroin, streblin and zingibain are obtained from *Actinidia spp.*, *Ananas comosus*, *Ficus carica sylvestris*, *Cucumis melo*, *Solanum dubium*, *Ficus spp.*, *Bromelia hieronymi*, *Lactuca sativa*, *Onopordum acanthium*, *Oryza sativa*, *Carica papaya*, *Maclura pomifera*, *Cirsium vulgare*, *Salpichroa origanifolia*, *Streblus asper*, and *Zingiber officinale*, respectively. Neriifolin and neriifolin S are obtained from *Euphorbia neriifolia* [43, 44]; religiosin, religiosin B [47, 48], and religiosin C from *Ficus religiosa* [49]; and caricain from *Carica papaya* [117].

Protease extracts were obtained from *Albizia lebbeck* [61], *Balanites aegyptiaca* [65], *Centaurea calcitrapa* [53–55], *Foeniculum vulgare* [66], *Helianthus annuus* [61], *Jacaratia corumbensis* [62], *Moringa oleifera* [63], *Onopordum turcicum* [60], *Silybum marianum* [58, 59], *Solanum elaeagnifolium* [64], and *Withania coagulans* [56, 57]. Sun et al. [118] isolated three serine proteases from tomato (*Lycopersicum esculentum* Mill.) fruit and Li et al. [119] prepared tamarillin from tamarillo fruit (*Cyphomandra betacea*).

Many of the plant proteases are obtained from plants that grow in tropical or subtropical, geographically remote areas. These plants are subjected to various internal and external stresses, which affect the quality and quantity of proteases. Additionally, the selection of high-yielding varieties is difficult due the long cultivation periods between planting and harvesting, leading to the expensive products. Thus, the development of alternative and complimentary techniques to the extraction of proteases from whole plant sources is quite important from socioeconomic point of view [120]. An alternative to this is the plant cell culture using in vitro technologies. These techniques can be used to produce large quantities of proteases which are homogenous in nature and thus can be easily commercialized [4].

3 Production

Proteases are required by plants for various physiological and developmental processes from the beginning up to death of a plant. They are present in all kinds of plant tissues and, thus, can be extracted from their natural sources or can be prepared using in vitro techniques [4].

Table 1 Plant proteases in food industry

Protease	Source	Function/ application	Reference
Cardosins and cyprosins	*Cynara cardunculus*	Milk clotting activity	[8–24]
Cynarase	*Cynara scolymus*	Milk clotting activity	[25–30]
Cardosin	*Cynara humilis*	Milk clotting activity	[10]
Onopordosin	*Onopordum acanthium*	Milk clotting activity	[31]
Oryzasin	*Oryza sativa*	Milk clotting activity	[32]
Procirsin	*Cirsium vulgare*	Milk clotting activity	[33]
Ficin	*Ficus racemosa*	Milk clotting activity	[34]
Caprifig coagulant	*Ficus carica sylvestris*	Milk clotting activity	[35]
Ginger protease, zingibain	*Zingiber officinale*	Milk clotting activity	[36, 37]
Actinidin	*Actinidia chinensis,*	Milk clotting activity	[38–41]
	Actinidia deliciosa		
Cucumisin	*Cucumis melo*	Milk clotting activity	[42]
Neriifolin	*Euphorbia neriifolia*	Milk clotting activity	[43]
Neriifolin S	*Euphorbia neriifolia*	Milk clotting activity	[44]
Dubiumin	*Solanum dubium*	Milk clotting activity	[45, 46]
Religiosin	*Ficus religiosa*	Milk clotting activity	[47]
Religiosin B	*Ficus religiosa*	Milk clotting activity	[48]
Religiosin C	*Ficus religiosa*	Milk clotting activity	[49]
Streblin	*Streblus asper*	Milk clotting activity	[50]
Lettucine	*Lactuca sativa*	Milk clotting activity	[51]
Hieronymain	*Bromelia hieronymi*	Milk clotting activity	[52]
Protein extract	*Centaurea calcitrapa*	Milk clotting activity	[53–55]
Protein extract	*Withania coagulans*	Milk clotting activity	[56, 57]

(*continued*)

Table 1 (continued)

Protease	Source	Function/ application	Reference
Protein extract	*Silybum marianum*	Milk clotting activity	[58, 59]
Protein extract	*Onopordum turcicum*	Milk clotting activity	[60]
Protein extract	*Albizia lebbeck*	Milk clotting activity	[61]
Protein extract	*Helianthus annuus*	Milk clotting activity	[61]
Protein extract	*Jacaratia corumbensis*	Milk clotting activity	[62]
Protein extract	*Moringa oleifera*	Milk clotting activity	[63]
Protein extract	*Solanum elaeagnifolium*	Milk clotting activity	[64]
Protein extract	*Balanites aegyptiaca*	Milk clotting activity	[65]
Protein extract	*Foeniculum vulgare*	Milk clotting activity	[66]
Actinidin	*Actinidia chinensis*, *Actinidia deliciosa*	Meat tenderizing activity	[41, 67–71]
Bromelain	*Ananas comosus*	Meat tenderizing activity	[69, 72]
Ficin	*Ficus carica*	Meat tenderizing activity	[71, 73]
Papain	*Carica papaya*	Meat tenderizing activity	[69, 74–76]
Zingibain	*Zingiber officinale*	Meat tenderizing activity	[69, 75, 77]
Protein extract	*Cucumis trigonus*	Meat tenderizing activity	[77]
Protein extract	*Asparagus officinalis*	Meat tenderizing activity	[70]
Protein extract	*Calotropis procera*	Meat tenderizing activity	[78]
Protein extract	*Pyrus pyrifolia*	Meat tenderizing activity	[79] [71]
Protein extract	*Actinidia arguta*	Meat tenderizing activity	[80]
Papain	*Carica papaya*	Bioactive peptide production	[81–97]
Bromelain	*Ananas comosus*	Bioactive peptide production	[81, 86, 90, 91, 93, 95, 96, 98–102]
Ficin	*Ficus spp.*	Bioactive peptide production	[83]

(*continued*)

Table 1 (continued)

Protease	Source	Function/ application	Reference
Actinidin	*Actinidia spp.*	Bioactive peptide production	[103]
Zingibain	*Zingiber officinale*	Bioactive peptide production	[103]
Salpichroin	*Salpichroa origanifolia*	Bioactive peptide production	[104]
Pomiferin	*Maclura pomifera*	Bioactive peptide production	[105–107]
Cyprosin, Cardosins, Cynarases	*Cynara cardunculus*	Bioactive peptide production	[108–110]
Onopordosin	*Onopordum acanthium*	Bioactive peptide production	[111]
Bromelain	*Ananas comosus*	Flour modification	[112]
Papain	*Carica papaya*	Flour/dough modification	[113–116]
Caricain	*Carica papaya*	Dough modification	[117]

3.1 Production from Natural Sources

Plant proteases can be extracted from natural sources by aqueous maceration of various plant organs such as flowers, seeds, roots, and leaves [121]. The crude extract thus obtained may be further purified to obtain partially purified enzyme or pure enzyme depending upon the degree of purification. Precipitation with ammonium sulfate is an effective way to produce substantial amounts of active proteases [8].

A protease tamarillin was extracted from tamarillo fruit (*Cyphomandra betacea* Cav.) and purified by ammonium sulfate precipitation and diethylaminoethyl-Sepharose chromatography. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis showed that the peak with the highest protease activity consisted of one protein of molecular mass ca. 70 kDa. The protease showed optimal activity at pH 11 and 60 °C [119]. Beka et al. [65] isolated protease extract from *Balanites aegyptiaca* fruit pulp. These fruits were disinfected, husked, and soaked in different buffers and saline solutions. The extracts obtained were diafiltered, concentrated, and decolored with active charcoal.

Gagaoua et al. [37] isolated zingibain from *Zingiber officinale* rhizomes. Chopped ginger rhizomes were homogenized with 50 mM sodium phosphate buffer (pH 7.0) containing 10 mM L-cysteine. The homogenate thus obtained was stirred continuously for 45 min at 4 °C and then filtered. The filtrate was centrifuged and concentrated using ammonium sulfate up to 80% saturation. After an overnight dialysis of the precipitate, the dialyzed extract was subjected to three-phase

partitioning purification system. The enzyme was found to be exclusively partitioned in the aqueous phase. The enzyme exhibited maximal proteolytic activity at a temperature of 60 °C and pH 7.0.

3.2 In Vitro Production

The plant cells are totipotent and thus are able to produce the same chemical compounds in vitro and in vivo. The yield of enzymes differs between the two processes [122]. Generally, the yield of plant proteases obtained in vitro is lower than in vivo conditions [4]. Different plant organs produce proteases with different activities [123].

Different in vitro techniques such as callus and cell suspension cultures have been used to produce proteases from various plant tissues by several authors. Proteases were produced from *Mirabilis jalapa* culture [124], cell suspension culture of *Centaurea calcitrapa* [54, 125], and callus culture of *Silybum marianum* [126] and *Cynara cardunculus* [127]. Proteases from *Hohenbergia penduliflora* were produced using micropropagation technique by Perez et al. [123]. The molecular cloning, expression, and characterization of procirsin from the flowers of *Cirsium vulgare* were studied by Lufrano et al. [33]. Feijoo-Siota et al. [128] produced recombinant progaline B, an aspartic protease from *Galium verum* and expressed in the yeast *Pichia pastoris.*

Callus tissue cultures were obtained from cotyledons of *Cynara cardunculus*. Calli were subcultured every month using Gamborg's B5 medium supplemented with dichloro-phenoxyacetic acid (1 mg/l), benzyladenine (0.1 mg/l), ascorbic acid (5 mg/l), and citric acid (5 mg/l). The cultures were maintained at 24 °C and in the dark [127]. Cell suspension of *Centaurea calcitrapa* were homogenized in 50 mM Tris–HCl, pH 8.1, 1 mM EDTA, and 3 mM mercaptoethanol (buffer A), at 4 °C, and sonicated. The homogenate was centrifuged at 4 °C, to produce the crude extract [125].

In vitro techniques offer many advantages. Production of plant proteases by in vitro techniques leads to higher enzyme yields and minimizes the extraction procedures used in extraction from natural sources. Furthermore, these techniques reduce the effects of climate and seasonal changes and also the heterogeneity of enzymes produced from different parts of plant [4].

4 Applications

4.1 Cheese Production

Plant proteases have the ability to coagulate milk proteins and thus have been utilized as milk clotting enzymes in cheesemaking for centuries. These proteases are used as crude or in purified form; they are a substitute to the calf rennet. Plant proteases are a better alternative to calf rennet because of the limited availability and

high price of calf rennet, religious factors, diet, or ban on recombinant calf rennet in some countries. Most of the proteases used as milk clotting enzymes are aspartic proteases, but proteases belonging to cysteine and serine groups have also been reported to have milk clotting activity under certain conditions [121].

Plant proteases are used for making different varieties of cheese in Mediterranean, West African, and southern European countries. Spain and Portugal are the largest producers of cheeses using plant proteases from *Cynara* spp. [129]. Portuguese Serra and Serpa cheese and Spanish Los Pedroches cheese, La Serena cheese, Torta del Casar cheese, Los Ibores cheese, and Flor de Guía cheese are prepared with proteases from *Cynara* spp. [121]. The traditional cheeses in West African countries are prepared using proteases from *Calotropis procera* [129].

A large number of plant proteases have been isolated from various plant sources and studied for milk clotting activity (Table 1). The proteases from *Cynara cardunculus* were reported to have milk clotting activity. Sanjuan et al. [11] investigated the effect of animal and plant protease (*Cynara cardunculus*) on the physicochemical properties of Los Pedroches cheese prepared from ewes' milk and reported that moisture, protein, and water activity were higher, while fat and soluble nitrogen were lower in the cheeses prepared with animal rennet compared with cheeses prepared with plant protease. Silva et al. [12] studied the milk clotting activity of proteases (cardosins A and B), prepared from *Cynara cardunculus* flowers, and chymosin and reported that the highest specific milk clotting activity was shown by chymosin followed by cardosin B.

Tejada et al. [15] studied the effect of animal rennet and plant coagulant obtained from *Cynara cardunculus* on the sensory properties of Murcia al Vino cheese prepared from goat milk and reported that the cheeses prepared with rennet showed less odor and taste intensities, exhibited a bit clearer color, and were harder and grainier but less creamy than the cheeses made with plant coagulant. In another study, Tejad et al. [16] reported that the proteolysis was more intense, and after 60 days of ripening, it was higher in cheeses prepared with plant coagulant than with animal rennet. The use of plant coagulant for goat cheese manufacture might accelerate ripening, and, thus, a cheese with different sensory characteristics may be prepared [16].

Galan et al. [17] studied the effect of two different concentrations of plant coagulant from *Cynara cardunculus* in comparison with calf rennet on cheese prepared from sheep milk and reported that higher concentration of plant coagulant resulted in higher casein hydrolysis in cheese compared with lower concentration after 2 days of ripening. The plant coagulant improved the sensory properties of cheese than calf rennet. Pino et al. [19] studied the effect of plant coagulant extracted from *Cynara cardunculus* and calf rennet on cheese prepared from goat milk cheese during ripening and reported that no significant differences were found between the compositions of cheeses made with these two types of coagulants. But the plant coagulant resulted in higher amounts of pH 4.6-soluble nitrogen, greater breakdown of α_s-casein and formation of hydrophobic peptides, and higher ratio of hydrophobic/hydrophilic peptides throughout the ripening than calf rennet.

Ben Amira et al. [21] prepared a protease extract from *Cynara cardunculus*, at different pH, and reported that the best milk clotting properties and highest enzyme content were shown by the extract extracted at pH 3. In another study, Ben Amira et al. [23] studied the effect of the ripening stage and the lyophilization of *Cynara cardunculus* flowers on their chemical composition, enzymatic activities of extracts, and technological properties of cheese curds and reported that flowers harvested at the middle of ripening stage and lyophilized produce plant coagulant with better clotting properties, resulting in higher yield, moisture, and texture parameters of curd.

Liburdi et al. [24] developed a technically friendly enzyme bioreactor by optimizing the immobilization procedure of calf rennet and plant coagulant from *Cynara cardunculus* on CLEA® magnetic supports. The developed technique may be used to produce soft cheeses by a continuous milk coagulation process. In another study, Esposito et al. [30] isolated aspartic proteases from alpine thistle flowers and immobilized it on polyacrylic support. The enzyme showed an optimal pH of 5.0, a value very similar to the one generally used for milk clotting during cheesemaking, and exhibited a satisfactory stability over time.

Llorente et al. [26] studied the protease activity in crude extracts from different parts of the globe artichoke (*Cynara scolymus*) and reported that mature flowers are the main source of proteolytic and milk clotting enzymes. According to Chazarra et al. [29], the rennet strength of *Cynara scolymus* flower extract increased hyperbolically with increase in concentration of calcium, and the concentration was saturated at 50 Mm. Llorente et al. [27] used *Cynara scolymus* flower extract and animal rennet for the production of Gouda-type cheeses from bovine milk and reported that the cheese yield was equal to that produced with animal rennet. Also, chemical and sensory properties of the cheeses prepared with plant and animal coagulants were similar. Overproteolysis and background flavor development can be prevented by extending the brining process (40 h) of the cheeses prepared with plant coagulant.

Salvador et al. [55] isolated and purified aspartic proteases from *Centaurea calcitrapa* seeds during germination and reported that the purified enzyme has an optimum pH range of 3.5–4.5, using FTC-hemoglobin as a substrate and an optimum temperature at 52 °C. The clotting time of seed extracts was similar to that of flower extracts. The extract may be used for the production of special cheeses requiring weak coagulants. Egito et al. [61] studied the milk clotting activity of seed extracts from albizia (*Albizia lebbeck*) and sunflower (*Helianthus annuus*) and reported that albizia seed extract showed higher specific clotting activity than sunflower seed extract. The albizia and sunflower extract hydrolyzed κ-casein at the Lys116-Thr117 bond and at the Phe105-Met106 bond, respectively. Ahmed et al. [45, 46] prepared and purified a protease dubiumin from *Solanum dubium* seeds. The protease showed high milk clotting activity and coagulated skimmed milk to form a white and firm curd. The ratio of milk clotting activity to the proteolytic activity of dubiumin was comparable to those of calf and microbial rennet.

Vairo-Cavalli et al. [59] studied the effect of proteases isolated from flowers of *Silybum marianum* (L.) on degradation of caprine and ovine caseins. The proteases degraded the caprine α_{s1} to a lower percentage than ovine α_s but the caprine

β-caseins to higher percentage than ovine β-caseins. The major cleavage site in ovine α_{s1}-casein was Leu99-Arg100.

Duarte et al. [62] extracted and purified a protease from the root latex of *Jacaratia corumbensis* O. kuntze and reported that the purified protease showed the highest milk clotting activity and higher optimal pH in comparison to the crude extract. Both the crude and purified proteases showed the maximum clotting at 55 °C. Faccia et al. [35] studied the effect of plant coagulant extracted from caprifig latex on artisanal-type Cacioricotta cheese (traditional Italian cheese) in comparison with the industrial type prepared with calf rennet. Higher concentrations of nitrogen fractions and peptides were observed in artisanal-type cheese prepared with the plant coagulant than in the industrial type.

Bruno et al. [52] isolated a protease named as hieronymain from unripe fruits of *Bromelia hieronymi* and reported that caseinolytic activity and milk clotting activity was 3.3 Ucas/ml and 40 ± 0.2 IMCU/ml, respectively, at 30 °C and pH 6.5. Whey proteins, bovine serum albumin, and α-lactalbumin were quickly degraded after 30 min, while β-lactoglobulin was considerably degraded only after 60 min at 50 °C. Cheeses prepared with hieronymain and chymosin showed similar sensory attributes as analyzed by a taste panel. Nestor et al. [64] prepared protease extracts from ripe *Solanum elaeagnifolium* berries and reported that these extracts showed lower milk clotting activities than rennin or chymosin but produced firm gels under acidic conditions. The cheeses prepared with these extracts were softer than cheeses manufactured with rennin or chymosin. Thus, these extracts can be suitable for the preparation of filata-type cheeses as well as cream cheeses.

Withania coagulans fruit has traditionally been used as milk clotting agent in cheesemaking. Protease extracts were prepared from *Withania coagulans* fruits [56, 57]. Pezeshki et al. [56] reported that severe proteolysis occurred in cheeses prepared with protease from *Withania coagulans* in comparison to cheeses prepared with animal and fungi coagulants. The α_{s1}-caseins and β-caseins were absent in cheeses prepared with *Withania coagulans* protease extract. The plant protease led to an increase in soluble nitrogen in 12% trichloroacetic acid (SNTCA) during ripening of cheeses. This may be due to higher proteolytic activity of *Withania coagulans* protease extracts as compared to other coagulants. Salehi et al. [57] reported that the protease prepared from *Withania coagulans* fruits showed optimal activity at temperature 65 °C and pH 5.5. The activity of the plant protease was reduced by NaCl and $CaCl_2$ and thus may be suitable for producing low salt content cheeses.

A milk coagulating protease was isolated and purified from ginger rhizomes [36, 37]. The purified enzyme showed maximum activity at pH 5.5 and at a temperature of about 60 °C. This enzyme showed higher specificity toward α_s -casein followed by β- and κ-casein, and thus the purified protease may be used as a rennet substitute in the dairy industry [36].

Brutti et al. [31] isolated a protease onopordosin from fresh *Onopordum acanthium* flowers and used it to prepare semihard-type cheese from bovine milk. It was reported that the sensory quality of cheese prepared with onopordosin was similar to that of commercial cheeses. Pontual et al. [63] reported caseinolytic and milk clotting activities from *Moringa oleifera* flowers and reported that the

precipitated protein fraction promoted extensive cleavage of κ-casein and low level of α_s- and β-casein hydrolysis.

Grozdanovic et al. [39] reported that three kiwifruit extracts prepared from a single fruit pulp showed significantly different levels of active actinidin, depending on the extraction buffer used. The extract prepared at pH 5.0 showed the best milk clotting properties, with a higher ratio of clotting activity to proteolytic activity than purified actinidin. This extract produced a casein coagulum clearly separated from the whey proteins and was shown to be stable at room temperature for up to 2 months. In another study Puglisi et al. [40] used aqueous solution of kiwi juice for manufacture of mozzarella cheese and reported that the aqueous solution exhibited high levels of milk clotting activity and a cheese yield of 10.6% was obtained. No bitterness was found in the prepared cheeses. Zhang et al. [41] studied the milk clotting activity and protease activity of actinidin from seven Chinese kiwifruit cultivars and reported that the highest milk clotting activity and protease activity were shown by actinidin from Xuxiang cultivar.

Beka et al. [65] prepared an extract from *Balanites aegyptiaca* fruit pulp and reported that this extract contains two types of proteases, an aspartic protease and a serine protease, with an optimum activity at pH 5.0 and pH 8.0, respectively, and an optimal temperature at 50 °C. The aspartic protease from the *B. aegyptiaca* extract mainly involved in milk clotting conditions, like other plant proteases, is more thermostable than bovine chymosin.

Bey et al. [66] prepared protease extracts from fennel stems and leaves and reported that milk clotting activity of the extracts depends on coagulation temperature, pH, and calcium concentration in milk. The proteases remain active and stable in the pH ranging from 6 to 7.5 and temperatures ranging from 40 to 60 °C.

Most of the plant coagulants cause excessive proteolysis and thus result in lower cheese yield and defects in flavor and texture [51]. Therefore, new plant coagulants are being continuously investigated so that the increasing global demand for diversified and high-quality cheese production may be fulfilled [36, 121].

4.2 Meat Tenderization

Tenderness is an important attribute of meat that affects its acceptability. There are many methods used for tenderizing meat and may be either chemical or physical methods. Meat tenderization by protease treatment is one of the popular methods used in meat industry. Proteases extracted from different plant sources have been widely used in meat tenderization [41, 68, 69, 71, 75–78, 80].

Naveena et al. [77] studied the effect of proteases from *Cucumis trigonus* and *Zingiber officinale* rhizome on buffalo meat tenderness in comparison with papain. The meat pieces from *Biceps femoris* muscles were marinated with these proteases leading to an increase in protein solubility and reduction in shear force values compared to control. Extensive proteolysis and reduction in the number of protein bands were observed in enzyme-treated samples. The sensory values were higher for enzyme-treated samples in comparison to control.

Liu et al. [68] injected kiwifruit juice in freeze–thaw abused porcine meat and reported that shear force values increased two times after one freeze–thaw cycle but reduced after additional freeze–thaw treatments. The kiwifruit juice injection decreased the pH of meat from 5.6 to 5.2 and enhanced the cooking loss from 21% to 30%. The decrease in the shear force values improved tenderness of meat more than two times after kiwifruit juice treatment. Myosin degradation was observed in kiwifruit juice-treated meat samples.

Ha et al. [69] investigated the effect of different plant proteases (papain, bromelain, actinidin, and zingibain) on beef proteins and reported significant differences in protease activity. Myofibril proteins were most effectively hydrolyzed by the actinidin while connective tissue proteins by the zingibain. Thus, these enzymes have the potential for specific tenderizing applications. In another study, Ha et al. [70] reported that the protease activity of kiwifruit protease extract was more effective at hydrolyzing beef (myofibrillar and collagen) proteins than the asparagus protease extract. The two protease extracts hydrolyze myofibrillar and collagen proteins differently. Thus, these proteases can be used to improve the tenderness of specific cuts of meat, based on their intrinsic protein composition.

Enzyme extract prepared from *Calotropis procera* latex was applied to different meat samples (pork, beef and chicken), and it was observed that moisture content decreased in all the enzyme-treated samples. Firmness and toughness values of the meat samples decreased significantly with the increased concentration of enzyme extract. The enzyme extract had no significant effect on water holding capacity and cooking yield of the treated samples. Protein solubility and TCA-soluble peptides content increased in all the treated samples. The enzyme extract resulted in extensive proteolysis in the treated samples [78].

Abdel-Naeem and Mohamed [75] investigated the effect of ginger extract, papain, and their mixture on camel meat burger patties and reported that the ginger, papain, and their mixture increased the collagen solubility and sensory scores and decreased the shear force values, significantly. Ginger extract led to extensive fragmentation of myofibrils, while papain extract resulted in the degradation of connective tissue. Also, these extracts improved the lipid stability of treated burger patties during storage.

Choe et al. [79] prepared a crude extract from Asian pear and reported its meat tenderization activity. In another study, Nam et al. [71] investigated the effect of purified pear protease on beef myofibrillar proteins, individually and in combination with fig and kiwifruit proteases, and reported that pear protease has a good proteolytic activity on beef myofibrillar proteins, especially in combination with purified kiwifruit protease.

Wang et al. [80] investigated the effect of *Actinidia arguta* fruit protease on meat tenderness and reported that the protease was effective in tenderizing beef and decomposing actomyosin. Zhang et al. [41] studied the effect of actinidin from seven Chinese kiwifruit cultivars on pork and rabbit meat and reported that actinidin treatment improved the tenderness in pork and rabbit meat. The shear force was decreased by more than half in pork and rabbit meat using the purified actinidin at a dosage of 0.5 mg/100 g muscle.

Plant proteases in combination with other techniques can have promising effects on meat tenderness. Barekat and Soltanizadeh [76] studied the effect of papain enzyme alone or in combination with ultrasonication and reported that the application of enzyme, either alone or in combination with ultrasound, significantly reduced the filtering residue, Warner–Bratzler shear force, and textural parameters. The highest proteolytic activity and tenderness were observed using the combined treatment at ultrasonic power of 100 W for 20 min.

4.3 Bioactive Peptide Production

Bioactive peptides are specific protein fragments that have a positive effect on body functions and may influence health [130]. These are short sequences of amino acids (~2–30) with a low molecular weight that may be generated from both animal [94, 104, 131] and plant sources [88, 86, 103, 132]. These peptides are inactive within the sequence of the parent protein but become active and exert a positive impact on body functions once released. The amino acid composition and sequence of a bioactive peptide determines its activity [133].

Bioactive peptides have high potential to be used as nutraceuticals and functional foods to improve health and decrease the chances of disease occurrence. These peptides have positive effect on the immune, cardiovascular, nervous, and intestinal systems [134]. These peptides act as antimicrobials and antioxidants [135–137]. They have cholesterol-lowering effect and antihypertensive, antithrombotic, immunomodulatory [138, 139], and anticariogenic properties [140].

Bioactive peptides are produced during food processing or from food by-products by microbial fermentation or by chemical/enzymatic hydrolysis using proteolytic enzymes derived from animals, microorganisms, or plants [141, 142]. Enzymatic hydrolysis is the most common process used for bioactive peptide production. Plant proteases have been used for bioactive peptide production from both the plant and animal sources. The plant proteases such as papain, bromelain, ficin, actinidin, zingibain, salpichroin, pomiferin, cyprosin, cardosins, cynarases, and onopordosin have been used to produce bioactive peptides.

Bah et al. [94] reported that plant proteases (papain and bromelain) generated hydrolysates of plasma of various animals (deer, sheep, and pig) with a lower yield of soluble peptides compared with fungal proteases. The hydrolysates produced with plant proteases showed lower DPPH radical scavenging, oxygen radical scavenging capacity, and ferric reducing antioxidant power in comparison with those produced with fungal proteases. In another study, Bah et al. [95] prepared protein hydrolysates from red blood cell fractions separated from the blood of various animals (deer, sheep, pig, and cattle) using plant and fungal proteases and reported that papain-generated hydrolysates from red blood cell fractions exhibited higher ferric reducing antioxidant power and oxygen radical absorbance capacity compared to those generated with bromelain and fungal proteases. Bah et al. [96] reported that the hydrolysates of cattle plasma generated with fungal protease had higher antioxidant activities than hydrolysates generated with papain and bromelain.

Rocha et al. [104] used a protease from *Salpichroa origanifolia* fruits to hydrolyze whey protein concentrates and reported that this protease hydrolyzed α-lactalbumin protein with a higher affinity than β-lactoglobulin. The fraction containing peptides with a molecular mass below 3 kDa demonstrated a strong DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging effect.

4.4 Flour/Dough Modification

Enzymatic modification of food components is an effective means to improve their techno-functional properties. Proteases are used in the baking industries to hydrolyze gluten for making gluten-free products or improve various other functional properties of the flour such as modifications of dough handling properties, gluten elasticity, and texture accompanying shorter mixing time and larger loaf volume [143]. Some of the plant proteases such as papain, bromelain, and caricain have been used in flour/dough modification in baking industry.

Tanabe et al. [112] used bromelain to hydrolyze wheat glutenin IgE epitope and produce hypoallergenic wheat flour. The bread prepared from hypoallergenic wheat flour showed higher sensory scores than the control bread made from soft flour.

Chen et al. [113] studied the effects of the papain hydrolysis on wheat flour pasting properties and reported that papain hydrolysis significantly affected the pasting properties of flour by changing the structural characteristics, amylase activity, and exothermic transition, especially during the early stage of hydrolysis. The pasting parameters (peak and setback) significantly decreased with the increase in papain concentration. Pasting temperature and pasting time increased significantly with the increase in papain concentration. In another study, Yang et al. [114] studied the effect of papain on fresh whole wheat dough browning index and rheology and reported that papain had no effect on carotenoid content of dough. The higher papain concentration (0.010%, w/w) decreased the polyphenol oxidase activity, which may be due to either binding or hydrolysis of the specific amino acid sites of polyphenol oxidase. Papain reduced the browning rate of whole wheat dough as compared to the control dough. Rheological investigations revealed that papain led to decreases of elastic (G') and viscous modulus (G'') of doughs and this may be due to the degradation of flour protein and liberation of amylolytic enzymes by papain.

Hatta et al. [115] reported that papain increased the specific volume of gluten-free rice breads and decreased crumb hardness compared with control breads. Scanning electron microscopy revealed many fine bubble cells in the crumb of the papain-treated rice breads. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of protein in the rice batter revealed that the concentration of low molecular weight proteins (less than 10 kDa) increased with the use of papain compared to control rice batter. The small protein aggregates were formed through disulfide bonds, and this network helped to retain CO_2 gas during the fermentation process. This resulted in an increase in the specific volume and a decrease in the crumb hardness of gluten-free rice bread. Li et al. [116] optimized and evaluated the effect of different enzymes on wheat flour and reported that alcalase and papain had greater ability to reduce

gliadin content of wheat flour than flavourzyme, pepsin, trypsin, or α-chymotrypsin. The sequential treatment of wheat flour by alcalase–papain was more effective in decreasing gliadin content than single enzyme treatment. The optimized conditions of sequential enzymatic treatment resulted in complete removal of gliadin in the flour extract, showing lowest IgE binding, and thus may be used for preparing low allergenic wheat products.

Buddrick et al. [117] investigated the effect of enzyme caricain to reduce gliadin content in whole wheat flour and prepare gluten-free bread. The authors reported that the caricain was more effective in reducing the gliadin content in treated breads compared to control breads.

5 Conclusion

Proteases hydrolyze protein molecules into peptides and amino acids. In recent decades, interest in plant proteases has grown rapidly. Plants provide an attractive source of plant proteases. They are present in all kinds of plant tissues and, thus, can be extracted from their natural sources or can be prepared using various cell culture techniques. The most widely used plant proteases are papain, bromelain, ficin, actinidin, zingibain, and cardosins. Plant proteases are being used in dairy processing, meat tenderization, bioactive peptide production, and baking industry.

References

1. Sumantha A, Larroche C, Pandey A (2006) Microbiology and industrial biotechnology of food-grade proteases: a perspective. Food Technol Biotechnol 44(2):211–220
2. Palma JM, Sandalio LM, Corpas FJ, Romero-Puertas MC, McCarthy I, Del Rio LA (2002) Plant proteases, protein degradation, and oxidative stress: role of peroxisomes. Plant Physiol Biochem 40:521–530
3. Rawlings ND, Barrett AJ, Bateman A (2010) MEROPS: the peptidase database. Nucleic Acids Res 38:D227–D233
4. Gonzalez-Rabade N, Badillo-Corona JA, Aranda-Barradas JS, Oliver-Salvador MC (2011) Production of plant proteases in vivo and in vitro–a review. Biotechnol Adv 29:983–996
5. Schaller A (2004) A cut above the rest: the regulatory function of plant proteases. Planta 220:183–197
6. Van der Hoorn RAL (2008) Plant proteases: from phenotypes to molecular mechanisms. Annu Rev Plant Biol 59:191–223
7. Uhlig H (1998) Industrial enzymes and their applications. Willey, New York
8. Barros RM, Ferreira CA, Silva SV, Malcata FX (2001) Quantitative studies on the enzymatic hydrolysis of milk proteins brought about by cardosins precipitated by ammonium sulfate. Enzym Microb Technol 29:541–547
9. Esteves CLC, Lucey JA, Pires EMV (2002) Rheological properties of milk gels made using coagulants of plant origin and chymosin. Int Dairy J 12:427–434
10. Esteves CLC, Lucey JA, Hyslop DB, Pires EMV (2003) Effect of gelation temperature on the properties of skim milk gels made from plant coagulants and chymosin. Int Dairy J 13:877–885

11. Sanjuan E, Millan R, Saavedra P, Carmona MA, Gomez R, Fernandez-Salguero J (2002) Influence of animal and vegetable rennet on the physiochemical characteristics of Los Pedroches cheese during ripening. Food Chem 78:281–289
12. Silva SV, Allmere T, Malcata FX, Andrén A (2003) Comparative studies on the gelling properties of cardosins extracted from *Cynara cardunculus* and chymosin on cow's skim milk. Int Dairy J 13:558–564
13. Silva SV, Malcata FX (2005) Studies pertaining to coagulant and proteolytic activities of plant proteases from *Cynara cardunculus*. Food Chem 89:19–26
14. Low YH, Agboola S, Zhao J, Lim MY (2006) Clotting and proteolytic properties of plant coagulants in regular and ultrafiltered bovine skim milk. Int Dairy J 16:335–343
15. Tejada L, Abellan A, Cayuela JM, Martinez-Cacha A (2006) Sensorial characteristics during ripening of the Murcia al Vino goat's milk cheese. The effect of the type of coagulant used and the size of the cheese. J Sens Stud 21:333–347
16. Tejada L, Abellan A, Cayuela JM, Martinez-Cacha A, Fernandez-Salguero J (2008) Proteolysis in goats' milk cheese made with calf rennet and plant coagulant. Int Dairy J 18:139–146
17. Galan E, Prados F, Pino A, Tejada L, Fernandez-Salguero J (2008) Influence of different amounts of vegetable coagulant from cardoon *Cynara cardunculus* and calf rennet on the proteolysis and sensory characteristics of cheeses made with sheep milk. Int Dairy J 18:93–98
18. Agboola SO, Chan HH, Zhao J, Rehman A (2009) Can the use of Australian cardoon (*Cynara cardunculus* L.) coagulant overcome the quality problems associated with cheese made from ultrafiltered milk? LWT Food Sci Technol 42:1352–1359
19. Pino A, Prados F, Galan E, McSweeney PLH, Fernandez-Salguero J (2009) Proteolysis during the ripening of goats' milk cheese made with plant coagulant or calf rennet. Food Res Int 42:324–330
20. Ordiales E, Martin A, Benito MJ, Hernandez A, Ruiz-Moyano S, Cordoba MG (2012) Technological characterisation by free zone capillary electrophoresis (FZCE) of the vegetable rennet (*Cynara cardunculus*) used in "Torta del Casar" cheese-making. Food Chem 133:227–235
21. Ben Amira A, Makhlouf I, Petryt RF, Francis F, Bauwens J, Attia H, Besbes S, Blecker C (2017) Effect of extraction pH on techno-functional properties of crude extracts from wild cardoon (*Cynara cardunculus* L.) flowers. Food Chem 225:258–266
22. Ben Amira A, Mokni A, Yaich H, Chaabouni M, Besbes S, Blecker C, Attia H (2017) Technological properties of milk gels produced by chymosin and wild cardoon rennet optimized by response surface methodology. Food Chem 237:150–158
23. Ben Amira A, Blecker C, Richel A, Arias AA, Fickers P, Francis F, Besbes S, Attia H (2018) Influence of the ripening stage and the lyophilization of wild cardoon flowers on their chemical composition, enzymatic activities of extracts and technological properties of cheese curds. Food Chem 245:919–925
24. Liburdi K, Spinelli SE, Benucci I, Lombardelli C, Esti M (2018) A preliminary study of continuous milk coagulation using *Cynara cardunculus* flower extract and calf rennet immobilized on magnetic particles. Food Chem 239:157–164
25. Llorente BE, Brutti CB, Natalucci CL, Caffini NO (1997) Partial characterization of a milk clotting proteinase isolated from artichoke (*Cynara scolymus* L., Asteraceae). Acta Farm Bonaer 16:37–42
26. Llorente BE, Brutti CB, Caffini NO (2004) Purification and characterization of a milk-clotting aspartic proteinase from globe artichoke (*Cynara scolymus* L.). J Agric Food Chem 52:8182–8189
27. Llorente BE, Obregon WD, Aviles FX, Caffini NO, Vairo-Cavalli S (2014) Use of artichoke (*Cynara scolymus*) flower extract as a substitute for bovine rennet in the manufacture of gouda type cheese:characterization of aspartic proteases. Food Chem 159:55–63
28. Sidrach L, Garcia-Canovas F, Tudela J, Rodriguez-Lopez JN (2005) Purification of cynarases from artichoke (*Cynara scolymus* L.): enzymatic properties of cynarase A. Phytochemistry 66:41–49

29. Chazarra S, Sidrach L, Lopez-Molina D, Rodriguez-Lopez JN (2007) Characterization of the milk-clotting properties of extracts from artichoke (*Cynara scolymus* L) flowers. Int Dairy J 17:1393–1400
30. Esposito M, Di Pierro P, Dejonghe W, Mariniell L, Porta R (2016) Enzymatic milk clotting activity in artichoke (*Cynara scolymus*) leaves and alpine thistle (*Carduus defloratus*) flowers. Immobilization of alpine thistle aspartic protease. Food Chem 204:115–121
31. Brutti CB, Pardo MF, Caffini NO, Natalucci CL (2012) *Onopordum acanthium* L. (Asteraceae) flowers as coagulating agent for cheesemaking. LWT Food Sci Technol 45:172–179
32. Asakura T, Watanabe H, Keiko A, Soichi A (1997) Oryzasin as an aspartic proteinase occurring in rice seeds: purification, characterization, and application to milk-clotting. J Agric Food Chem 45:1070–1075
33. Lufrano D, Faro R, Castanheira P, Parisi G, Verissimo P, Vairo-Cavalli S, Simoes I, Faro C (2012) Molecular cloning and characterization of procirsin, an active aspartic protease precursor from *Cirsium vulgare* (Asteraceae). Phytochemistry 81:7–18
34. Devaraj KB, Gowda LR, Prakash V (2008) An unusual thermostable aspartic protease from the latex of *Ficus racemosa* (L.). Phytochemistry 69:647–655
35. Faccia M, Picariello G, Trani A, Loizzo P, Gambacorta G, Lamacchia C, Di Luccia A (2012) Proteolysis of Cacioricotta cheese made from goat milk coagulated with caprifig (*Ficus carica sylvestris*) or calf rennet. Eur Food Res Technol 234:527–533
36. Hashim MM, Mingsheng D, Iqbal MF, Xiaohong C (2011) Ginger rhizome as a potential source of milk coagulating cysteine protease. Phytochemistry 72:458–464
37. Gagaoua M, Hoggas N, Hafid K (2015) Three phase partitioning of zingibain, a milk-clotting enzyme from *Zingiber officinale* Roscoe rhizomes. Int J Biol Macromol 73:245–252
38. Katsaros GI, Tavantzis G, Taoukis PS (2010) Production of novel dairy products using actinidin and high pressure as enzyme activity regulator. Innov Food Sci Emerg Technol 11:47–51
39. Grozdanovic MM, Burazer L, Gavrovic-Jankulovic M (2013) Kiwifruit (*Actinidia deliciosa*) extract shows potential as a low-cost and efficient milk-clotting agent. Int Dairy J 32:46–52
40. Puglisi I, Petrone G, Lo Piero AR (2014) A kiwi juice aqueous solution as coagulant of bovine milk and its potential in mozzarella cheese manufacture. Food Bioprod Process 92:67–72
41. Zhang B, Sun Q, Liu HJ, Li SZ, Jiang ZQ (2017) Characterization of actinidin from Chinese kiwifruit cultivars and its applications in meat tenderization and production of angiotensin I-converting enzyme (ACE) inhibitory peptides. LWT Food Sci Technol 78:1–7
42. Uchikoba T, Kaneda M (1996) Milk-clotting activity of cucumisin, a plant serine protease from melon fruit. Appl Biochem Biotechnol 56:325–330
43. Yadav RP, Patel AK, Jagannadham MV (2011) Purification and biochemical characterization of a chymotrypsin-like serine protease from *Euphorbia neriifolia* Linn. Process Biochem 46:1654–1662
44. Yadav RP, Patel AK, Jagannadham MV (2012) Neriifolin S, a dimeric serine protease from *Euphorbia neriifolia* Linn.: purification and biochemical characterisation. Food Chem 132:1296–1304
45. Ahmed IAM, Morishima I, Babiker EE, Mori N (2009) Characterisation of partially purified milk-clotting enzyme from *Solanum dubium* Fresen seeds. Food Chem 116:395–400
46. Ahmed IAM, Morishima I, Babiker EE, Mori N (2009) Dubiumin, a chymotrypsin-like serine protease from the seeds of *Solanum dubium* Fresen. Phytochemistry 70:483–491
47. Kumari M, Sharma A, Jagannadham MV (2010) Decolorization of crude latex by activated charcoal, purification and physico-chemical characterization of Religiosin, a milk-clotting serine protease from the latex of *Ficus religiosa*. J Agric Food Chem 58:8027–8034
48. Kumari M, Sharma A, Jagannadham MV (2012) Religiosin B, a milk-clotting serine protease from *Ficus religiosa*. Food Chem 131:1295–1303
49. Sharma A, Kumari M, Jagannadham MV (2012) Religiosin C, a cucumisin-like serine protease from *Ficus religiosa*. Process Biochem 47:914–921

50. Tripathi P, Tomar R, Jagannadham MV (2011) Purification and biochemical characterisation of a novel protease streblin. Food Chem 125:1005–1012
51. Lo Piero AR, Puglisi I, Petrone G (2002) Characterization of "lettucine", a serine-like protease from *Lactuca sativa* leaves, as a novel enzyme for milk clotting. J Agric Food Chem 50:2439–2443
52. Bruno MA, Lazza CM, Errasti ME, López LMI, Caffini NO, Pardo MF (2010) Milk clotting and proteolytic activity of an enzyme preparation from *Bromelia hieronymi* fruits. LWT Food Sci Technol 43:695–701
53. Domingos A, Cardos PC, Xue ZT, Clemente A, Brodelius PE, Pais MS (2000) Purification, cloning and autoproteolytic processing of an aspartic proteinase from *Centaurea calcitrapa*. Eur J Biochem 267:6824–6831
54. Reis PM, Lourenço PL, Domingos A, Clemente AF, Pais MS, Malcata FX (2000) Applicability of extracts from *Centaurea calcitrapa* in ripening of bovine cheese. Int Dairy J 10:775–780
55. Salvador SM, Novo C, Domingos A (2006) Evaluation of the presence of aspartic proteases from *Centaurea calcitrapa* during seed germination. Enzym Microb Technol 38:893–898
56. Pezeshki A, Hesari J, Zonoz AA, Ghambarzadeh B (2011) Influence of *Withania coagulans* protease as a vegetable rennet on proteolysis of Iranian UF white cheese. J Agric Sci Technol 13:567–576
57. Salehi M, Aghamaali MR, Saajedi RH, Asghari SM, Jorjani E (2017) Purification and characterization of a milk-clotting aspartic protease from *Withania coagulans* fruit. Int J Biol Macromol 98:847–854
58. Vairo-Cavalli S, Claver S, Priolo N, Natalucci C (2005) Extraction and partial characterization of a coagulant preparation from *Silybum marianum* flowers. Its action on bovine caseinate. J Dairy Res 72:271–275
59. Vairo-Cavalli S, Silva SV, Cimino C, Malcata FX, Priolo N (2008) Hydrolysis of caprine and ovine milk proteins, brought about by aspartic peptidases from *Silybum marianum* flowers. Food Chem 106:997–1003
60. Tamer MI (1993) Identification and partial purification of a novel milk-clotting enzyme from *Onopordum turcicum*. Biotechnol Lett 13:427–432
61. Egito AS, Girardet JM, Laguna LE, Poirson C, Molle D, Miclo L, Humbert G, Gaillard JL (2007) Milkclotting activity of enzyme extracts from sunflower and albizia seeds and specific hydrolysis of bovine κ-casein. Int Dairy J 17:816–825
62. Duarte AR, Duarte DMR, Moreira KA, Cavalcanti MTH, de Lima-Filho JL, Porto ALF (2009) *Jacaratia corumbensis* O. Kuntze a new vegetable source for milk-clotting enzymes. Braz Arch Biol Technol 52(1):1–9
63. Pontual EV, Carvalho BEA, Bezerra RS, Coelho LCBB, Napoleao TH, Paiva PMG (2012) Caseinolytic and milk-clotting activities from *Moringa oleifera* flowers. Food Chem 135:1848–1854
64. Nestor GM, Rubi CGD, Hector JC (2012) Exploring the milk-clotting properties of a plant coagulant from the berries of *S. elaeagnifoliun* var. Cavanillies. J Food Sci 71:89–94
65. Beka RG, Krier F, Botquin M, Guiama VD, Donn P, Libouga DG, Mbofung CM, Dimitrov K, Slomianny MC, Guillochon D, Vercaigne-Marko D (2014) Characterisation of a milk-clotting extract from *Balanites aegyptiaca* fruit pulp. Int Dairy J 34:25–31
66. Bey N, Debbebi H, Abidi F, Marzouki MN, Salah AB (2018) The non-edible parts of fennel (*Foeniculum vulgare*) as a new milk-clotting protease source. Ind Crop Prod 112:181–187
67. Toohey ES, Kerr MJ, van de Ven R, Hopkins DL (2011) The effect of a kiwi fruit based solution on meat traits in beef m. semimembranosus (topside). Meat Sci 88:468–471
68. Liu C, Xiong YL, Rentfrow GK (2011) Kiwifruit protease extract injection reduces toughness of pork loin muscle induced by freeze-thaw abuse. LWT-Food Sci Technol 44:2026–2031
69. Ha M, Bekhit AEDA, Carne A, Hopkins DL (2012) Characterisation of commercial papain, bromelain, actinidin and zingibain protease preparations and their activities toward meat proteins. Food Chem 134:95–105

70. Ha M, Bekhit AEDA, Carne A, Hopkins DL (2013) Characterisation of kiwifruit and asparagus enzyme extracts, and their activities toward meat proteins. Food Chem 136:989–998
71. Nam SH, Kim YM, Walsh MK, Yim SH, Eun JB (2016) Functional characterization of purified pear protease and its proteolytic activities with casein and myofibrillar proteins. Food Sci Biotechnol 25(S):31–39
72. Chaurasiya RS, Sakhare PZ, Bhaskar N, Hebbar HU (2015) Efficacy of reverse micellar extracted fruit bromelain in meat tenderization. J Food Sci Technol 52:870–880
73. Ramezani R, Aminlari M, Fallahi H (2003) Effect of chemically modified soy proteins and ficin-tenderized meat on the quality attributes of sausage. J Food Sci 68:85–88
74. Akpan IP, Omojola AB (2015) Quality attributes of crude papain injected beef. J Meat Sci Technol 3:42–46
75. Abdel-Naeem HH, Mohamed HM (2016) Improving the physico-chemical and sensory characteristics of camel meat burger patties using ginger extract and papain. Meat Sci 118:52–60
76. Barekat S, Soltanizadeh N (2017) Improvement of meat tenderness by simultaneous application of high-intensity ultrasonic radiation and papain treatment. Innov Food Sci Emerg Technol 39:223–229
77. Naveena BM, Mendiratta SK, Anjaneyulu ASR (2004) Tenderization of buffalo meat using plant proteases from *Cucumis trigonus* Roxb (Kachri) and *Zingiber officinale* roscoe (ginger rhizome). Meat Sci 68:363–369
78. Rawdkuen S, Jaimakreu M, Benjakul S (2013) Physicochemical properties and tenderness of meat samples using proteolytic extract from *Calotropis procera* latex. Food Chem 136:909–916
79. Choe IS, Park YJ, Ishioroshi M, Samejima K (1996) A new protease in Korean pears as meat tenderizer. Anim Sci Technol 67:43–46
80. Wang J, Liu H, Wang H, Cui M, Jin Q, Jin T, Cui F, Cui T, Liang C, Kim B, Li G (2016) Isolation and characterization of a protease from the *Actinidia arguta* fruit for improving meat tenderness. Food Sci Biotechnol 25(4):1059–1064
81. Lee JS, Yoo MA, Koo SH, Baek HH, Lee HG (2008) Antioxidant and ACE inhibitory activities of soybean hydrolysates: effect of enzyme and degree of hydrolysis. Food Sci Biotechnol 17:873–877
82. Chen YC, Chang HS, Wang CT, Cheng FY (2009) Antioxidative activities of hydrolysates from duck egg white using enzymatic hydrolysis. Asian-Aust J Anim Sci 22:1587–1593
83. Udenigw CC, Lin YS, Hou WC, Aluko RE (2009) Kinetics of the inhibition of renin and angiotensin I-converting enzyme by flaxseed protein hydrolysate fractions. J Funct Foods 1:199–207
84. Chen C, Chi YJ (2012) Antioxidant, ACE inhibitory activities and functional properties of egg white protein hydrolysate. J Food Biochem 36:383–394
85. Gu RZ, Liu WY, Lin F, Jin ZT, Chen L, Yi WX, Lu J, Cai MY (2012) Antioxidant and angiotensin I-converting enzyme inhibitory properties of oligopeptides derived from blackbone silky fowl (*Gallus gallus domesticus* Brisson) muscle. Food Res Int 49:326–333
86. Guo X, Zhang J, Ma Y, Tian S (2013) Optimization of limited hydrolysis of proteins in rice residue and characterization of the functional properties of the products. J Food Process Preserv 37:245–253
87. Luo HY, Wang B, Li ZR, Chi CF, Zhanga QH, He GY (2013) Preparation and evaluation of antioxidant peptide from papain hydrolysate of *Sphyrna lewini* muscle protein. LWT-Food Sci Technol 51:281–288
88. Margatan W, Ruud K, Wang Q, Markowski T, Ismail B (2013) Angiotensin converting enzyme inhibitory activity of soy protein subjected to selective hydrolysis and thermal processing. J Agric Food Chem 61:3460–3467
89. Lafarga T, Aluko RE, Rai DK, O'Connor P, Hayes M (2016) Identification of bioactive peptides from a papain hydrolysate of bovine serum albumin and assessment of an antihypertensive effect in spontaneously hypertensive rats. Food Res Int 81:91–99

90. Zarei M, Ebrahimpour A, Abdul-Hamid A, Anwar F, Saari N (2012) Production of defatted palm kernel cake protein hydrolysate as a valuable source of natural antioxidants. Int J Mol Sci 13:8097–8111
91. Zarei M, Ghanbari R, Tajabadi N, Abdul-Hamid A, Bakar FA, Saari N (2016) Generation, fractionation, and characterization of iron-chelating protein hydrolysate from palm kernel cake proteins. J Food Sci 81:C341–C347
92. Hayes M, Mora L, Hussey K, Aluko RE (2016) Boarfish protein recovery using the pH shift process and generation of protein hydrolysates with ACE-I and antihypertensive bioactivities in spontaneously hypertensive rats. Innov Food Sci Emerg Technol 37:253–260
93. Gajanan PG, Elavarasan K, Shamasundar BA (2016) Bioactive and functional properties of protein hydrolysates from fish frame processing waste using plant proteases. Environ Sci Pollut Res 23:24901–24911
94. Bah CSF, Bekhit AEDA, Carne A, McConnell MA (2015) Production of bioactive peptide hydrolysates from deer, sheep and pig plasma using plant and fungal protease preparations. Food Chem 176:54–63
95. Bah CSF, Carne A, McConnell MA, Mros S, Bekhit AEDA (2016a) Production of bioactive peptide hydrolysates from deer, sheep, pig and cattle red blood cell fractions using plant and fungal protease preparations. Food Chem 202:458–466
96. Bah CSF, Bekhit AEDA, McConnell MA, Carne A (2016b) Generation of bioactive peptide hydrolysates from cattle plasma using plant and fungal proteases. Food Chem 213:98–107
97. Abdel-Hamid M, Otte J, De Gobba C, Osman A, Hamad E (2017) Angiotensin I converting enzyme inhibitory activity and antioxidant capacity of bioactive peptides derived from enzymatic hydrolysis of buffalo milk proteins. Int Dairy J 66:91–98
98. Liu Z, Dong S, Xu J, Zeng M, Song H, Zhao Y (2008) Production of cysteine-rich antimicrobial peptide by digestion of oyster (*Crassostrea gigas*) with alcalase and bromelin. Food Control 19:231–235
99. Wang B, Li ZR, Chi CF, Zhang QH, Luo HY (2012) Preparation and evaluation of antioxidant peptides from ethanol-soluble proteins hydrolysate of *Sphyrna lewini* muscle. Peptides 36:240–250
100. Bordbar S, Anwar F, Ebrahimpour A, Saari N, Hamid AA, Manap MYA (2013) The improvement of the endogenous antioxidant property of stone fish (*Actinopyga lecanora*) tissue using enzymatic proteolysis. BioMed Res Int. https://doi.org/10.1155/2013/849529
101. Elevarasan K, Kumar VN, Shamasundar BA (2014) Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (*Catla catla*) as influenced by the nature of enzyme. J Food Process Preserv 38:1207–1214
102. Medeiros V, Rainha N, Paiva L, Lima E, Baptista J (2014) Bovine milk formula based on partial hydrolysis of caseins by bromelain enzyme: better digestibility and angiotensin-converting enzyme-inhibitory properties. Int J Food Prop 17:806–817
103. Teh SS, Bekhit AEDA, Carne A, Birch J (2016) Antioxidant and ACE-inhibitory activities of hemp (*Cannabis sativa* L.) protein hydrolysates produced by the proteases AFP, HT, pro-G, actinidin and zingibain. Food Chem 203:199–206
104. Rocha GF, Kise F, Rosso AM, Parisi MG (2017) Potential antioxidant peptides produced from whey hydrolysis with an immobilized aspartic protease from *Salpichroa origanifolia* fruits. Food Chem 237:350–355
105. Corrons MA, Bertucci JI, Liggieri CS, Lopez LMI, Bruno MA (2012) Milk clotting activity and production of bioactive peptides from whey using *Maclura pomifera* proteases. LWT-Food Sci Technol 47:103–109
106. Corrons MA, Liggieri CS, Trejo SA, Bruno MA (2017) ACE-inhibitory peptides from bovine caseins released with peptidases from *Maclura pomifera* latex. Food Res Int 93:8–15
107. Bertucci JA, Liggieri CS, Colombo ML, Vairo-Cavalli SE, Bruno MA (2015) Application of peptidases from *Maclura pomifera* fruit for the production of active biopeptides from whey protein. LWT-Food Sci Technol 64:157–163

108. Tavares T, Montiero KM, Possenti A, Pintado ME, Carvalho JE, Malcata FX (2011) Anti-ulcerogenic activity of peptide concentrates obtained from hydrolysis of whey proteins by proteases from *Cynara cardunculus*. Int Dairy J 21:934–939
109. Tavares TG, Malcata FX (2012) The Portuguese paradox: why do some inhabitants of Portugal appear to live so long when their diet is based on whey cheese? Food Chem 131:727–729
110. Tavares T, Spindola H, Longato G, Pintado ME, Carvalho JE, Malcata FX (2013) Anti-nociceptive and anti-inflammatory effects of novel dietary protein hydrolysate produced from whey by proteases of *Cynara cardunculus*. Int Dairy J 32:156–162
111. Silva SV, Pihlanto A, Malcata FX (2006) Bioactive peptides in ovine and caprine cheeselike systems prepared with proteases from *Cynara cardunculus*. J Dairy Sci 89:3336–3344
112. Tanabe S, Arai S, Watanabe M (1996) Modification of wheat flour with bromelain and baking hypoallergenic bread with added ingredients. Biosci Biotechnol Biochem 60(8):1269–1272
113. Chen JS, Tian JC, Deng ZY, Zhang YX, Feng SL, Yan ZC, Zhang XY, Yuan Q (2012) Effects of papain hydrolysis on the pasting properties of wheat flour. J Integr Agric 11(12):1948–1957
114. Yang T, Bai Y, Wu F, Yang N, Zhang Y, Bashari M, Jin Z, Xu X (2014) Combined effects of glucose oxidase, papain and xylanase on browning inhibition and characteristics of fresh whole wheat dough. J Cereal Sci 60:249–254
115. Hatta E, Matsumoto K, Honda Y (2015) Bacillolysin, papain, and subtilisin improve the quality of gluten-free rice bread. J Cereal Sci 61:41–47
116. Li Y, Yu J, Goktepe I, Ahmedna M (2016) The potential of papain and alcalase enzymes and process optimizations to reduce allergenic gliadins in wheat flour. Food Chem 196:1338–1345
117. Buddrick O, Cornell HJ, Small DM (2015) Reduction of toxic gliadin content of wholegrain bread by the enzyme caricain. Food Chem 170:343–347
118. Sun J, Wang M, Cao J, Zhao Y, Jiang W (2010) Characterization of three novel alkaline serine proteases from tomato (*Lycopersicum esculentum* mill.) fruit and their potential application. J Food Biochem 34:1014–1031
119. Li Z, Scott K, Hemar Y, Zhang H, Otter D (2018) Purification and characterisation of a protease (tamarillin) from tamarillo fruit. Food Chem. https://doi.org/10.1016/j.foodchem.2018.02.091
120. DiCosmo F, Misawa M (1995) Plant cell and tissue culture: alternatives for metabolite production. Biotechnol Adv 13:425–453
121. Shah MA, Mir SA, Paray MA (2014) Plant proteases as milk-clotting enzymes in cheesemaking: a review. Dairy Sci Technol 94:5–16
122. Tamer MI, Mavituna F (1996) Protease from callus and cell suspension cultures of *Onopordum turcicum* (Compositae). Biotehcnol Lett 18:361–366
123. Perez A, Laudat T, Mora M, Carvajal C, Aragon C, Gonzalez J, Escalona M, Daquinta M, Trujillo R, Hernandez M, Lorenzo JC (2013) Micropropagation of *Hohenbergia penduliflora* (A. Rich.) Mez. for sustainable production of plant proteases. Acta Physiol Plant 35:2525–2537
124. Tamer MI, Mavituna F (1997) Protease from freely suspended and immobilized *Mirabilis jalapa*. Process Biochem 32:195–200
125. Raposo S, Domingos A (2008) Purification and characterization milk-clotting aspartic proteases from *Centaurea calcitrapa* cell suspension cultures. Process Biochem 43:139–144
126. Cimino C, Cavalli SV, Spina F, Natalucci C, Priolo N (2006) Callus culture for biomass production of milk thistle as a potential source of milk-clotting peptidases. Electron J Biotechnol 9:237–240
127. Oliveira A, Perira C, Soares da Costa D, Teixeira J, Fidalgo F, Pereira S, Pissarra J (2010) Characterization of aspartic proteinases in *C. cardunculus* L. callus tissue for its prospective transformation. Plant Sci 178:140–146
128. Feijoo-Siota L, Rama JLR, Sanchez-Perez A, Villa TG (2018) Expression, activation and processing of a novel plant milk-clotting aspartic protease in *Pichia pastoris*. J Biotechnol 268:28–39

129. Roseiro LB, Barbosa M, Ames JM, Wilbey RA (2003) Cheesemaking with vegetable coagulants-the use of *Cynara* L. for the production of ovine cheeses. Int J Dairy Technol 56:76–85
130. Kitts DD, Weiler K (2003) Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. Curr Pharm Des 9:1309–1323
131. Di Bernardini R, Harnedy P, Bolton D, Kerry J, O'Neill E, Mullen AM, Hayes M (2011) Antioxidant and antimicrobial peptidic hydrolysates from muscle protein sources and by-products. Food Chem 124(4):1296–1307
132. Maestri E, Marmiroli M, Marmiroli N (2016) Bioactive peptides in plant-derived foodstuffs. J Proteome 147:140–155
133. Lafarga T, Hayes M (2014) Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. Meat Sci 98:227–239
134. Udenigwe CC, Aluko RE (2012) Food protein-derived bioactive peptides: production, processing, and potential health benefits. J Food Sci 71:R11–R24
135. Arruda MS, Silva FO, Egito AS, Silva TMS, Lima-Filho JL, Porto ALF, Moreira KA (2012) New peptides obtained by hydrolysis of caseins from bovine milk by protease extracted from the latex *Jacaratia corumbensis*. LWT-Food Sci Technol 49:73–79
136. Dabrowska A, Szoltysik M, Babij K, Pokora M, Zambrowicz A, Chrzanowska J (2013) Application of Asian pumpkin (*Cucurbita ficifolia*) serine proteinase for production of biologically active peptides from casein. Acta Biochim Pol 60:117–122
137. Memarpoor-Yazdi M, Asoodeh A, Chamania J (2012) A novel antioxidant and antimicrobial peptide from hen egg white lysozyme hydrolysates. J Funct Foods 4:278–286
138. Hosomi R, Fukunaga K, Arai H, Kanda S, Nishiyama T, Yoshida M (2012) Fish protein hydrolysates affect cholesterol metabolism in rats fed non-cholesterol and high cholesterol diets. J Med Food 15:299–306
139. Memarpoor-Yazdi MA, Ahmad A, Chamani J (2012) Structure and ACE-inhibitory activity of peptides derived from hen egg white lysozyme. Int J Pept Res Ther 18:353–360
140. Mazorra-Manzano MA, Ramirez-Suarez JC, Yada RY (2017) Plant proteases for bioactive peptides release: a review. Crit Rev Food Sci Nutr. https://doi.org/10.1080/10408398.2017.1308312
141. Korhonen H, Pihlanto A (2006) Bioactive peptides: production and functionality. Int Dairy J 16:945–960
142. Mora L, Sentandreu MA, Koistinen KM, Fraser PD, Toldra F, Bramley PM (2009) Naturally generated small peptides derived from myofibrillar proteins in serrano dry-cured ham. J Agric Food Chem 57(8):3228–3234
143. Woods FC, Bruinsma BL, Kinsella JE (1980) Note on the effects of protease from *Saccharomyces-carlsbergensis* on dough strength. Cereal Chem 57:290–293

Part III

Lipids and Their Biological Activity

Bioactive Lipids

17

Luis Vázquez, Marta Corzo-Martínez, Pablo Arranz-Martínez, Elvira Barroso, Guillermo Reglero, and Carlos Torres

Contents

L. Vázquez · M. Corzo-Martínez · P. Arranz-Martínez · E. Barroso · C. Torres (✉)
Department of Production and Characterization of Novel Foods, Institute of Food Science Research (CIAL,CSIC-UAM), Madrid, Spain
e-mail: luis.vazquez@uam.es; marta.corzo@uam.es; pablo.arranz@uam.es; Elvira.barroso@uam.es; carlos.torres@uam.es

G. Reglero
Department of Production and Characterization of Novel Foods, Institute of Food Science Research (CIAL,CSIC-UAM), Madrid, Spain

Department of Production and Development of Foods for Health, IMDEA-Food Institute, CEI (UAM-CSIC), Madrid, Spain
e-mail: guillermo.reglero@uam.es

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_58

Abstract

Historically, lipids have been considered just as a source of energy for our bodies and as the basic unit of membranes. The discovery of the platelet-activating factor in 1979 as one of the first biologically active phospholipids was a relevant landmark in this field. Since then, some unique biological activities have been assigned to every single lipid class. For example, lipids, as small hydrophobic molecules, are extraordinary as chemical messengers to send information between organelles and to other cells. Additionally, polar lipids that contain hydrophobic and hydrophilic regions can interact distinctly with membrane proteins modulating their activities, while glycosphingolipids including their structure complex carbohydrates can play important roles in the immune system. Therefore, in this chapter, many relevant biological functions of some lipid classes from dietary sources will be extensively reviewed.

Keywords

Free fatty acids · Glyceryl ethers · Phospholipids · Isoprenoids · Phenolic lipids · Lipid delivery systems

1 Introduction

For many years, fats have been associated with dangerous substances to our health and consequently should be minimized or avoided in our diet. Fortunately, more and more people recognize that fats are essential ingredients for food palatability. More interestingly, some lipids such as cholesterol have been recognized as essential for the production of hormones, vitamin D, and bile salts, among other physiological functions. Additionally numerous health benefits have been attributed to unsaturated fats from seafood. However, the essential role that lipids play in life similar to proteins and genes is not broadly understood. Very few people are aware that fat is the most relevant part of our brain and plays an extremely important role in all other soft tissues. Lipids are studied by nutritionists who try to find out the relationship between fats in our diet and the composition of different organs and tissues. Biochemists investigate synthesis and breakdown of lipids to produce energy and building material for cells. However, lipids have not been extensively recognized as essential molecules in cell membranes. Lipids form fascinating and intriguing structures as a consequence of basic physical principles of self-organization that governs interaction among many molecules. In this scenario, water plays a key role functioning as the definite biological solvent. The unique properties of water oblige lipid molecules to self-assemble and to produce subtle structures. Consequently, lipids, in aqueous medium, form lipid bilayers and membranes. Lipid membranes can be considered as extended thin layers with the thickness of only two molecules. These structures constitute the backbone of all biological membranes which are omnipresent in all living cells [1].

Dietary bioactive lipids, from a food science point of view, can be generically defined as lipids with specific roles on health. Their function can be related to

physical or chemical properties of these molecules. Numerous evidences point out that lipid classes provide health benefits based on mainly two mechanisms: (1) changing the fatty acid composition of different tissues or (2) promoting cell signaling pathways [2]. Some health benefits can be attributed to short- to medium-chain fatty acids, although most evidences suggest that the most important bioactive lipids are polyunsaturated fatty acids (PUFAs). PUFAs are precursors in the biosynthesis of cellular hormones (eicosanoids) and various signaling compounds with relevant roles in human health [3].

Recently, more and more interest for natural substances with beneficial activity to humans has been observed. Among these substances, those with strong antioxidant activity have brought a lot of attention because oxidative stress induced by multiple factors is the main cause of many pathological conditions. However, many phenolic compounds are mainly hydrophilic which decreases their application in oil-based formulations and emulsions. For that reason, sources, production, and formulation of more lipophilic derivatives of these phenolic compounds will be also discussed in this chapter [4].

2 Neutral Lipids: Fatty Acids

The term "fatty acid" refers to any aliphatic monocarboxylic acids able to be liberated by hydrolysis from naturally existing fats and oils. "Neutral fats" are mono-, di-, or tri-esters of glycerol containing one, two, or three fatty acids and are termed monoacylglycerides, diacylglycerides, and triacylglycerides, respectively [5]. Fats and oils consisting of triglycerides are one of the main components of every living organism, and they are also important constituents of the human diet. Bioactivity and nutritional value of these fats depend on the length and number of unsaturated bonds of the fatty acid chains that they contain. Depending on the length, fatty acids are classified into long-chain fatty acids (LCFAs) with >12 carbons, medium-chain fatty acids (MCFAs) with 6–12 carbons, and short-chain fatty acids (SCFAs) which contain <6 carbon atoms [6]. Their absorption into the human digestive system will depend to a large extent on the stereospecific distribution of the fatty acids in the triglyceride molecule. Long-chain saturated fatty acids located at the outer positions sn-1 and sn-3 have limited absorption, and this may influence lipemia and serum cholesterol levels [7].

2.1 Essential Fatty Acids and Ratio Omega-6/Omega-3

Polyunsaturated fatty acids (PUFAs) have aroused great interest based on their beneficial properties, so there is a trend toward obtaining foods rich in this type of fatty acids. These fatty acids are generally classified into omega-3 fatty acids and omega-6 fatty acids, depending on the position of the first double bond counting from the methylene extreme of the hydrocarbon chain. A number of information sources suggest that humans evolved on a diet whose ratio of omega-6 to omega-3

essential fatty acids was close to 1 [8]. Due to the modern western diets, food processing, and agribusiness, the intake of omega-6 PUFAs has increased, and the omega-3 PUFAs have decreased, leading to a ratio around 20:1 [9]. Although the percentage of total calories ingested from fat is decreasing, nowadays the prevalence of overweight and obesity has increased which represent a major public health problem. The excessive intake of omega-6 PUFAs and very high omega-6/omega-3 ratio contribute to the development of several diseases, including obesity; cardiovascular, autoimmune, and inflammatory diseases; and cancer, whereas increased levels of omega-3 show suppressive effects. High amounts of omega-6 fatty acids, linoleic acid (LA) and arachidonic acid (AA), compete with omega-3 PUFA absorption [10]. There is also a competition for desaturation and elongation enzymes between LA and α-linolenic acid (ALA) [11]. Moreover, anti-inflammatory mediators are cleaved from omega-3 fatty acids, while omega-6 fatty acids provide precursors for pro-inflammatory metabolites [457]. Most of the health benefits associated with omega-3 fatty acids are attributable to the long-chain polyunsaturated fatty acids (LC PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [12].

It has been established that consumption of omega-3 PUFAs ameliorates the risk of cardiovascular disease [13], produces a decrease in blood pressure, and shows a positive association with serum high-density lipoprotein (HDL) and inverse association with very-low-density lipoprotein (VLDL) and triglyceride [14]. Besides, it is associated with lower risk of glucose tolerance and non-insulin-dependent diabetes mellitus [15].

On the other hand, EPA, DHA, and lately docosapentaenoic acid (DPA, 22:5n-3) have shown beneficial effects in a variety of neurodegenerative and neurological conditions [16, 17]. They exert positive effects on neuronal membrane properties (such as membrane fluidity and permeability) and increase brain phospholipid levels, phosphatidylserine, phosphatidylethanolamine (PE), and phosphatidylinositol [18]. They are also promoters of differentiation of neural stem cells via metabolic oxidation [19, 20].

A variety of studies have investigated the relationship of neuroprotection and risk of cognitive decline with the fatty acid composition in the blood (EPA, DPA, and DHA). Specifically, EPA has been associated with slower cognitive decline [21], depressive symptom risk, and dementia risk in the elderly [22]. Inflammation has an important effect in learning and memory parameters. In this line, DHA have shown to reduce inflammatory cytokine release, and EPA change the pro-inflammatory profile of Alzheimer patient's cells to a healthy cell profile, reducing the IL-6/IL-10 ratio [23, 24].

The most widely used natural source of EPA and DHA is fish oil. This has led to overexploitation of the seas by the fishing industry, which has seriously affected a large number of marine species. Algae aquaculture could also provide a new important source for omega-3 PUFAs [25]. Alternatively, stearidonic acid (SDA, 18:4 omega-3) is of great interest, since its endogenous conversion to EPA and DHA has been shown to be very efficient [26, 27]. Natural sources of SDA are the seed oils of corn gromwell, hemp, blackcurrant, and cyanobacterium *Spirulina*.

Furthermore, there is a variety of plants high in SDA, including some of the families Grossulariaceae, Caryophyllaceae, Primulaceae, and Boraginaceae (such as echium), that could serve for oil extraction to human consumption and to supplementation of livestock feeds, in order to increase the omega-3 PUFA content in dairy products and meat [28]. Recently, refined oils extracted from *Echium L.* species have been positively evaluated as a novel food ingredient by the EFSA (European Food Safety Authority) because of their high concentrations of SDA and other PUFAs such as alpha-linolenic acid (ALA, 18:3 omega-3) and gamma-linolenic acid (GLA, 18:3 omega-6) [29]. GLA is a metabolic precursor of AA, which exerts pro-inflammatory activities; however this conversion in humans is slow and gives rise to the production of anti-inflammatory eicosanoids [30]. In addition, the combination of GLA and EPA appears to enhance the anti-inflammatory activity, for competition with the AA cascade, and it could also be useful in prostate and breast cancer prevention or cancer-cachexia [31–33].

2.2 Monounsaturated Fatty Acids

Other fatty acids that have recently shown interesting health effects are omega-7 fatty acids such as palmitoleic acid (C16:1 n-7). Doses of 300 mg/kg in the form of free fatty acid have shown to reduce body weight, improved hyperglycemia, hypertriglyceridemia, and insulin sensitivity in mice fed during 4 weeks, when compared with the same doses of palmitic acid (16:0 n-9). Furthermore, palmitoleic acid administration suppressed pro-inflammatory gene expressions and lipogenic genes in the liver [34]. We can find it in animal fats, marine oils, and vegetable oils (such as macadamia nut oil and hawthorn oil).

Moreover, extensive epidemiological studies have shown that the omega-9 fatty acid such as oleic acid (C18:1 n-9) decreased incidence of coronary heart diseases, metabolic syndrome, and cancer [35] and regulates many biological processes. Oleic acid is a monounsaturated fatty acid present mostly in olive oil and other vegetable oils such as pecan oil, macadamia oil, peanut oil, canola oil, and sunflower oil and seed oils such as sesame oil, poppy-seed oil, and avocado oil. It is also present in animal fats of turkey or chicken and other animals fed on grass. Oleic acid is not an essential fatty acid; it can be formed by de novo synthesis in humans [36] and from other fatty acids such as stearic (C18:0).

2.3 Saturated Fatty Acids

Consumption of saturated fats in Western countries is high, and their intake, especially of palmitic acid (C16:0) and myristic acid (C14:0), is related to a higher incidence of cardiovascular diseases. After palmitic acid, stearic acid is the most consumed saturated fatty acid in Western countries with a total of 26% of consumption. Stearic acid is ingested through the consumption of animal fat derived from pork or beef, as well as vegetable fat such as coconut or soybean oil [37]. Compared

with other saturated fatty acids, stearic acid lowers LDL cholesterol, has no effect on HDL levels, and lowers total cholesterol [38, 39]. According to [40], this beneficial effect of stearic on cholesterol is based on its malabsorption in the body and its reduced solubility after being released from the triglyceride by pancreatic lipase [41]. In addition, this fatty acid is rapidly transformed to oleic acid in the liver during its metabolism. Unlike stearic acid, palmitic acid raises total cholesterol mainly by increasing LDL levels, which is attributed to its ability to suppress the expression of LDL receptors. This increase in total cholesterol by palmitic acid is also attributed to its slower passage to oleic acid, since it must be first elongated to stearic acid [40]. On the contrary, stearic acid is used to synthesize hypocaloric fats considering its low digestibility and high excretion. An example of hypocaloric fats recognized as novel food is Salatrim®, which is composed of triglycerides with random positional distribution of fatty acids; it contains LCFAs (mainly stearic) and SCFAs, such as propionic or butyric acid [42].

On the other hand, capric acid (10:0) and lauric acid (12:0) have been shown to possess antimicrobial and anti-inflammatory properties [43, 44]. In addition, lauric acid has been also associated with positive effects on cardiovascular system related to the increase of HDL levels [45] and decrease of blood pressure in rats [46]. Moreover, lauric acid has shown to prevent the prostatic hyperplasia and exert inhibitory effects in colon cancer, endometrial cancer, and breast cancer cells [47], and consumption of virgin coconut oil as a natural source of these two fatty acids has shown to improve quality of life in patients during chemotherapy [48]. Lauric acid is also present in other plant oils, seeds, and fruits and in breast milk [49, 50].

Recent studies on myristic acid have shown contradictory effects on its impact on health. Although it has always been considered detrimental to health [51–53], a group of authors has shown that myristic acid increases diacylglycerol kinase levels and improves glucose uptake into cells [54]. This raises the question regarding the potential role of myristic acid on the prevention of type 2 diabetes. These fatty acids are presented in high concentration in high-fat dairy products which could explain the diabetes-protective properties associated with their intake [55].

2.4 Medium- and Short-Chain Fatty Acids

Medium-chain fatty acids (MCFAs) are minor dietary components. However, their intake provides a quick source of energy because they are easily digested and absorbed. Triacylglycerides with three molecules of MCFAs are quickly absorbed and transported to the mitochondria where they serve as combustible; therefore they do not accumulate as body fat. MCFAs do not pass through the enterocyte and do not require the formation of chylomicrons; they are directly transported by the portal system [56].

Several studies have confirmed the potential of MCFA to reduce body fat; they suppress fat deposition improving thermogenesis and fat oxidation in both humans and animals [57–59]. Triglycerides enriched in MCFA are attractive for use in

low-calorie products [60]. In addition, it has been reported that MCFAs preserve insulin sensitivity in patients with type 2 diabetes [59].

Triacylglycerides with MCFAs are used as part of liquid diet formulas suitable for patients with digestive problems or conditions that demand fluid restriction, such as burn injury, postoperative cancer, AIDS, respiratory distress, cystic fibrosis, and hepatic or renal disease [61]. A relevant example of this type of fatty acid is the case of lauric acid (C12:0). As previously mentioned, this compound as well as its monoester monolaurin possesses numerous antibacterial and antiviral activities [62]. It is reported to be effective against *Staphylococcus aureus*, *Enterococcus faecalis*, *Mycobacterium terrae*, *Streptococcus agalactiae*, and *Listeria monocytogenes* [63] and against viruses such as herpes simplex-1, HIV, cytomegalovirus, vesicular stomatitis, measles, and visna virus [64]. Myristic acid (C14:0), capric acid (C10:0), and caprylic acid (C8:0) can also be virucidal and bactericidal [62].

SCFAs, mainly acetate, propionate, and butyrate, are microbial end products generated by fermentation of fiber from the intestinal microbiota. Their presences inside the intestine have shown a positive influence on intestinal barrier function. Disruption of intestinal barrier function can lead to the development of many diseases such as inflammatory bowel disease, obesity, and HIV infection. Thus SCFAs play an important role in human physiology affecting neuroimmune function, nutrition, and protection from pathogen colonization [65–67]. SCFAs also modulate the secretion of hormones including peptide YY and leptin and cell differentiation and proliferation [68, 69].

The association of the plasma and colonic SCFAs with metabolic syndrome has been extensively documented. Recently, the relation between SCFAs and regulation of energy homeostasis have been studied [70, 71]. Many of the SCFAs produced in the gut are used as host energy source, and this could represent 10% of our caloric requirements per day [72]. Acetate and butyrate are involved in the synthesis of cholesterol and palmitate in the liver and propionate in the gluconeogenesis [73]. It is estimated that 90% of SCFAs produced in the colon are rapidly and efficiently absorbed [74]. Furthermore, acetate and propionate are energy substrate for peripheral tissues and butyrate for the colonic epithelium [75].

Focusing on SCFA pathway and signaling, it has been reported that the free fatty acid receptors, GPR43 and GPR41, can regulate host energy homeostasis in the gut and adipose tissues [76, 77]. Mice deficient in GPR43 are obese following a normal diet, while mice overexpressing GPR43 remain lean even with a high-fat diet [78]. Thus gut microbiota and SCFAs could affect the development of obesity, diabetes, and insulin resistance. These disorders are characterized by the low presence of specific microbial groups (*Akkermansia muciniphila* among them) and SCFAs, leading to alteration on glucose, lipid, and energy homeostasis, gut barrier dysfunction, and low-grade inflammation [79]. Therefore SCFAs are playing significant roles in several inflammatory mechanisms including atherosclerosis [80] and also in cancer [81]. The anti-inflammatory effect of butyrate in the colon, which is produced by some microbial groups such as *Faecalibacterium prausnitzii* [82] or *Ruminococcus* [83] and other butyrate-producing bacteria, has been extensively reported. SCFAs are involved in the pathophysiology of neuroimmune and

immune-inflammatory disease having effect on T cell differentiation, cytokine expression, epithelial barrier function, and the recruitment of leucocytes to areas of inflammatory activity [84]. Chronic intestinal inflammation increases the development of colon cancer. SCFAs confer protection in carcinogenesis. Propionate and butyrate were observed to induce apoptosis and inhibit proliferation on cancer cells [85]. SCFAs have also reduced colitis and allergic asthma in animal models [86–88]. Indeed treatments with SCFAs in ulcerative colitis patients have shown to ameliorate colitis [89].

In order to drive the metabolic activity of microbiota toward fermentation of carbohydrates, the intake of dietary fibers and prebiotics (nondigestible carbohydrates) is essential [90]. These nondigestible carbohydrates, such as xylans, cellulose, inulin, fructooligosaccharides, or resistant starch, are fermented by the anaerobic colonic bacteria to generate energy for microbial growth, moderate concentrations of SCFAs in the colon, as well as peripheral circulation [66, 91, 92]. There are other strategies such as supplementation with specific probiotic strains (live microbiota) to promote production of butyrate, other SCFAs, or lactate that is also implicated in the cross-feeding mechanism between colonic bacteria [93, 94]. However, when SCFAs are produced fast and in large amounts or under special unhealthy conditions, they could disrupt barrier function and instead exert beneficial effects [95, 96]. Hence, it is necessary to study the appropriate concentrations of these SCFAs to provide health benefits in each situation.

2.5 Trans-Fatty Acids and Conjugated Fatty Acids

It has been established that consumption of trans-fatty acids (TFAs) increased the risk of heart disease [97]. But there are TFAs originated from different sources which produce diverse effects in human health. Nonnatural or industrial TFAs (iTFAs) are mainly formed by hydrogenation in order to achieve the conversion of liquid oils into semisolid or plastic fats. These newly formed fats have interesting technological characteristics but include different proportions of TFAs [98]. iTFAs are rich in elaidic acid (*trans*-9-18:1) and *trans*-10-18:1, and their intake has shown to stimulate atherosclerosis in humans [99]. However, some natural TFAs (nTFAs), such as vaccenic acid (*trans*-11-18:1), have shown to protect against atherosclerosis [99]. Vaccenic acid is produced inside the digestive tract of ruminant animals becoming the main trans-isomer of oleic acid (18:1) in the fat of dairy and beef products [100]. Nevertheless ruminant TFAs (rTFAs) are in low proportion in food (4–8% of total fatty acids) [101], whereas iTFAs are present in shortenings and pastries and can reach up to 61% of total fatty acids [102]. Several studies focus on the role of different trans-isomers on lipid metabolism. Comparison of the effects of trans-isomers of 18:1 in vitro has shown that *trans*-9 isomer upregulated the lipogenic genes and enhanced total cell fatty acids, whereas isomers *trans*-11 and *trans*-13 did not have this effect [103]. The same authors have observed that C18:1 *trans*-9 and *trans*-10 increased cholesteryl ester content and triglyceride in cultured liver cells compared to trans-isomers 11, 14, and 15 [104]. Moreover, trans-isomers 6, 9,

and 10 upregulated genes related to cholesterol and fatty acid synthesis. A meta-analysis of studies on iTFA and nTFA did not show association between coronary heart disease (CHD) and nTFA intake (0.5–1.9 g/day), whereas intake of iTFA (1.3–5 g/day) showed to increase the risk of CHD [105]. In the same line, another meta-analysis of observational studies has reported an increase in CHD (30%) and in the risk of CHD mortality (28%), but not in type 2 diabetes, associated with 1.8% of total energy intake of iTFA. However, rTFA intake had no effect on CHD, and ruminant C16:1 *trans*-9 showed inversely association with type 2 diabetes [106]. Another review of 29 and 6 treatments with intake of equivalent range of iTFA and rTFA did not find significant difference between the TFA from either source and the effects on LDL/HDL ratio [107]. Further studies are needed in order to clarify these contradictions, but it seems clear that rTFA intake does not increase the risk of CVD and may exert certain beneficial effects on health.

The fatty acid composition of milk and dairy products is very important for its effect on human health and depends on the cow diet, the metabolism inside the digestive tract of ruminant animals, and the mammary metabolism [100]. Briefly, after lipolysis of dietary lipids, bacteria inside the rumen produce, via biohydrogenation of unsaturated fatty acids, different isomers (*cis* and *trans*) of 18:1, non-conjugated 18:2, conjugated linoleic acids (CLA), and conjugated linolenic acids (CLnA) [108]. The two isomers of CLA (*cis*-9, *trans*-11 and *trans*-10, *cis*-12) and their mixture have demonstrated a variety of biological activities, among them reduction of body fat, prevention of atherosclerosis, modulation of immune and inflammatory responses, and anticancer effects [109–111].

Conjugated fatty acids also have diverse origin. Rumenic acid (*cis*-9, *trans*-11 18:2) is the predominant CLA isomer in ruminant milk fat (75–90%) [112], followed by *trans*-10, *cis*-12 and *trans*-7, *cis*9. Rumenic acid is basically a product of the endogenous synthesis by the action of the enzyme γ-desaturase on the mammary gland, which is used as a substrate vaccenic acid (*trans*-11 C18:1). Other preparations of CLA are produced by chemical synthesis via alkaline isomerization of sunflower or safflower oils (rich in 18:2n-6) containing equally proportions of c18:2 *trans*-10, *cis*-12 and c18:2 *cis*-9, *trans*-11. Human studies have demonstrated that C18:2 *trans*-10, *cis*-12 is implicated in lipolysis and fat oxidation (catabolic effect) and C18:2 *cis*-9, *trans*-11 isomer has anti-inflammatory and anabolic effects [113]. Supplementation with 6 g/day of CLA for 12 weeks improved symptoms of inflammatory bowel disease in humans [114]. However, more studies to compare the form of administration of CLA as triacylglycerols (TAG) or free fatty acids (FFA) are necessary [115]. Studies in animal models have shown an increase of lean body mass and reduction of fat deposition associated with the isomer *trans*-10, *cis*-12 rather than *cis*-9, *trans*-11 [116]. However, the effects of individual isomers on body weight have not been shown in human, although modulation of insulin resistance [117, 118] was observed. On the other hand, effects of CLA mixture intake during 6 months (3.2–3.4 g/day) have been reported to slightly decrease body fat mass in humans [119]. Regarding regulation of blood pressure, the finding of a meta-analysis of eight human studies did not report favorable effect of CLA [120]. In addition, several studies have reported inhibitory effects of CLA on cell proliferation

of cancer and induction of apoptosis and antimutagenesis in rats and cell lines [121–123]. However, a systemic review in humans has shown a weak inverse relation between CLA dietary intake and breast cancer risk [124].

Conjugated linolenic acid (CLnA) is the term for the isomers of octadecatrienoic acid (18:3) with three conjugated double bonds. CLnA are present in milk fat and mainly in seed oils [125] such as pomegranate, which contains *cis*-9, *trans*-11, *cis*-13 C18:3 (punicic acid), bitter melon with *cis*-9, *trans*-11, *trans*-13 C18:3 (α-eleostearic acid), or pot marigold containing *trans*-8, *trans*-10, *cis*-12 C18:3 (calendic acid) [126]. Studies in rat and mouse models and in human cells have indicated that isomers of conjugated eicosapentaenoic acid, conjugated non-adecadienoic acid, and CLnA (mostly *cis*9, *trans*11, *cis*15 isomer) possess anti-adipogenic, anti-inflammatory, anticarcinogenic, and immune-modulating properties. Several recent studies have also reported the health benefits of pomegranate seed oil (PSO) consumption, rich in punicic acid. After 2 weeks of feeding obese mice with PSO (2 ml/kg/day), a reduction of pro-inflammatory cytokines (interleukin-6) and tumor necrosis factor-α in plasma was observed. The treatment showed anti-inflammatory properties and also improved insulin sensitivity [127]. Punicic acid from PSO has also shown in vivo anti-inflammatory effects by limiting neutrophil activation and decreasing lipid peroxidation [128]. Schubert et al. [129] have reported that polyphenols and pomegranate seed oil, in a fermented juice, retard prostaglandin synthesis and oxidation, promote apoptosis of breast cancer cells, and inhibit breast cancer cell invasion and proliferation. Diet supplemented with PSO has inhibited colonic adenocarcinomas in rats. The inhibition of colonic tumors was related to an increase in the content of CLA in liver and colonic mucosa lipid fraction. In the nontumor mucosa, an increase of peroxisome proliferator-activated receptors after administration of PSO was observed [130]. Toi et al. [131] have also reported significant potential of PSO and other pomegranate fractions for down-regulation of angiogenesis in vitro and in vivo. Białek et al. [126] have reported that pomegranate seed oil intake leads to an increment in the content of *cis*-9, *trans*-11 CLA in the liver of rats and decreases the activity of the enzymes Δ5- and Δ6-desaturase more than other CLA supplements. However, other studies of lipid profile and insulin resistance associated with exercise in healthy humans did not show significant effect on insulin resistance and lipid profiles, except for HDL [132]. Further investigations are needed in order to demonstrate all these positive effects in humans.

2.6 Branched-Chain Fatty Acids

Branched-chain fatty acids (BCFAs) are mostly saturated fatty acids with one or more methyl branches on the carbon chain. Most of them are at terminal (iso) or next to the terminal (anteiso) methyl group [133, 134]. Overall, BCFAs are found in ruminant milk and adipose tissue of animals such as cows, sheep, and goats. They are presumably produced and utilized as membrane lipids by microorganisms of the rumen. The composition of BCFAs varies depending on the animal and its diet

[133, 135, 136]. Those with chains of 14–18 carbons are typically found in the fat of beef and dairy products [137]. A study of the US retail cow milk supply has shown that BCFA constitutes 2% w/w of the milk fat [138]. Amounts of BCFA in lanolin, wool wax, exceed 40% [139]. Although less studied than PUFA omega-3s, BCFAs are also present in fish. Wang et al. [140] have reported that mean BCFA content in edible muscle across 27 freshwater fish species analyzed was 1.0 ± 0.5% of total fatty acids, being 1.8 ± 0.7% in fish skin. Most of the BCFAs found were anteiso-15:0, iso-15:0, iso-16:0, anteiso-17:0, and iso-17:0.

BCFAs are also synthesized by the skin in humans, and they constitute almost one third of the fatty acids in the vernix (fatty film covering the fetus in utero). The fetuses swallow the vernix suspended in the amniotic fluid, exposing their gut to BCFA. They are also natural components of colostrum and breast milk (>1.5%w/w) [141–143]. Moreover, BCFAs are important components of the membranes of *Lactobacillus* and *Bifidobacterium*, bacterial genera present in the gastrointestinal tract of newborns [144–146].

Ongoing research revel that BCFAs are bioactive compounds with positive impact in the development of the intestinal microbiota, and anticancer effects have been also attributed to them [147–149]. A recent study with neonatal rat pup model has shown that BCFAs reduce necrotizing enterocolitis incidence by more than 50%, influence microbiota composition, and are selectively incorporated into the membrane lipids of the intestine [138].

2.7 Edible Cold-Pressed Oils

In recent years, a variety of edible cold-pressed oils obtained from fruits and seeds of different plants in the market are increasing. These oils have advantages compared to those obtained by other technologies of extraction or refining, since their characteristics and flavor are not altered by the increase of temperature or the use of solvents [150]. Thus, they present a large number of intact bioactive components including polyunsaturated fatty acids, free and esterified sterols, tocopherols and tocotrienols, squalene, carotenoids and chlorophylls, and various phenols and triterpene alcohols. These compounds are characterized by possessing anti-inflammatory properties, reducing oxidative stress, and positively modulating the immune system, as well as chemopreventive and neuroprotective activities [150]. In this chapter, many of the bioactive qualities of several of these compounds have been reviewed, but further research is needed in both nutrition and pharmacology areas to better document their functionality and contribution to health [151]. In addition, legislative aspects regarding their identity, denomination, and certification must be defined, as well as clarifying issues related to authenticity, safety, presence of contaminants, rancidity, susceptibility to light and heat (for proper storage and use in cooking), and naturally health claims [152].

Some examples of the best known bioactive-rich cold-pressed oils are virgin olive oil, an important ingredient of the Mediterranean diet, whose health benefits are linked to the presence of monounsaturated oleic acid, squalene, triterpenes, and

bioactive phenolic compounds. Among the bioactive phenolic compounds, those that are raising greater attention interest are tyrosol, hydroxytyrosol, ligstroside and oleuropein, and dialdehydic forms of elenolic acid. It is recognized for their antioxidant, cardiovascular, anti-inflammatory, neuroprotective, and chemotherapeutic effects and the disease-related gene modulation; cold-pressed sesame oil, typical of Asian cuisine, is rich in polyunsaturated fatty acids, vitamin E, and phenolic compounds (such as gamma-tocopherol, sesamin, and sesamolin). Among their biological activities, we find reduction of oxidative stress, liver oxidative damage protection, and decreased cholesterol level in the blood; flaxseed oil is not classified under the category of "cooking oils" whereby it has to be added just before serving. This oil contains α-linolenic acid, sterols, carotenoids, and a tocochromanol plastochromanol-8. When it is enriched with particulates, it contains lignans which are recognized for its protective effect against colon, skin, and prostate cancer; *Camelina* seed oil is suitable for cooking due to its resistance to heat. It is rich in gamma-tocopherol, alpha-linolenic acid, and polar phenolic and possesses antioxidant, anti-inflammatory, and immune system stimulation properties. Virgin argan oil is rich in monounsaturated fatty acids and linoleic and oleic acids and also contains squalene, tocopherols, spinasterol, and schottenol (a bioactive sterol). It is suitable for cooking and frying and in salad dressings; it is very useful in Moroccan cooking; cold-pressed coconut oil contains antioxidants such as phenolic compounds and alpha-tocopherol. It is not resistant to heat due to its high content of medium-chain triacylglycerols; walnut oil is used as uncooked condiment; it is rich in omega-6 and omega-3 fatty acids. Rapeseed oil contains brassicasterol and plastochromanol-8. Pumpkin seed oil is rich in linoleic and oleic acids, phenolic compounds, and low amounts of gallic acid; *Echium*, black currant, and hempseed oils contain high amounts of stearidonic acid, whose health benefits have been previously described; evening primrose seed and borage oils are rich in gamma-tocopherol and gamma-linolenic acid; macadamia seed oil is rich in alpha-tocopherol and monounsaturated fatty acids. It is very resistant to heat and shelf-stable; avocado oil can be used for frying and cooking. It is rich in monounsaturated fatty acids, chlorophylls, tocopherols, and carotenoids [152–154].

At present, they are also being investigated for their anti-inflammatory activity and high content in tocopherols, carotenoids, alpha-linolenic acid, squalene, and phenolic antioxidants and oils from other seeds such as blackberry, amaranth, black caraway apple, black currant, boysenberry, cardamom, cranberry, blueberry, raspberry, strawberry grape, marionberry, sea buckthorn, poppy, cumin, hemp, *Pistacia atlantica*, coriander, parsley, and carrot [155, 156].

3 Polar Lipids

The biological activity of phospholipids is mainly based on their amphiphilic properties that self-assemble the polar heads and the hydrophobic hydrocarbon chains to create bilayers which are the keystones to build cells' and organelles' membranes [157]. Phosphatidylcholine is the main phospholipid in the external layer

of cellular membranes, basic to warrant their function and integrity. Besides its role in membranes, phosphatidylcholine is also implicated in the hepatic secretion of VLDL, with an important function in cholesterol and lipid distribution to organs and tissues. Another role of phosphatidylcholine is the formation of digestive micelles in the intestinal lumen, as a part of the biliary secretions, promoting the absorption of lipids. Phosphatidylethanolamine is more abundant in the internal membrane leaflet and in mitochondria, with a relevant role in growth and stability. Phosphatidylethanolamine is also a precursor of glycosylphosphatidylinositol and helps protein binding to the membrane. This last activity can be explained by the small size of PE, which confers stability to the membrane [158].

After hydrolysis of phospholipids, the formed lysophospholipids participate in diverse biological activities related to their molecular structures. Multiple activities have been attributed to lysophosphatidylethanolamine [159]. Lysophosphatidylinositol has been used as a cancer biomarker [160]. Lysophosphatidylserine [161] participates in signal transduction related to activation of neutrophil, and it could also have anti-inflammatory properties. Finally, lysophosphatidylcholine [162] could be used in rheumatoid arthritis treatments and is also involved in DNA methylation preferentially in the central nervous system [163].

Plasmalogen activities have not been fully described, but its deficiencies have been correlated with peroxisomal disorders associated with mental retardation, deafness, or adrenal dysfunction [164]. Moreover, plasmalogens have also potential as biomarker in different diseases related to aging, such as Alzheimer, oxidative stress, and inflammation [165, 166].

The role of some phospholipids in some organelles should be also considered. In this sense, the main phospholipids in mitochondrial membrane are phosphatidylethanolamine and phosphatidylcholine. One unique property of these membranes is the presence of cardiolipin, a tetraacyl phospholipid. One of the most important activities of cardiolipin in mitochondria is the promotion and organization of the respiratory chain. Besides, its role is also relevant in keeping fluidity and osmosis [167]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the main responsible of oxidative stress. This situation occurs when these species are produced in excess and the cell is unable to destroy them with the help of natural antioxidants [168–171]. In addition, ROS can also oxidize cardiolipin. This newly restructured cardiolipin is related to mitochondria dysfunction associated with multiple pathologies such as diabetes, obesity, heart failure, hyperthyroidism, neurodegeneration, and aging. All of these diseases are highly related to oxidative stress, low levels of cardiolipin, and also anomalous high content of DHA in cardiolipin [172]. Another important activity induced by fatty acid oxidation products is apoptosis with the participation of the mitochondrial permeability transition pores (MPTP) [173, 174].

It is well known that many cell and membrane protein functions are related to membrane lipids [175]. This protein function regulation by membrane lipids involves mechanisms ranging from specific and non-specific interactions between proteins and membrane lipids [176]. In general terms, membrane lipids can be categorized in two broad groups [2]:

1. Lipids that bind to points of recognition of specific proteins, acting as ligands, such as platelet-activating factor that can be grouped to the G protein-coupled receptor family [177]. This category is based on a well-known protein-small molecule interaction without any role on the collective membrane properties. Such specific interaction is the case of polyphosphoinositide interactions with specific proteins [178–181]. Another example of specific regulation takes place with membrane lipids that are cell signaling precursors – such as arachidonic acid in prostaglandin biosynthesis [182].
2. Lipids that, by altering their biophysical properties, have an impact on membrane and membrane protein functions and organization [183, 184].

Membranes behave as macrostructures with a collective physical property such as thickness, curvature, or elasticity [185–188]. An example of non-specific regulation of membrane protein function by the membrane lipids occurs by hydrophobic coupling between both molecules. Protein activity is modulated by the hydrophobic thickness and intrinsic curvature of the lipids at the bilayer [188], indicating that changes in bilayer physical properties can modify membrane protein conformation and function [189].

Sphingolipids (SLs) are ubiquitous constituents of cell membranes in many pro- and eukaryotic organisms. In vertebrates, they are also found in lipoproteins (especially LDL) and other lipid-rich structures, such as epidermis. For a long time, SLs were considered to play primarily structural roles in the membrane formation and fluidity regulation. Likewise, SLs have long been recognized as important structural components of the epidermis, securing the epidermal permeability barrier [190]. However, over the last decades, intensive research on SL metabolism and function has recently evidenced that SLs function as effector molecules in several cell processes and have key roles in stimulus−/agonist-mediated signaling pathways involved in cell response modulation. Hence, sphingomyelin participates in regulatory functions by interaction with specific proteins and serves as a receptor for the intake of transferrin, promoting the incorporation of iron to the cells. On the other hand, the most important property of sphingomyelin is its implications in rafts together with cholesterol, in both cell membranes and lipoproteins. It has been recently proposed that metabolism of sphingomyelin and cholesterol is closely related. Sphingomyelin could be responsible for cholesterol distribution in cells [191, 192]. Sphingomyelin has been extensively used as a chemopreventive agent, taking into account its activities as messenger in development, growth, differentiation, and apoptosis of human cells.

SLs exert their effects through two general mechanisms:

1. Biophysical mechanism (based on lipid-lipid interactions), whereby SLs, mainly sphingomyelin and glycosphingolipids, and cholesterol spontaneously self-associate and fuse into SL-enriched platforms (also referred to as microdomains or rafts). The formation of these microdomains has consequences to cell signaling by altering membrane structure and, subsequently, the interaction of protein membrane receptors with the cell membrane bilayer [193–198].

2. Biochemical mechanism (based on lipid-protein interactions), whereby SLs act as first and second messengers by interacting with several target proteins or receptors in a number of cell signaling pathways [199, 200].

In addition, a comprehensive understanding of bioactive SL function requires further knowledge of the metabolic organization of enzymes of SL metabolism and their interrelationships, as well as the compartmentalization of SL-mediated signaling pathways, the metabolic transformation of bioactive SLs, and the integration or coordination of overall responses. A number of studies have shown that many pathways of SL metabolism constitute an interconnected network of specialized and compartmentalized enzymes that not only regulates the levels of individual biologically active molecules but also their interconversion and, ultimately, the balance among them.

Ceramides (Cer), which are composed of sphingosine (Sph) (core of all SLs) esterified to a fatty acyl chain via an amide linkage [201, 202], are the center of SL biosynthesis and catabolism and precursors of complex SLs (Fig. 1a). Briefly, Cer can be produced through the de novo pathway (from serine and palmitate by the action of the serine palmitoyl transferase (SPT), ceramide synthases 1–6 (CerS1–6), and desaturase (DES)) and the hydrolysis of complex SLs, especially sphingomyelin (SM), by several sphingomyelinases (SMases). In SL biosynthetic reactions, Cer is primarily used for the synthesis of more complex SLs, including ceramide-1-phosphate (C1P), through phosphorylation by ceramide kinase (CK) [203]; sphingomyelin (SM), by transferring a phosphocholine head group from phosphatidylcholine, through the action of SM synthases (SMS) [204]; or glycosphingolipids (GSLs), through glycosylation (mainly with glucose and galactose) by glucosyl- and galactosyl-Cer synthases (GCS) [205]. In the catabolic pathway, glycosphingolipids are cleaved to glucosylceramide (GlcCer) and galactosylceramide (GalCer), which are subsequently hydrolyzed by specific β-glucosidases and galactosidases to release Cer [206]. Cer can also be metabolized by ceramidases (CDases) [207–209] to yield sphingosine (Sph), which in turn is phosphorylated by sphingosine kinases (SKs) to generate sphingosine-1-phosphate (S1P). S1P can be cleared by the action of specific phosphatases that regenerate Sph or by the action of a lyase that cleaves S1P into ethanolamine-1-phosphate and a C_{16}-fatty-aldehyde [201]. The many pathways of sphingolipid metabolism constitute an interconnected network that not only regulates the levels of individual biologically active molecules but also their interconversion and, ultimately, the balance among them.

Most enzymes of sphingolipid metabolism show specific subcellular localization(s) (Fig. 1b). This could dictate distinct functional responses, since, coupled with the poor solubility of SLs in cells, it imposes clear restrictions on the subcellular localization of bioactive lipids. Thus, in the absence of specific mechanisms of SL transport, the site of generation of a bioactive SL is likely to dictate its site of action. Among all of these SLs, the most extensively studied so far have been sphingosine (Sph), ceramides (Cer), and sphingosine-1-phosphate (S1P). Cer and Sph are reported to act as tumor-suppressor lipids involved in both intracellular and extracellular processes. The long-chain amino alcohol sphingosine (Sph) was the first

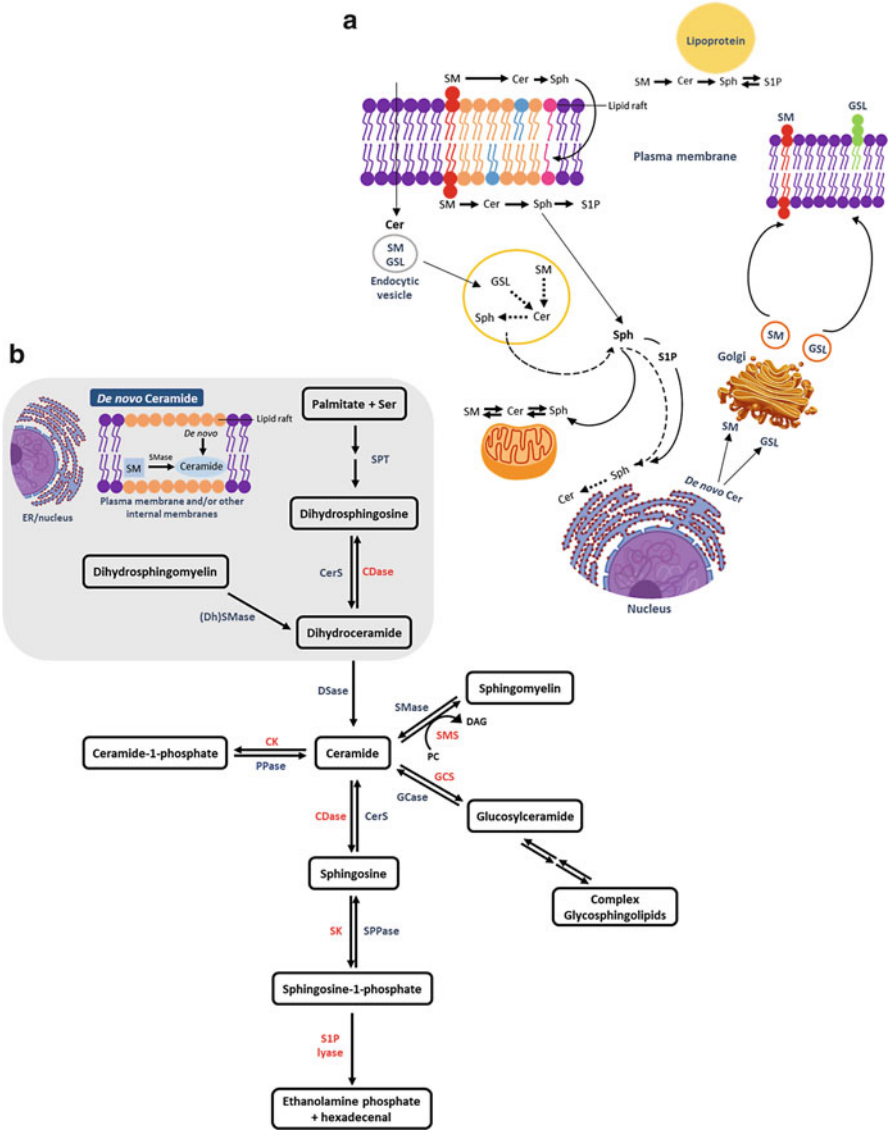


Fig. 1 Sphingolipid metabolism. (**a**) Sphingolipid biosynthesis. (**b**) Sphingolipid localization

SL metabolite to be identified. Sph has been connected with cellular processes such as inducing cell cycle arrest and apoptosis by modulation of protein kinases and other signaling pathways. It has roles in regulating the actin cytoskeleton and endocytosis and has been shown to inhibit protein kinase C (PKC) [210]. Kinase targets for sphingoid bases including pkh1p and pkh2p have been found in yeast, indicating functions in regulating endocytosis, cell cycle arrest, and protein

synthesis [211]. Sphingosine is, along with other sphingoid bases (sphinganine or dihydrosphingosine), the core of all sphingolipids. The simplest SLs, ceramides (Cer), are composed of a sphingoid base esterified to a fatty acyl chain via an amide linkage [201, 202]. The length of the fatty acyl chain (from C14 to C28) conjugated to the sphingoid backbone characterizes the different species of ceramide, which have shown diverse biological functions.

Many different ceramide species have been observed in different human tissues (including brain, skeletal muscle, skin, testicular, leukocyte, cardiomyocyte, and hepatocyte tissues), differing in relative abundance, fatty acid composition, and, hence, biological function [212, 213]. Ceramides often exert their effect via biophysical mechanism by forming ceramide-rich platforms that modify both receptor- and stress-mediated cell signaling and, hence, may influence various disease states and neurotransmission [214–217]. Thus, for example, Kolesnick and Gulbins have recently proposed a mechanism by which FasL/Fas interaction leads to caspase-8-dependent activation of acid sphingomyelinase, with the resultant ceramide formation capable of orchestrating raft reorganization into large cell-surface microdomains where the proteins of the death-inducing signaling complex of Fas can oligomerize thereby amplifying or modifying cell signaling [218]. Moreover, it is believed that ceramide-rich platforms serve to cluster the receptors for the pathogen, and the negative membrane curvature induced by ceramide facilitates cellular invasion of pathogens, including several microbes, parasites, and viruses [219–223]. Often the end result of ceramide-mediated cellular entry of pathogens is containment and/or inactivation of the pathogen. Moreover, ceramides may also influence membrane permeability via interactions with ion channels [224].

In addition, Cer may act as second messengers in a number of stress stimulus-mediated signaling pathways by regulating specific protein targets, mainly phosphatases (as PP1A and PP2A) [225] and kinases (as protein kinase C (PKC) z [226], raf-1 [227], and the kinase suppressor of Ras [228]). In this way, ceramides play a role in multiple vital cell processes such as cancer cell growth, differentiation, senescence (by telomerase inhibition and suppression of key mitogenic pathways [229]), and apoptosis [200, 230]. Many cytokines, chemotherapeutic agents, and other stress-causing agonists (such as heat stress, ultraviolet (UV), ionizing radiation, DNA damage, and ligation of death receptors) result in an increase of endogenous ceramide levels through de novo synthesis and/or the hydrolysis of sphingomyelin [231, 232] (Fig. 1a). Reciprocally, decreased levels of endogenous ceramide caused by increased expression of glucosylceramide synthase (GCS), which clears ceramide levels by incorporating it into glucosylceramide, result in the development of a multidrug resistance phenotype in many cancer cells [233]. In contrast to the actions of ceramide, S1P is emerging as a key regulator of proliferation, inflammation, vasculogenesis, and resistance to apoptotic cell death [234]. Many growth factors, such as epidermal growth factor and platelet-derived growth factor, as well as the cytokines TNF-α and IL-1, activate SK1 acutely, resulting in transient elevations in the levels of S1P. S1P is then secreted from the cell and acts either in a paracrine or autocrine manner to engage specific transmembrane hepta-helical G-protein-coupled receptors (GRCR). In mammals,

S1P receptors are widely expressed and are thought to regulate important physiological actions, such as immune cell trafficking, vascular development, vascular tone control, cardiac function, and vascular permeability, among others. In addition, S1P may participate in various pathological conditions. For example, S1P has been implicated as an important mediator in autoimmunity, transplant rejection, cancer, angiogenesis, vascular permeability, female infertility, and myocardial infarction [235].

Sphingolipids other than ceramide and S1P are emerging as candidate bioeffector molecules. These include C1P, which has roles in activation of phospholipase A2 [236, 237], release of arachidonic acid in response to interleukin-β (41), regulation of vesicular trafficking, phagocytosis [238], macrophage degranulation [239], and mitogenesis [240]. Dihydroceramide, which had been shown to be inactive in apoptosis, has been implicated in mediating the growth inhibitory actions of fenretinide (a retinoid analogue used in the treatment of neuroblastoma), which has been shown to inhibit the activity of the desaturase [241, 242]. Lysosphingomyelin as been shown to induce multiple cellular effects, possibly mediated by binding to specific GPCRs [243]. Finally, glucosylceramide (GluCer) has been implicated in resistance to chemotherapeutic agents [244] and may serve as the endogenous cargo for the P-glycoprotein transporter MDR1 [245]. Another property of GSLs, such as monoglycerides and gangliosides, with application in health sciences is their protective effect against certain pathogens. As commented above, several microorganisms, microbial toxins, and viruses link themselves to the cells using SL-enriched platforms. Therefore, when such compounds are present in the diet, they compete for the active union sites and help in the elimination of pathogens from the intestine [246]. Since microbial adherence is the first step in infection [247], site competition may have a protective effect against pathogens from food sources.

In addition to GSLs, other complex lipids that have attracted a lot of attention because of their significant biological and technological functions are glycoglycerolipids [248]. They are distinguished from GSLs by their lipid moieties, having glycans linked to the C-3 hydroxyl of diacylglycerol, and are very minor constituents of most animal tissues, other than the testes. Several biological activities, such as anti-algal, antiviral, antitumor, and anti-inflammatory, have been attributed to galactolipids [248]. Monogalactosyl diacylglycerols and digalactosyl diacylglycerols have shown inhibitory activity of DNA polymerase, antitumor promotion, inhibition of cancer cell proliferation, and antiangiogenesis properties [249–251]. In addition, hydrolyzed glycolipids have higher anticancer activity than the non-hydrolyzed form [249]. The anti-inflammatory activity of monogalactosyl diacylglycerols and digalactosyl diacylglycerols is lower as the fatty acid saturation degree is increased [251]. Apart from that, digalactosyl diacylglycerols have also been proposed for controlling the appetite [252].

GSLs are widely distributed in microbes and plants. Due to their amphipathic properties, they are collectively known as biosurfactants (BS) [253]. BS offer several advantages as compared to their surfactant homologues derived from petroleum, due to their low toxicity, high biodegradability, environmentally friendly, high foaming capacity, and high selectivity and specificity at extreme temperatures, pH, and saline

conditions. They are also synthesized from renewable sources and have a low critical micellar concentration and high surface activity and biological activity, which is important in the therapeutic and biomedical (antimicrobial) fields. Therefore the interest in BS has increased considerably in recent years, as well as their potential application in different industries as there is a higher concern for the protection of the environment and sustainability [254, 255].

The oral application of dietary glycerophospholipids with a specific fatty acid composition has the potential to cause defined alterations of the fatty acid composition of membrane phospholipids within a certain cell type. As a consequence, cellular functions, including signaling and transport, as well as the activity of membrane-bound enzymes, could be modulated by dietary phospholipids and hence contribute to their health benefits [256]. Recently, lipid replacement therapy has surged as a natural medicine approach to restore function in cell membranes and organelles by replacing damaged membrane lipids [257]. Besides, the importance of polar head groups from membrane lipids in protein-lipid interactions is also relevant to the coupling of hydrophobic complementary structures, such as diacylglycerol chains from phospholipids and proteins [258]. This coupling can be perturbed by oxidation of the acyl chains from diacylglycerols. This oxidation and subsequent disorder can promote enzyme activation [184, 259]. The proposed mechanism is the change produced in the hydrocarbon chain packing and interruption of hydrophobic bounding. This hydrophobic match can be facilitated by the conformational state of the lipids or by selecting the optimum lipid species for that particular hydrophobic coupling [260–262]. In order to reduce phospholipid oxidation and degradation that take place along ingestion, digestion, and adsorption, phospholipids utilized in oral lipid replacement therapy should be protected from acid pH of the stomach, alterations by bile salts, and breakage by pancreatic enzymes and microflora. This task can be achieved by complexation of phospholipids with certain fructooligosaccharides, which insert in the phospholipid micelles and prevent interactions with other molecules [263, 264]. Another important feature of phospholipids is that enrichment of plasma membranes of the colonic mucosa with these molecules protects from different pathogenic conditions such as ulcerative colitis and other chronic inflammatory. The proposed mechanism for that is the hydrophobic barrier they provide, which regulates signaling pathway involved in different conditions such as inflammation [265]. Lipid replacement therapy has applicability in fatigue associated with cancer and cancer therapies [266, 267]. In this sense, a nutritional supplement called NTFactor [268] has been successfully utilized in cancer-associated fatigue that was reduced by 30% in an 8-week treatment. The combination of lifestyle and lipid replacement therapy could be a very efficient treatment to improve mitochondrial fusion and decrease inflammation and oxidative stress, becoming a powerful remedy in noncommunicable prevalent diseases. As an example, a pilot trial utilizing lipid replacement therapy has shown metabolism changes in the direction of reducing body mass and moderating appetite [269]. In this sense, alterations in fatty acid metabolism and glucose have an influence in insulin resistance and metabolic syndrome [270]. Considering that metabolic syndrome has been associated with the excessive presence of reactive oxygen species (ROS) and

reactive nitrogen species (RNS) in membranes, lipid replacement therapy could restore big oxidized membrane fragments in blood lipoproteins. New dietetic patterns in conjunction with the administration of membrane phospholipids can remove oxidized cholesterol and phospholipids from HDL and LDL [271].

4 Glyceryl Ethers

Glycerol-based ether lipids (Fig. 2) are normally minor constituents of cell membranes of mammals, and its presence is major in the liver of some fish of the Chondrichthyes class [272]. Different bioactive properties have been attributed to 1-*O*-alkyl-sn-glycerols (AKGs). The oral administration of AKG reduced the tumor growth and the number of pulmonary metastases of mice inoculated with 3LL cells [273]. AKG with hydrocarbon chains of 18:1, 14:0, and 16:1 showed the most active antiproliferative effect, compared to 16:0 and 12:0, inducing a reduction of the tumor blood vessel endothelial marker, suggesting an antiangiogenic effect. AKGs reduced also the major angiogenesis stimulator, basic fibroblast growth factor (bFGF), on endothelial cell proliferation [274] and influenced endothelial cell growth, without showing cytotoxic effects, decreasing the cell proliferation [275].

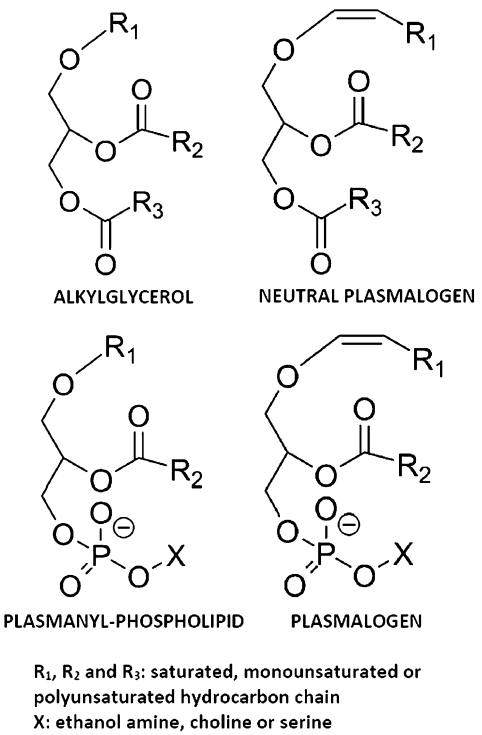


Fig. 2 Main glyceryl ether chemical structures

The antiproliferative effect of AKG was also tested in human mammary carcinoma (MCF7), ovarian carcinoma (OVP10), and prostate cancer (DU45, LnCap) cell lines, showing an increased percentage of apoptotic cells of ovarian and prostate carcinoma and a significant reduction in the number of prostatic cells [276, 277]. A methoxy-substituted alkylglycerol inhibited the growth of three human colon cancer cell lines (Moser, HT29, and HCT116) [278]. AKG supplementation was able to reduce Walker 256 tumor cell growth proportionately to the increase of lipid peroxidation, apoptosis, and reduction of cancer cell proliferative capacity. Additionally, the cachexia state, associated to patients of cancer and other progressive illnesses, was reversed with the intake of AKG [279, 280]. The prophylactic administration of AKG on patients affected by cancer can reduce the side effects and complex injuries derived from the radiation treatments, in particular leucopenia, thrombocytopenia [281], and fistulas [282].

Cytotoxic macrophages are activated by AKG, enhancing Fc-receptor-mediated phagocytosis, increasing humoral immune response, and delaying hypersensitivity reactions [283]. Regardless the carbon chain length, AKG stimulates the production of IL-12, a cytokine involved in the activation of Th1 responses [284], and IL-2 [285], enhancing the proliferation and activation of lymphocyte B, whereas levels of IL-4 and IL-10, involved in the activation of Th2 responses, are decreased [286]. Levels of C1q are also raised with the administration of AKG [287]. The intake of AKG to very old people before surgical treatment induces a significant increase of WBC, lymphocytes, IgM, and IgA in the postoperative period [288]. The administration of AKG to gestating and lactating mammals induces a positive effect on the immune system of the litter, with higher concentrations of IgG, erythrocytes, and hemoglobin in their blood and a modification on the lipid profile and immune properties of the mother's milk [289, 290].

Short-chain AKGs have the ability to increase blood-brain barrier permeability, allowing therapeutic molecules to cross it [291–293]. Even the penetration of high-molecular-weight materials, such as albumin and antibodies, into the brain could be significantly increased [292, 294]. The opening of the blood-brain barrier by the action of AKG is short and reversible and does not affect tight junction [295].

A recent experimental study has shown that unsaturated AKGs can promote a decrease in the high-fat-induced obesity and improve the insulin resistance in rats [296]. Likewise, the administration of AKG in obese patients can reduce the total cholesterol levels as well as complements 3 and 4, associated with higher risk of metabolic syndrome [297].

The in vitro treatment of spermatozoa improves their motility and fertility, related to PAF metabolism, resulting in a better fertilization performances when used for artificial inseminations [298]. In vivo studies have shown similar effects in motility and velocity of sperm after the oral intake of AKG [299].

AKG have been reported to exhibit antibacterial activity against several strains by releasing or activating proteases, such as the enzyme autolysin [300], increasing their activity with 1-O-chains from C8:0 to C12:0 and decreasing with longer 1-O-chains [301].

5 Isoprenoids

Isoprenoids are considered one of the most diverse families of compounds produced by biological systems; approximately 40,000–70,000 isoprenoid molecules are known, including sterols, carotenoids, and quinines [302–305]. Among their biological activities are maintenance of membrane fluidity, electron transport, protein prenylation, and cellular development. They are also utilized as fragrances and essential oils, antibacterial and antifungal agents, and highly valuable pharmaceuticals and fuel alternatives [304, 305].

According to the number of isoprene (C5) units that they contain, terpenes are classified in hemiterpenoids (C5), such as isopentenols; monoterpenes (C10), such as menthol and camphor; and sesquiterpenes (C15), such as zingiberene (ginger). These are the major constituents of herbs and spices. Other sesquiterpenes and diterpenes (C20) are pheromones, defenses, and signal transduction substances [306–308]. The roles of isoprenoids with higher molecular weight are membrane stabilization (such as cholesterol and other C30 compounds) and photoreception (such as carotenoids and other C40 compounds).

Many terpenoids have been found to exhibit potent biological activity, with several of them in development or in use therapeutically. Taxol, a diterpene extracted from the Pacific yew, is extremely effective in the treatment of certain cancers (ovarian, breast, lung and neck, bladder and cervix, melanoma, and Kaposi's sarcoma) [305, 309, 310]. A range of medicinal diterpenoid compounds (i.e., phorbol esters and the related casbanes, lathyranes, jatrophanes, and ingenanes) are solely produced in *Euphorbiaceae* and *Thymelaeaceae* species [311] from casbene and neocembrene diterpene backbones [312]. These diterpenoids have gained interest due to unique anticancer, anti-HIV, vascular relaxing, neuroprotective, anti-inflammatory, or immunomodulatory activities [311, 313–316]. The monoterpene limonene and related derivatives are believed to inhibit farnesylation of the growth-promoting protein RAS, inhibiting malignant cell proliferation [317–319]. The ability to produce terpenoid drugs in microbes could significantly reduce their production costs, reduce pressure on unsustainable plant-derived sources, and increase their chances of reaching clinical trials and the market.

Tocopherols and tocotrienols also known as tocochromanols are a group of compounds with vitamin E activity with numerous biological activities essential for human nutrition. They are comprised of different molecules: α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol [320]. Vitamin E is incorporated in plasma through chylomicrons. The transformation of chylomicrons to remnant particles is the mechanism to distribute the absorbed vitamin E to circulating lipoproteins and tissues [321]. On the other side, newly absorbed dietary lipids are incorporated into nascent very-low-density lipoproteins in the liver. This organ controls the release of α-tocopherol into blood plasma. This release is mediated by α-tocopherol transport protein (TTP). When TTP is absent, tocopherol is not secreted back into the plasma, and excess vitamin E is metabolized and excreted by the bile.

TTP is responsible of α-tocopherol transport to vital organs but is poorly effective on tocotrienols [322]. Surprisingly, in some tissues, the level of tocotrienol is higher

than that of tocopherols pointing out the presence of an alternative tocotrienol transport system in vivo [323].

Emulsification is a technique broadly utilized to improve absorption of hydrophobic drugs. One of the most well-known formulations are SEDDS (self-emulsifying drug delivery systems) [324–326]. In addition, soft gelatin capsules (Tocovid Suprabio™, Carotech Inc., NJ) of tocotrienol have been also developed. A study utilizing Tocovid Suprabio™ has studied the postabsorptive fate of the different tocotrienol isomers associated with lipoprotein subfractions in humans [327]. α-Tocotrienol concentrations in supplemented individuals are distributed among 3 μM in blood plasma, 1.7 μM in LDL, 0.9 μM in triglyceride-rich lipoprotein, and 0.5 μM in HDL. The plasma concentration of α-tocotrienol observed in SEDDS is two to three times higher than that previously reported in using generic supplements [328, 329].

Considering structural similarities in all eight tocols, it is logical to think they should have comparable antioxidant efficacy. However, current studies indicate that some members of the vitamin E family possess unique biological functions not shared by other family members. For example, α-tocopherol may inhibit platelet adhesion apart from its antioxidant properties. In addition, α-tocopherol has shown anti-inflammatory, antineoplastic, and natriuretic functions related to specific binding interactions [330]. Tocotrienols' main peculiarity is the presence of three trans-double bonds in the hydrocarbon tail. These unsaturations in the isoprenoid side chain provide a unique conformation [331]. For this reason, α-tocotrienol is much more flexible producing greater curvature stress on phospholipid membranes. It has been described that α-tocotrienol possesses numerous functions that are not shared by α-tocopherol [332]. Hence, tocotrienol inhibits the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, key enzyme in cholesterol biosynthesis [333, 334]. This activity is not shared by tocopherols [335]. In addition, tocotrienol, but not tocopherol, reduces oxidative damage in proteins [336].

It is well known that pure and mixed isoprenoids possess anticancer activity [337]. In strict sense, it should be indicated that tocotrienols, but no tocopherols, are isoprenoids. Regarding the neuroprotection activity, α-tocotrienol seems to be the most potent isoform [332, 338–340], and γ- and δ-tocotrienols have been considered as the most potent anticancer isoform of all tocotrienols.

One special group of isoprenoid derivatives are sterols with relevant functions in eukaryotes, including higher plants [341]. Two predominant types of sterols, cholesterol and ergosterol, are found in animal and fungal cells, respectively [342]. One of the principal functions of sterols is the structure ordering of the biological membranes [343]. Different biophysical studies of the structure and physicochemical properties of membrane sterols have indicated that cholesterol shields negative charges reducing the net membrane surface charge [344]. It also contributes to closer packing of hydrocarbon chains in the gel phase and increasing membrane microviscosity and decreasing its permeability. Membrane permeability is affected by free sterols at different extents. The highest permeability reduction is attained with cholesterol followed by campesterol, β-sitosterol, and stigmasterol [345]. Some studies have attributed to β-sitosterol and campesterol, a role in ordering fatty acid

chains at the membrane, which have an influence on water and ion permeability and also on the activity of some membrane proteins [346].

Sterols also act as important regulatory molecules. They are hormone precursors, regulating plant growth and development [342, 347, 348]. Moreover, sterols are implicated in the formation of specific lipid membrane microdomains called "lipid rafts," with an important role in cells as transmembrane signal transduction and enzyme anchoring [349, 350].

Sterols in plants are present in three different chemical forms: free sterols, esterified with fatty acids (sterol esters), steryl glycosides, and acylated steryl glycosides, also known as the carbohydrate derivatives of sterols. Much higher proportion of free sterols is found in plants compared to sterol esters. It has been established that the main role of sterol esterification is to maintain the physiological level of sterols in cell membranes [351].

β-Sitosterol has been extensively utilized in drugs and dietary supplements for restoring lipid and cholesterol level in humans. Plant sterols and saponins combined have shown hypolipidemic and angioprotective activities [352]. Another biological activity attributed to β-sitosterol is inhibition of cancer cell proliferation besides other pharmacological properties such as anti-hypercholesterolemic [353], anti-inflammatory, and antiangiogenic [354–356]. A proposed mechanism of β-sitosterol is the activation of the sphingomyelin cycle which increases the ceramide production associated with apoptosis in various cancer cells, such as human colon cancer cells [357], human prostate cancer cells [358], human leukemic cells [359], human stomach cancer cells [360], and human breast cancer cells [361]. It has been recently published that stigmasterol, sitosterol, campesterol, and brassicasterol may be involved in the regulation of lipid metabolism and the pathogenesis of dementia [362, 363].

The chemical form of phytosterols plays an essential role on their activity, since it is well known that crystalline phytosterols are not soluble in the bile and, therefore, are unable to reduce cholesterol absorption [364]. On the contrary, development of formulations of free phytosterols with several emulsifiers has produced significant reduction in cholesterol absorption and LDL cholesterol [64]. Esterification of phytosterols with fatty acids increases their solubilization in the oily phase and consequently their activity [365]. Differently, phytosterol glycosides have amphipathic structure, which give rise to questions about their solubilization in bile and mechanism of action. Glycosylation is very common in many cell compounds [366], and for these compounds, bioavailability is the main concern. For example, more than 80% of phytosterols in potatoes are found as glycosides [367]. In a study based on a single dose, it was observed that the measured reduction of cholesterol absorption was similar to phytosterol glycosides and phytosterol esters [368].

A recent meta-analysis trying to elucidate if plant sterols reduce plasma concentrations of fat-soluble vitamins and carotenoids indicates that both plant sterols and stanols reduce hydrocarbon carotenoid concentrations (β-carotene, α-carotene, and lycopene), differently affect oxygenated carotenoid concentrations (not reduction in zeaxanthin and β-cryptoxanthin but not in lutein), and have no influence on tocopherol, retinol, and vitamin D plasmatic concentrations [369].

Finally, a recent study concerning to phytosterol oxidation products points out the relevance of the assessment of their potential adverse effects [370].

6 Phenolipids

Phenolic lipids are a class of natural products composed of a phenolic head group and an aliphatic side chain. These compounds are secondary metabolites that are not essential for cell growth but can assist against possible stress conditions of the producing organisms, such as infection, wounds, and UV radiation. Phenolic lipids mainly occur in plants but also in fungi and bacteria. Originally, its production was attributed to plant families of Anacardiaceae, Ginkgoaceae, Myristicaceae, and Orchidaceae, but later studies showed that these compounds can be produced by bacteria and mycobacteria pathogens. Besides, the synthesis of phenolic lipids by several other plant families was also reported [371].

Chemically, phenolic lipids can be considered as derivatives of mono- and dihydroxy phenols that generally contain a catechol, a resorcinol, or a hydroquinone nucleus alkylated by a carbon chain, mostly C11–C17 (see Fig. 3) [372].

Although phenolic lipids can be toxic, causing different allergic issues, they have been the subject of much attention due to other physiological properties. Phenolic lipids show amphiphilic properties, caused by the simultaneous presence of a hydrophilic phenol and a lipophilic alkyl chain in the same molecule. In aqueous media, they can be organized in micellar structures [373]. They can also incorporate into phospholipid membranes forming hydrogen bonds with these compounds. As consequence, phenolic lipids have the potential to affect biophysical properties of cell membranes, including many cellular metabolic processes, fluidity, charge and mobility of phospholipids, and the activity of membrane-bound enzymes [372]. Stasiuk and Kozubek extensively reviewed the various biological activities of phenolic lipids [373]. In addition, the possible participation of these compounds as antimutagens was suggested, since they can exert protection of cells against carcinogenesis and serve as cytotoxic and antitumor agents by causing apoptosis [374–376]. Moreover, due to their interaction with proteins and/or on their membrane-disturbing properties, the ability of these compounds to inhibit bacterial, fungal, protozoan, and parasite development besides the activity of several enzymes has been reported [377–380].

Among short-chain alkylphenols, the phenylpropanoids are present in essential oils and include isochavicol and derivatives, which displayed an interesting antiplasmodial activity [381].

The group of catechol lipids includes urushiol, which is found in plants of the family Anacardiaceae, especially *Toxicodendron* spp., and it is well known by its allergic properties. The likelihood and severity of allergic reaction to urushiol are dependent on the degree of unsaturation of the alkyl chain [382]. Similar compounds with a catechol nucleus esterified with a fatty acid (palmitic, stearic, and oleic acids) have been synthesized for their lipophilic antioxidant properties. All derivatives showed better radical-scavenging capacities than α-tocopherol and ascorbyl

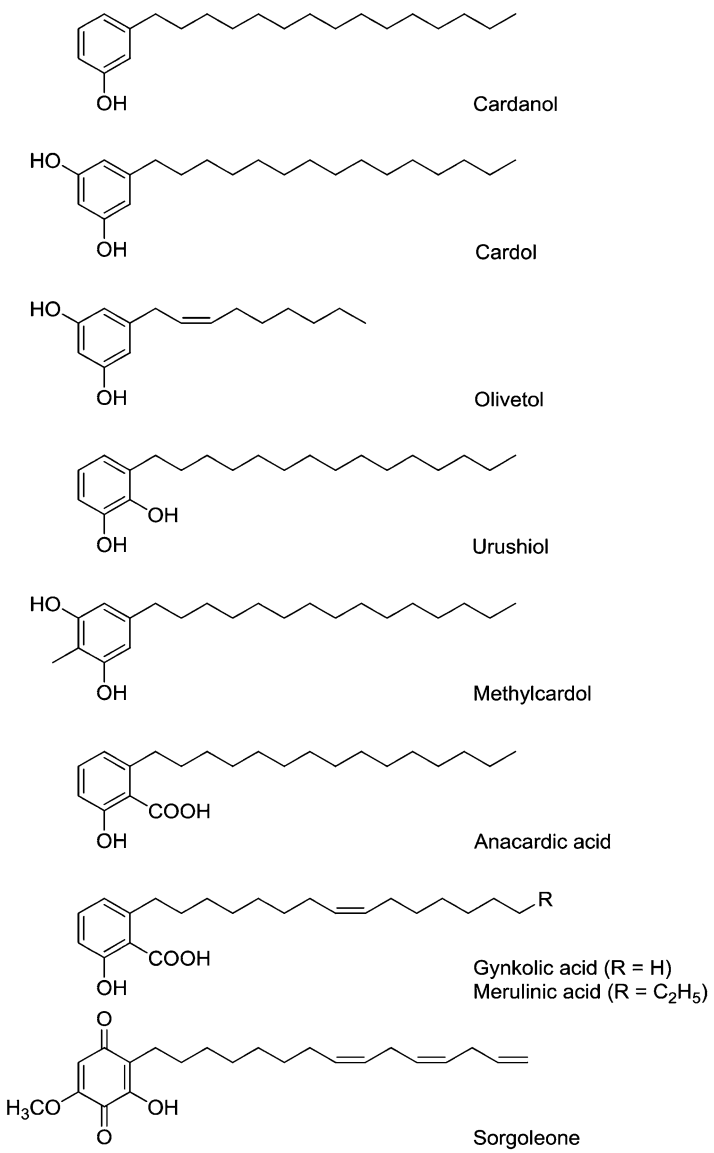


Fig. 3 Structural diversity of phenolic lipids [372]

palmitate [383]. Bonediol, an alkyl catechol from the Mayan medicinal plant *Bonellia macrocarpa*, showed to have antiproliferative and antiestrogenic activities. Additionally, bonediol could induce oxidative stress and activation of detoxification enzymes. For these reasons, it may serve as a potential chemopreventive treatment with therapeutic potential against prostate cancer [384].

The acid form of urushiol leads to the group of anacardic acids, which also cause an allergic skin rash on contact. These molecules consisted of a salicylic acid substituted with an alkyl chain (saturated or unsaturated) that has 15 or 17 carbon atoms. Moreover, anacardic acids from cashew nut shell liquid, a Brazilian natural substance, have antimicrobial and antioxidant activities and modulate immune responses and angiogenesis. The 15 carbon unsaturated side chain compound found in the cashew plant is lethal to Gram-positive bacteria [385]. In a study in mice, all doses of anacardic acids improved the antioxidant enzyme activities and decreased vascular adhesion molecule in vessels. With doses of 50 mg/kg of anacardic acids, animals showed decreased levels of neutrophils and tumor necrosis factor in the lungs and bronchoalveolar lavage fluid. Thus, it was demonstrated that this supplementation has a potential protective role on oxidative and inflammatory mechanisms in the lungs [386]. In this field, Hamad and Mubofu extensively reviewed the potential biological applications of bio-based anacardic acids and their derivatives [387].

Alkylresorcinols, also known as resorcinolic lipids, are phenolic lipids composed of long aliphatic chains and resorcinol-type phenolic rings [388]. Alkylresorcinols are relatively rare in nature, with the main known sources being wheat, rye, barley, triticale (cereal grasses), *Ginkgo biloba*, several other Anacardiaceae (*Anacardium occidentale*), mango latex and peel, and some species of bacteria. In these plants, resorcinol lipids were also related to strong allergic responses. Alkylresorcinols can also be used for specific applications, for example, 4-hexylresorcinol is a food additive (E-586) used as an antioxidant and color-stabilizing agent [389]. Bioactive properties of alkylresorcinols usually based their ability to integrate into membranes and inhibit enzymes. A wide number of in vitro studies have been carried out with alkylresorcinols, ranging from induction of apoptosis, inhibition of lipoxygenases, and cleavage of DNA to triglyceride reduction in adipocytes [390]. Alkylresorcinols were shown to have anticancer activities. Hence, in vitro studies presented inhibition of human colon, breast, lung, central nervous system, adenocarcinoma, hepatocarcinoma, cervix squamous carcinoma, and ovarian cancer cell lines, at micromolar alkylresorcinol concentration. Model studies suggest a high cytotoxicity of alkylresorcinols toward cancer cells; however, intervention studies to confirm their preventive action are needed [391].

Hydroquinone lipids are phenolic lipids with an aromatic ring belonging to the hydroquinone group linked to a straight carbon chain. Embelin and sorgoleone and their derivatives are included in this group of natural compounds reporting bioactive properties. Embelin was used in traditional Asian medicine as anti-vomiting and antidiarrhea agent [392], whereas sorgoleone can act as inhibitor of mitochondrial respiration and photosynthesis, and it has been used as natural herbicide [393].

In addition to the aforementioned compounds, other types of phenolic esters can also be found in nature. Phenolic compounds constitute a heterogeneous group that consist of simple phenols and polyphenols as well as their derivatives including phenolic acids, stilbenes, lignans, and flavonoids, by far the largest group of phenolics. Hence, for example, alkyl esters of ferulic and/or p-coumaric acids are present in soybean, cereals, propolis, periderm of potato, latex of sweet potato, parts

of *Artemisia assoana*, and leaves of *Larix kaempferi*. In addition, pods of *Piliostigma thonningii*, roots of *Tanacetum longifolium*, and wax of gala apples contain phenolic fatty acid esters of p-coumaryl alcohol, and docosyl caffeate can be found in the halophytic plant *Halocnemum strobilaceum* [394].

In this regard, acylated flavonoids were reported to protect glycosides from enzymatic degradation, to enhance pigment solubility in water, and to increase affinity to cell membranes (resulting in better penetration), antioxidant activity, enzyme inhibitory, and antiproliferative, cytogenetic, and antimicrobial properties. The enhanced hydrophobicity of these molecules results in a higher degree of penetration through the cell membrane, which permits them to interact with multiple cellular or molecular targets leading to their various biological activities [395].

It should to be noted that apart from natural sources, phenolic lipids can also be obtained from their corresponding phenolic compounds by modification techniques. Hence, different strategies were intended to produce these new molecules with potential use in technological applications or with targeted physiological properties.

One of the main purposes of these modifications is to improve the antioxidant activity of phenolic compounds in a variety of media. Redox properties of phenolic compounds promote their antioxidant activity, since they can act quenching singlet and triplet oxygen, neutralizing free radicals, or decomposing peroxides [396]. The polarity of the environment, in particular, strongly affects the antioxidant activity of phenolic antioxidants [397]. According to the antioxidant polar paradox, hydrophilic antioxidants tend to be more active in bulk oils or nonpolar media, whereas lipophilic antioxidants would inhibit lipid oxidation more efficiently in emulsions since they have more affinity for the oil-water interfaces [398]. Because phenolic antioxidants are highly polar, its lipophilization becomes a crucial step in the design of new antioxidant additives and drugs. Basically, lipophilization consists of the esterification of a lipophilic moiety (fatty acid or fatty alcohol) to a given substrate resulting in new more surface-active molecules [399]. Until now, numerous lipophilized antioxidants have been synthesized. Modification of a wide range of phenolic acids, flavonoids, tocopherols, triacylglycerols, or phospholipids has been performed to obtain lipophilized phenolics, also known as *phenolipids* [400]. As with ones found in nature, these structured lipids possess a saturated and/or polyunsaturated hydrocarbon chain and an aromatic ring bearing one or more hydroxyl or methoxyl substitutes, attached via an ester bond [401].

These molecules, with an appropriate lipophilicity, have shown improved bioavailability in vivo over their polar analogues. As reported with natural phenolic lipids, they enhance miscibility and incorporation into lipid phases and lipocarriers and can also be used in micellar, emulsified, and liposomal systems, so they open potential new applications for drug delivery systems and food, nutraceutical, pharmaceutical, and cosmetic industries [389].

It should be noted that the selection of the critical chain length of the alkyl moiety is crucial in the design and use of lipophilic antioxidants. Although the polar paradox states that apolar antioxidants are more active in oil-in-water emulsions than their polar analogues, the antioxidant activity regarding alkyl chain length is actually defined by a nonlinear trend [398]. Hence, the efficiency of hydrophobic antioxidants

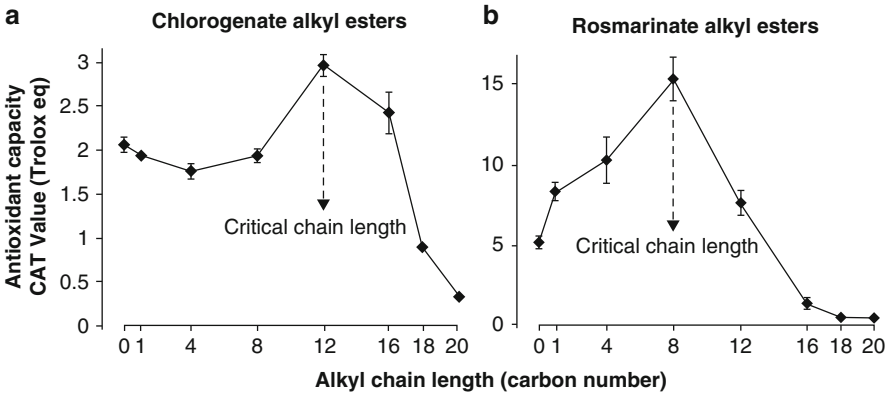


Fig. 4 Influence of the alkyl chain length of chlorogenate (**a**) and rosmarinate (**b**) alkyl esters on antioxidant activity in stripped tung oil-in-water emulsion [400]

increased with the alkyl chain length in emulsions and other more complex systems as liposomes, until a maximum is reached, but from that point, the antioxidant activity remarkably decreases, leading to a "cutoff" phenomenon [402]. Different studies established that esters of medium-chain length (C8–C12 alkyl chains) provided the highest activity [398]. Figure 4 shows two examples of different critical chain length providing the best antioxidant activity for chlorogenate and rosmarinate alkyl esters.

This nonlinear behavior can be explained because antioxidants with medium alkyl chain length tend to locate more at the oil-in-water interface, where antioxidants can provide greater protection against oxidation. Increasing the hydrocarbon chain could drive the antioxidant away from the interface into the emulsion droplet core, where the phenolics would be less efficient, which may explain the cutoff effect [403].

Apart from their wide use as food additives due to their antioxidant properties, it has been reported that, for example, propyl (E-310), octyl (E-311), and dodecyl (E-312) gallates also possess antifungal and antibacterial activities, essentially on Gram-positive bacteria [404]. Likewise, sapienic acid esters comprising hydroxybenzyl alcohol, tyrosol, and coniferyl alcohol exhibited some cytotoxicity toward different tested cell lines [389]. The effect of phenolipids on LDL oxidation has been of interest recently. Katsoura et al. reported that methyl, ethyl, and octyl ferulate had significantly higher antioxidant capacity toward LDL, HDL, and total serum oxidation. In this study, the protection effectiveness increased with the increasing length of the alkyl chain [405]. In this field, Shahidi's group lipase-catalyzed the synthesis of phenolipids to test the potential inhibitory effect of these compounds on LDL oxidation as well as radical-induced DNA cleavage [389, 401]. Moreover, Danihelová et al. reviewed the biological properties of lipophilic flavonoid derivatives (see Table 1) [406].

These functionalized antioxidants resulting from the grafting of lipid on a phenolic moiety can be prepared by a wide range of lipophilization strategies such as esterification, transesterification, amidation, and etherification. Normally, lipophilization of phenolic acids can be achieved by chemical, enzymatic, or chemo-

Table 1 Biological properties of lipophilic flavonoid derivatives [406]

Flavonoid	Substituent	Biological effect
Naringin, hesperidin, neohesperidin, hesperetin glucoside	Butyrate, decanoate, laurate	↑Antifungal
Rutin	Laurate, palmitate	↑↓Antioxidant
Chrysoeriol-7-*O*-β-D-(3″-E-pcoumaroyl)-glucopyranoside, chrysoeriol-7-[6″-*O*-acetyl-β-D-allosyl-(1→2)-β-D-glucopyranoside]	Vinyl laurate	↑Antioxidant, ↑antibacterial
Flavone, isoflavone	Prenyl, geranyl, dimethylallyl, methyl, methoxy	Antiproliferative
Flavone	Geranyl	Antiproliferative
Isorhamnetin-3-*O*-glucoside	Ethyl laurate, ethyl butyrate	↑Anticancer, ↓antioxidant
Quercetin	Tert-butylhydroxy	↑↓Antioxidant
Isoquercitrin	Butyrate, caproate, caprylate, decanoate, laurate, palmitate, stearate, oleate	↑↓Antioxidant, ↑anticancer
Flavane, thiaflavane	Methoxy	↑↓Antifungal
Rutin, phloridzin, esculin	Butyrate, caproate, caprylate, decanoate, laurate, myristate, palmitate, stearate, oleate, linoleate, linolenate, arachidonate, erucate	↑↓Antiprotease
Flavone	Methylethylamine, diethylamine, piperidine, pyrrolidine	Neuroprotective
Flavone	Alkyl	↑Anti-inflammatory
Rutin, naringin	Oleate, linolenate, linoleate	↑Anticancer
Quercetin	Alkyl	↑Antioxidant
Rutin	Butyrate, caproate, caprylate, decanoate, laurate, palmitate, stearate, oleate, linoleate, linolenate	↑Antioxidant
Biochanin A	Alkyl	↓Anti-inflammatory
Flavone	Methyl, naftyl, nitro, halogen	↑Anticancer
Chrysin	Methoxy, dodecoxy, diacetyl, methoxycinnamate	↑Anti-inflammatory
Flavone, isoflavone	Trifluoromethyl	↓Anticancer
Flavanone	Methoxy, benzyl	↑Anticancer
Flavone	Aliphatic and heterocyclic with N	↑↓Anticancer

enzymatic esterification of (a) the carboxylic acid group (−COOH) of phenolic acid with fatty alcohols or (b) the phenolic hydroxyl group (−OH) with fatty acids [403].

Although chemical esterifications are quite quick, cheap, and simple, they usually involve drastic conditions of temperature and pH, so they can promote deoxidation of phenolic compounds. In addition, as another drawbacks, chemical processes are

generally not selective and require many steps to remove residues and by-products [399]. On the other hand, enzymatic syntheses offer some advantages since they provide high selectivity, occur at mild conditions, are environmentally friendly, and require fewer purification steps. However, it should be taken into account that enzymatic processes may imply high costs, possible deactivation of the catalysts, and longer reaction times. Phenolic acids have been efficiently esterified with enzymes of the carboxylic ester hydrolase family, such as lipases, tannin acyl hydrolases, feruloyl esterases, and cutinases, by using different organic solvents or in a solvent-free media [407].

As aforementioned, synthetic lipophilization of phenolic acids with fatty alcohols can be used as a tool to produce these new amphiphile antioxidants. Figueroa-Espinoza et al. report some examples to obtain phenolipids from phenolic acids via chemical or enzymatic esterification at different conditions [407]. More particularly, Figueroa-Espinoza and Villeneuve extensively reviewed the background of the enzymatic esterification of phenolic acids as caffeic, cinnamic, ferulic, chlorogenic, gallic, etc. with fatty alcohols with different chain length [394].

The acylation of flavonoids, a class of phenolic compounds with a wide range of bioactive properties, has been the subject of much investigations, since it also permits to enhance their solubility in various media, stability, and antioxidant activity, among other physiological properties [408]. As with phenolic acids, acylation of flavonoids can be performed by using chemical catalysts [409]. However, apart from other advantages of enzymatic technology aforementioned, the regioselectivity of enzymes plays a crucial role in these processes, since it remarkably affects the functionalization of phenolic hydroxyl groups, which is responsible for the antioxidant activity of flavonoids. Chebil et al. extensively reviewed a multitude of investigations dealing with the regioselectivity and performance of the enzymatic acylation of flavonoids [410]. Several factors should be taken into account in order to enhance the performance of acylation, e.g., type, regioselectivity, and load of enzyme, nature of the flavonoid, acyl donor, operating conditions, etc. The reaction can be carried out using different organic solvents or almost solvent-free systems [411]. In this regard, both ionic liquids [412] and eutectic mixtures [413, 414] have also provided good properties as solvents, becoming an alternative solution for these enzymatic transformations.

Lipophilization with different acyl donors has also been applied to other polar antioxidants to increase its solubility and activity in nonpolar matrices. For example, ascorbic acid (vitamin C) has traditionally been hydrophobized by esterification or transesterification reactions with different aliphatic chains [415], mainly palmitic acid [416], or even with polyunsaturated fatty acids [417].

Last decades, there is a growing interest in the development of *structured lipids* with enhanced functional properties and health benefits. Structured lipids are originally triacylglycerols in which the composition and the distribution of fatty acids at the glycerol backbone are modified. The use of structured triacylglycerols seemed to be the most efficient way of delivering bioactive fatty acids, increasing their bioavailability, and thus enhancing their physiological effects [418]. Improvements in the solubility and miscibility of phenolic compounds in nonpolar systems can be attained upon their incorporation into triacylglycerols [419]. Hence, the structuring

of triacylglycerols with phenolic acids could potentially result in novel structured phenolic lipids becoming an interesting strategy for medical, nutraceutical, and food applications [420]. These novel structured molecules may provide a great number of beneficial properties by the combination of bioactive fatty acids (e.g., PUFA) and phenolic compounds in the same molecule [421]. Phenolic structured lipids were generally obtained via transesterification by acidolysis using enzyme catalysts. Acidolysis can be used to replace the existing fatty acids in the triacylglycerol molecule by other desired fatty acids, phenolic acids, or other organic acids [422].

It should be noted that Novozym 435 (*Candida antarctica* lipase) was the most widely used and showed the best catalysis performance [423]. As examples, this lipase was employed to effectively incorporate dihydrocaffeic acid in flaxseed oil [424], *p*-coumaric acid in seal blubber oil and menhaden oil [401], dihydrocaffeic acid and ferulic acid in trilinolein and trilinolenin [425], and cinnamic acid in triolein [426]. The same methodology was also used for acidolysis between flaxseed oil and selected phenolic acids (ferulic, *p*-coumaric, sinapic, etc.), including hydroxylated and/or methoxylated derivatives of cinnamic, phenyl acetic, and benzoic acids [419]. Besides, synthesis of phenolic lipids by transesterification of ethyl ferulate with castor oil [423], dihydroxyphenylacetic acid and dihydrocaffeic acid with krill oil [427], 3,4-dihydroxyphenyl acetic acid [421], and other selected phenolic acids [428] with flaxseed oil was affected in solvent-free media, using Novozym 435 from *Candida antarctica* as the biocatalyst. Supercritical carbon dioxide is a *green* solvent that was also used as solvent in the reaction medium for the acidolysis between flaxseed oil and ferulic acid [429].

The synthesis of structured phenolic lipids has great potential in the production of lipids with health benefits. On the one hand, it permits the combination of the antioxidant capacity of the phenolic compounds with the bioactive properties of specific fatty acids in the triglyceride molecule. On the other hand, the lipophilization of phenolic compounds occurs, improving the solubility, stability, and activity of these compounds in nonaqueous systems. For these reasons, structured phenolic lipids have potential applications in functional foods, nutraceuticals, pharmaceuticals, or cosmetics for health promotion and disease risk reduction.

7 Lipid Delivery Systems

As described above, a number of lipid-based compounds have shown in vitro and in vivo health benefits besides their normal nutritional role [430], so that their consumption is thought to promote human wellness and to reduce the risk of certain chronic diseases. Moreover, some of them also provide specific functional attributes to the product, as desirable color, flavor, or texture, and longer shelf life. Consequently, the interest of food and beverage industries in the incorporation of these compounds (called from now “lipophilic actives”) into commercial products is growing [431–433]. Moreover, many of the lipophilic actives covered in this book chapter are also applicable as nutraceuticals (therapeutic/preventive agents) and cosmetics.

However, application of lipophilic actives is often challenging due to their physicochemical properties, such as low chemical stability, poor water solubility, low oil solubility, high oil-water partition coefficient, high melting point, and crystalline state at room temperature [431, 434, 435]. Such characteristics make them incompatible with the aqueous matrix of food and beverage products and prone to physical, chemical, and biological degradation, either during product storage or during gastrointestinal (GI) digestion and systemic circulation (extensive metabolism and quick clearance). This may result in the generation of off-flavors or, even, potentially toxic reaction products, a poor and variable bioavailability, and the loss of in vivo bioactivity. All this limits industrial applicability of this kind of compounds, thereby highlighting the need of appropriately formulating them before their final application to improve these aspects.

Formulation strategies usually used by food, pharmaceutical, and cosmetic industries to overcome limitations of lipophilic actives include:

1. To mix them with other oil phase components (usually edible neutral oils) prior to preparing the product (conventional emulsion-based products) [436]. Neutral oils used for this purpose are usually TAG or terpene oils, which, moreover, make the product more palatable or desirable to consumers.
2. To modify their chemical structure, mainly by esterification [437].
3. To incorporate them into a suitable nanoparticle-based and microparticle-based delivery system before their final application [431, 432, 438].

The latter is the most commonly used strategy to efficiently incorporate lipophilic actives into commercial products and increase their in vivo efficacy after product ingestion or nutraceutical administration (usually by oral route). Preferably used delivery systems are those manufactured from food-grade ingredients (such as proteins, carbohydrates, lipids, and surfactants) and specifically designed for oral administration applications.

Among them, polymer-based delivery systems (PBDS) (as polymeric micelles, polymeric nanoparticles (PNPs), polymer-bioactive conjugates (PBCs), dendrimers, and biopolymer-based nanocarriers) have been popularly adopted. To a lesser extent, inclusion complexes with cyclodextrins and its derivatives as well as inorganic, hybrid, and other novel nanocarriers (as liquid crystals and lipid nanocapsules) are being currently used [439]. However, in recent years, an increased interest has been focused on the incorporation of lipophilic compounds into lipid-based delivery systems (LBDS), which has been shown to be one of the most powerful strategies for the formulation of these kinds of compounds [440, 441]. LBDS used for oral administration include vesicle systems (liposome and phospholipid complexes), lipid particulate systems (solid lipid particles (SLPs) and nanostructured lipid complexes (NLCs)), and emulsion-based systems (microemulsions (MEs), nanoemulsions (NEs), and self-emulsifying delivery systems (SEDSs)) [439].

No single delivery system is suitable for every lipophilic active. Due to their unique physicochemical characteristics, delivery systems must be carefully designed taking into account specific physicochemical properties of the lipophilic active to be

incorporated up (including physical state, solubility, partitioning, diffusion, interactions, optical characteristics, rheological properties, and stability) to overcome its specific challenges and, thus, reach the following goals [436]:

Before ingestion:

1. To readily disperse active compound in the aqueous matrix of food and beverage products.
2. To incorporate the active compound into food matrices without adversely affecting quality attributes of the product, such as appearance, texture, flavor, or stability.
3. To mask possible off-flavors of the lipophilic active (such as bitterness or astringency).
4. To efficiently protect the active ingredient against chemical, physical, or biological degradation, either during product storage, to avoid the generation of off-flavors and the loss of bioactivity. Knowledge of the degradation mechanism and the major factors that affect it (e.g., oxygen, light, pH, heat, enzyme activity, etc.) will facilitate the design of a more effective delivery system.
5. To improve the product storage and handling and extend product shelf life, which is directly related to goal 4.
6. To release the active ingredient (e.g., antimicrobial or antioxidant) at a particular site of action during food storage. For example, the physical location of an antioxidant within the food matrix is particularly important for determining its effectiveness, as it should be present as the site where lipid oxidation primarily occurs (within the oil, water, or interfacial regions). Appropriately designed delivery systems can be used to improve the efficacy of antioxidants in food and beverage products by delivering them to the appropriate site of action.

After ingestion of the fortified product or nutraceutical:

1. To enhance (or at least not adversely affect) the active compound bioavailability and reduce its inter- and intra-subject variability, leading to higher in vivo efficacy and more reproducible results in clinical assays. The Food and Drug Administration defines bioavailability as "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product, reach plasma and body tissues and becomes available at the site-of-action in an unchanged form." The impact of bioavailability is especially pronounced when the bioactive compound is intended for oral use, whereby gastrointestinal absorption constitutes the primary barrier between the active ingredient and systemic circulation. Delivery systems have shown to enhance oral bioavailability of lipophilic actives by improving their dispersion in GI tract (GIT) environment (which avoids their precipitation), protecting them against chemical (low pH in the stomach) and biological degradation (microbiota metabolism and enzymatic degradation) in the GIT, favoring their transportation until absorption area during GI digestion, and increasing GI wall permeability, which significantly enhances the intestinal absorption [439].

2. To enhance permeability of other mucosal membranes besides of GI membrane (such as the cornea, the nasal mucosa, the respiratory mucosa, and the stratum corneum of the epidermis) and the blood-brain barrier and increase the lipophilic active penetration into tumoral matrices [442, 443].
3. To increase the pharmaceutical stability of the active compound, thereby lengthening its systemic circulation time [444].
4. To release the active ingredient at a particular site of action (e.g., released in the mouth, stomach, small intestine, colon, or targeted organ/cells), at a controlled rate (sustained release), or in response to a specific environmental trigger (e.g., pH, ionic strength, temperature, or enzyme activity), which leads to targeted effects and increased in vivo efficacy.
5. To overcome multidrug resistance [445].
6. To enhance efficiency of co-delivery of lipophilic actives and therapeutic agents [446].
7. To reduce the effect of co-ingested food ingredients on pharmacokinetics of the active molecule [447].

As commented above, oral route is the most commonly used for the delivery of drugs and nutraceuticals (including lipophilic actives and other dietary bioactives). This is generally considered as the easiest and most convenient method since it is noninvasive, cost-effective, and less prone to side effects, such as injection-site reactions [448]. However, to overcome limitations in the oral administration of poor water-soluble compounds, parental (intravenous and intraperitoneal) and topical (transdermal, nasal, and ocular) administration routes can be used to increase dose precision and clinical efficacy. In recent years, topical delivery of bioactive compounds has also drawn great attention owing to its advantages over other administration routes and outstanding contribution in improving local action [151] or systemic absorption, which can minimize the first-pass hepatic metabolism [152]. Moreover, we should not forget that this administration route is the one used by cosmetic industry. These applications, however, also show several barriers that limit their use (including low skin permeation, injection-site reactions, short biological half-life, presystemic metabolism, or systemic toxicity [449]), and the use of nanocarriers has demonstrated to be also an efficient formulation strategy. In case of the parenteral route, most of the investigations have focused on utilizing nanocarriers (as liposomes, SLNs, NCLs, PNPs, and PBCs) to enhance bioactive efficiency through targeting of effects [450, 451], controlling drug release at site of action to minimize side effects [452, 453], or overcoming multidrug resistance [454]. For topical application, the incorporation of active compounds into nanocarriers (as MEs, liposomes, ethosomes, NLCs, PNPs, and PBCs) aims to enhance skin permeation and stability, lengthen systemic circulating time, and minimize metabolic degradation and systemic toxicity [439].

Table 2 summarizes the major types of lipophilic actives that need to be formulated, the specific challenges associated with their application, and the formulation strategies commonly used to overcome such challenges and increase their industrial applicability degree as therapeutics or in food fortification.

Table 2 Types of lipophilic actives commonly formulated

Lipophilic active	Challenge	Formulation strategy	Advantage of formulation
Neutral lipids			
• ω-3 PUFA (mainly ALA, EPA, and DHA) • CLA	• Low water solubility (aqueous matrix incompatibility) • Susceptible to lipid oxidation (problematic long-term storage, rancid off-flavors, and potentially toxic reaction products)	Use of LBDS (liposomes, spray-dried emulsions, multilayer emulsions, multiple emulsions) and filled hydrogel particles (complex coacervation)	• Easier incorporation into aqueous medium • Easier handling and storage • Prevention of lipid oxidation
Polar lipids			
Sphingolipids (ceramides)	• Low water solubility and in vivo bioavailability	• Use of LBDS (cationic pegylated liposomes) • Alteration of ceramide structure (e.g., cationic pyridinium, C_{16}-serinol, 4,6-diene-ceramide)	• More effective at crossing the cell membrane • Increased bioavailability/bioactivity • Targeting of antitumor effects • No side effects or lower toxicity to normal cells
Unsaponifiable lipids			
• Terpenes			
Essential oils	• Low water solubility • Some water solubility (instability by Ostwald ripening) • Low and variable bioavailability	• Use of LBDS (O/W emulsions, MEs, NEs, multilayer emulsions, multiple emulsions, and SLNs) • Esterification (e.g., citral acetate)	
	• Poor chemical stability (loss of bioactivity, off-flavors during storage) • Low molecular weight and high volatility (ease to loss of desirable flavors)		• Mask undesirable off-flavors • Easier incorporation into aqueous medium and handling • Easier handling and storage • Prevention of chemical degradation • Increased efficacy
Carotenoids	• Crystalline state at room temperature • Low oil solubility	• LBDS (O/W emulsions, surfactant micelles, MEs, NEs, NLCs), PBDS (PNPs and biopolymer-based nanocarriers), and CD inclusion complexes (vitamin A)	
Vitamin A (retinoid)	• Low water solubility • High melting point • Poor chemical stability • Low and variable bioavailability		
Vitamin E (tocopherols)		• Esterification (α-tocopherol acetate)	

• Sterols			
Vitamin D	• Low water solubility • Poor chemical stability • Low and variable bioavailability	• Use of LBDS (liposomes, O/W emulsions, surfactant micelles, NEs) and PBDS (PNPs and PBCs)	• Easier incorporation into aqueous medium and handling • Easier handling and storage • Prevention of chemical degradation • Increased efficacy
Phytosterols and phytostanols (stigmasterol, β-sitosterol, and campesterol)	• Low water solubility • Low oil solubility • High melting point • Poor chemical stability	• Esterification with PUFAs	
• Polyphenols			
Puerarin		LBDS (MEs, SMEDS, SLNs) and PBDS (dendrimers)	
Genistein		LBDS (MEs) and PBDS (dendrimers).	
Luteolin		LBDS (SMEDS, SNEDS)	
Silymarin		LBDS (SMEDS, NLCs), PBDS (PNPs), inorganic nanocarriers, and crystalline liquid nanocarriers	
EGCG	• Low water solubility • Extensive metabolism and quick clearance	LBDS (liposomes, PL-complexes, SMEDS, SNEDS)	• Increased bioavailability/bioactivity • Targeting of antitumor effects
Curcumin	• Low and variable bioavailability	LBDS (liposomes, S(N)EDS, NLCs) and PBDS (PNPs, PBCs, dendrimers)	• No side effects or lower toxicity to normal cells
Kaempferol		LBDS (PL-complexes)	
Quercetin		LBDS (MEs, PL-complexes[a]) and folate-modified lipid NCs	
Tyrosol		LBDS (phenolipid tyrosol-enriched lecithin)	
Resveratrol		LBDS (MEs, SLNs) and hybrid nanocarriers	

EGCG (−)-epigallocatechin-3-gallate, *LBDS* lipid-based delivery system, *PBDS* polymer-based delivery system, *SLN* solid lipid nanoparticle, *NLCs* nanostructured lipid complexes, *ME* microemulsion, *NE* nanoemulsion, *SMEDS* self-microemulsifying delivery systems, *SNEDS* self-nanoemulsifying delivery systems, *PNP* polymeric nanoparticle, *PBC* polymer-bioactive conjugate, *CD* cyclodextrin

[a]Phospholipid (PL)-complexes include phytosomes and phenolipids (quercetin-enriched lecithin)

It is worth mentioning that some of the bioactive lipids that have been discussed in the present book chapter can act, in turn, as efficient lipid-based delivery systems. This is the case, for example, of alkylglycerols (AKGs). AKGs, previously isolated from shark liver oil, have been used as efficient delivery systems of bioactive substances such as butyric acid [454] and hydroxytyrosol esters [455]. Likewise, ratfish liver oil, with an exceptionally high content in AKG, has been recently used to obtain a bioactive lipid-based delivery system with potential self-emulsifying properties by enzymatic glycerolysis [456]. In addition to all the advantages that the formulation of bioactive molecules with delivery systems offer, the use of bioactive AKG to design lipid-based delivery systems provides additional benefits, since its bioactivity could have an addition or even synergistic effect with the loaded bioactive compound, giving rise to highly bioefficient formulations.

Acknowledgments This study has been funded by the Comunidad Autónoma de Madrid (ALIBIRD, project number S2013/ABI-2728) and by Ministerio de Economia y Competitividad (project number AGL2016-76736-C3-1-R). Pablo Arranz-Martínez and Marta Corzo-Martínez also thank Ministerio de Economia y Competitividad and the European Social Fund: BES-2014-070395 for a predoctoral FPU grant and a Juan de la Cierva contract, respectively.

References

1. Mouritsen OG (2005) Prologue: lipidomics – a science beyond stamp collection. In: Life – as a matter of fat: the emerging science of lipidomics. Springer, Berlin/Heidelberg, pp 1–5. https://doi.org/10.10 07/3-540-27076-0_1
2. Escribá PV, González-Ros JM, Goñi FM, Kinnunen PKJ, Vigh L, Sánchez-Magraner L, Fernández AM, Busquets X, Horváth I, Barceló-Coblijn G (2008) Membranes: a meeting point for lipids, proteins and therapies. J Cell Mol Med 12(3):829–875. https://doi.org/10.1111/j.1582-4934.2008.00281.x
3. Aluko R (2012) Bioactive lipids. In: Functional foods and nutraceuticals. Springer, New York, pp 23–36. https://doi.org/10.1007/978-1-4614-3480-1_2
4. Roby MHH (2017) Synthesis and characterization of phenolic lipids, Ch. 04. In: Soto-Hernandez M, Palma-Tenango M, Garcia-Mateos MR (eds) Phenolic compounds – natural sources, importance and applications. InTech, Rijeka. https://doi.org/10.5772/66891
5. Svennerholm L (1977) The nomenclature of lipids. IUPAC-IUB Commission on Biochemical Nomenclature (CBN). Eur J Biochem 79:11–21
6. Layden BT, Angueira AR, Brodsky M, Durai V, Lowe WL (2013) Short chain fatty acids and their receptors: new metabolic targets. Transl Res 161(3):131–140
7. Bracco U (1994) Effect of triglyceride structure on fat absorption. Am J Clin Nutr 60(6):1002S–1009S
8. Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 233(6):674–688. https://doi.org/10.3181/0711-MR-311
9. Simopoulos AP (2001) Evolutionary aspects of diet and essential fatty acids. In: Fatty acids and lipids-new findings, vol 88. Karger Publishers, Basel, pp 18–27
10. Simopoulos A (2016) An increase in the Omega-6/Omega-3 fatty acid ratio increases the risk for obesity. Forum Nutr 8(3):128
11. Dumm INDG, Brenner RR (1975) Oxidative desaturation of α-linolenic, linoleic, and stearic acids by human liver microsomes. Lipids 10(6):315–317
12. Biesalski H-K (2005) Meat as a component of a healthy diet–are there any risks or benefits if meat is avoided in the diet? Meat Sci 70(3):509–524

13. Rangel-Huerta OD, Gil A (2017) Omega 3 fatty acids in cardiovascular disease risk factors: an updated systematic review of randomised clinical trials. Clin Nutr. https://doi.org/10.1016/j.clnu.2017.05.015
14. Bjerregaard P, Pedersen H, Mulvad G (2000) The associations of a marine diet with plasma lipids, blood glucose, blood pressure and obesity among the Inuit in Greenland. Eur J Clin Nutr 54(9):732
15. Ebbesson SO, Kennish J, Ebbesson L, Go O, Yeh J (1999) Diabetes is related to fatty acid imbalance in Eskimos. Int J Circumpolar Health 58(2):108–119
16. Dyall SC (2011) Methodological issues and inconsistencies in the field of omega-3 fatty acids research. Prostaglandins Leukot Essent Fatty Acids (PLEFA) 85(5):281–285
17. Kaur G, Cameron-Smith D, Garg M, Sinclair AJ (2011) Docosapentaenoic acid (22: 5n-3): a review of its biological effects. Prog Lipid Res 50(1):28–34
18. Cansev M, Wurtman R (2007) Chronic administration of docosahexaenoic acid or eicosapentaenoic acid, but not arachidonic acid, alone or in combination with uridine, increases brain phosphatide and synaptic protein levels in gerbils. Neuroscience 148(2):421–431
19. Yanes O, Clark J, Wong DM, Patti GJ, Sánchez-Ruiz A, Benton HP, Trauger SA, Desponts C, Ding S, Siuzdak G (2010) Metabolic oxidation regulates embryonic stem cell differentiation. Nat Chem Biol 6(6):411–417. http://www.nature.com/nchembio/journal/v6/n6/suppinfo/nchembio.364_S1.html
20. Katakura M, Hashimoto M, Okui T, Shahdat HM, Matsuzaki K, Shido O (2013) Omega-3 polyunsaturated fatty acids enhance neuronal differentiation in cultured rat neural stem cells. Stem Cells Int 2013:9. https://doi.org/10.1155/2013/490476
21. Samieri C, Feart C, Proust-Lima C, Peuchant E, Tzourio C, Stapf C, Berr C, Barberger-Gateau P (2011) Olive oil consumption, plasma oleic acid, and stroke incidence the three-city study. Neurology 77:418. https://doi.org/10.1212/WNL.0b013e318220abeb
22. Samieri C, Féart C, Letenneur L, Dartigues J-F, Pérès K, Auriacombe S, Peuchant E, Delcourt C, Barberger-Gateau P (2008) Low plasma eicosapentaenoic acid and depressive symptomatology are independent predictors of dementia risk. Am J Clin Nutr 88(3):714–721
23. Bazan NG, Molina MF, Gordon WC (2011) Docosahexaenoic acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. Annu Rev Nutr 31:321–351
24. Serini S, Bizzarro A, Piccioni E, Fasano E, Rossi C, Lauria A, Cittadini AR, Masullo C, Calviello G (2012) EPA and DHA differentially affect in vitro inflammatory cytokine release by peripheral blood mononuclear cells from Alzheimer's patients. Curr Alzheimer Res 9(8):913–923
25. Adarme-Vega TC, Lim DKY, Timmins M, Vernen F, Li Y, Schenk PM (2012) Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb Cell Factories 11(1):96. https://doi.org/10.1186/1475-2859-11-96
26. Surette ME, Edens M, Chilton FH, Tramposch KM (2004) Dietary echium oil increases plasma and neutrophil long-chain (n-3) fatty acids and lowers serum triacylglycerols in hypertriglyceridemic humans. J Nutr 134(6):1406–1411
27. Harris WS, Lemke SL, Hansen SN, Goldstein DA, DiRienzo MA, Su H, Nemeth MA, Taylor ML, Ahmed G, George C (2008) Stearidonic acid-enriched soybean oil increased the omega-3 index, an emerging cardiovascular risk marker. Lipids 43(9):805–811
28. Lenihan-Geels G, Bishop KS, Ferguson LR (2013) Alternative sources of omega-3 fats: can we find a sustainable substitute for fish? Forum Nutr 5(4):1301–1315
29. Kuhnt K, Degen C, Jaudszus A, Jahreis G (2012) Searching for health beneficial n-3 and n-6 fatty acids in plant seeds. Eur J Lipid Sci Technol 114(2):153–160
30. Kapoor R, Huang Y-S (2006) Gamma linolenic acid: an antiinflammatory omega-6 fatty acid. Curr Pharm Biotechnol 7(6):531–534
31. Mizock BA (2010) Immunonutrition and critical illness: an update. Nutrition 26(7):701–707
32. Olendzki BC, Leung K, Van Buskirk S, Reed G, Zurier RB (2011) Treatment of rheumatoid arthritis with marine and botanical oils: Influence on serum lipids. Evid Based Complement Alternat Med 2011:827286

33. Pottel L, Lycke M, Boterberg T, Foubert I, Pottel H, Duprez F, Goethals L, Debruyne PR (2014) Omega-3 fatty acids: physiology, biological sources and potential applications in supportive cancer care. Phytochem Rev 13(1):223–244
34. Yang Z-H, Miyahara H, Hatanaka A (2011) Chronic administration of palmitoleic acid reduces insulin resistance and hepatic lipid accumulation in KK-Ay mice with genetic type 2 diabetes. Lipids Health Dis 10(1):120. https://doi.org/10.1186/1476-511x-10-120
35. Lopez-Miranda J, Perez-Jimenez F, Ros E, De Caterina R, Badimon L, Covas MI, Escrich E, Ordovas JM, Soriguer F, Abia R, de la Lastra CA, Battino M, Corella D, Chamorro-Quirós J, Delgado-Lista J, Giugliano D, Esposito K, Estruch R, Fernandez-Real JM, Gaforio JJ, La Vecchia C, Lairon D, López-Segura F, Mata P, Menéndez JA, Muriana FJ, Osada J, Panagiotakos DB, Paniagua JA, Pérez-Martinez P (2010) Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaen and Cordoba (Spain) 2008. Nutr Metab Cardiovasc Dis 20:284. https://doi.org/10.1016/j.numecd.2009.12.007
36. Ducheix S, Montagner A, Polizzi A, Lasserre F, Régnier M, Marmugi A, Benhamed F, Bertrand-Michel J, Mselli-Lakhal L, Loiseau N (2017) Dietary oleic acid regulates hepatic lipogenesis through a liver X receptor-dependent signaling. PLoS One 12(7):e0181393
37. Valenzuela A, Delplanque B, Tavella M (2011) Stearic acid: a possible substitute for trans fatty acids from industrial origin. Grasas y Aceites 62:131–138
38. Emken EA (1994) Metabolism of dietary stearic acid relative to other fatty acids in human subjects. Am J Clin Nutr 60(6):1023S–1028S
39. Hunter JE, Zhang J, Kris-Etherton PM (2010) Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic review. Am J Clin Nutr 91(1):46–63
40. Grundy SM (1994) Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. Am J Clin Nutr 60(6):986S–990S
41. Fave G, Coste T, Armand M (2004) Physicochemical properties of lipids: new strategies to manage fatty acid bioavailability. Cell Mol Biol 50(7):815–832
42. Sørensen LB, Cueto HT, Andersen MT, Bitz C, Holst JJ, Rehfeld JF, Astrup A (2008) The effect of salatrim, a low-calorie modified triacylglycerol, on appetite and energy intake. Am J Clin Nutr 87(5):1163–1169
43. Huang W-C, Tsai T-H, Chuang L-T, Li Y-Y, Zouboulis CC, Tsai P-J (2014) Anti-bacterial and anti-inflammatory properties of capric acid against Propionibacterium acnes: a comparative study with lauric acid. J Dermatol Sci 73(3):232–240
44. Dayrit FM (2015) The properties of lauric acid and their significance in coconut oil. J Am Oil Chem Soc 92(1):1–15
45. Temme E, Mensink RP, Hornstra G (1996) Comparison of the effects of diets enriched in lauric, palmitic, or oleic acids on serum lipids and lipoproteins in healthy women and men. Am J Clin Nutr 63(6):897–903
46. Alves NFB, Queiroz TM, Almeida Travassos R, Magnani M, Andrade Braga V (2017) Acute treatment with Lauric acid reduces blood pressure and oxidative stress in spontaneously hypertensive rats. Basic Clin Pharmacol Toxicol 120(4):348–353
47. Lappano R, Sebastiani A, Cirillo F, Rigiracciolo DC, Galli GR, Curcio R, Malaguarnera R, Belfiore A, Cappello AR, Maggiolini M (2017) The lauric acid-activated signaling prompts apoptosis in cancer cells. Cell Death Dis 3:17063
48. Law KS, Azman N, Omar EA, Musa MY, Yusoff NM, Sulaiman SA, Hussain NHN (2014) The effects of virgin coconut oil (VCO) as supplementation on quality of life (QOL) among breast cancer patients. Lipids Health Dis 13(1):139
49. Silberstein T, Burg A, Blumenfeld J, Sheizaf B, Tzur T, Saphier O (2013) Saturated fatty acid composition of human milk in Israel: a comparison between Jewish and Bedouin women. Israel Med Assoc J 15(4):156–159
50. Silva RB, Silva-Junior EV, Rodrigues LC, Andrade LH, Silva SI, Harand W, Oliveira AF (2015) A comparative study of nutritional composition and potential use of some underutilized tropical fruits of Arecaceae. An Acad Bras Cienc 87(3):1701–1709

51. Zock PL, de Vries JH, Katan MB (1994) Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. Arterioscler Thromb Vasc Biol 14(4):567–575
52. Mensink RP, Zock PL, Kester AD, Katan MB (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 77(5):1146–1155
53. Katan MB, Zock PL, Mensink RP (1994) Effects of fats and fatty acids on blood lipids in humans: an overview. Am J Clin Nutr 60(6):1017S–1022S
54. Takato T, Iwata K, Murakami C, Wada Y, Sakane F (2017) Chronic administration of myristic acid improves hyperglycaemia in the Nagoya–Shibata–Yasuda mouse model of congenital type 2 diabetes. Diabetologia 60(10):2076–2083. https://doi.org/10.1007/s00125-017-4366-4
55. Ericson U, Hellstrand S, Brunkwall L, Schulz C-A, Sonestedt E, Wallström P, Gullberg B, Wirfält E, Orho-Melander M (2015) Food sources of fat may clarify the inconsistent role of dietary fat intake for incidence of type 2 diabetes. Am J Clin Nutr. https://doi.org/10.3945/ajcn.114.103010
56. Akoh CC (2017) Food lipids: chemistry, nutrition, and biotechnology. CRC Press, Boca Raton
57. Marten B, Pfeuffer M, Schrezenmeir J (2006) Medium-chain triglycerides. Int Dairy J 16(11):1374–1382
58. Zhang Y, Liu Y, Wang J, Zhang R, Jing H, Yu X, Zhang Y, Xu Q, Zhang J, Zheng Z (2010) Medium-and long-chain triacylglycerols reduce body fat and blood triacylglycerols in hypertriacylglycerolemic, overweight but not obese, Chinese individuals. Lipids 45(6):501–510
59. Nagao K, Yanagita T (2010) Medium-chain fatty acids: functional lipids for the prevention and treatment of the metabolic syndrome. Pharmacol Res 61(3):208–212. https://doi.org/10.1016/j.phrs.2009.11.007
60. Osborn H, Akoh C (2002) Structured lipids-novel fats with medical, nutraceutical, and food applications. Compr Rev Food Sci Food Saf 1(3):110–120
61. Vázquez L, Akoh CC (2010) Fractionation of short and medium chain fatty acid ethyl esters from a blend of oils via ethanolysis and short-path distillation. J Am Oil Chem Soc 87(8):917–928
62. Lieberman S, Enig MG, Preuss HG (2006) A review of monolaurin and lauric acid: natural virucidal and bactericidal agents. Altern Complement Ther 12(6):310–314
63. Ruzin A, Novick RP (2000) Equivalence of lauric acid and glycerol monolaurate as inhibitors of signal transduction in *Staphylococcus aureus*. J Bacteriol 182(9):2668–2671
64. Enig MG (1998) Lauricoils as antimicrobial agents: theory of effect, scientific rationale. Nutr Foods AIDS 17:81
65. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, Bäckhed F, Mithieux G (2014) Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell 156(1):84–96
66. Tremaroli V, Backhed F (2012) Functional interactions between the gut microbiota and host metabolism. Nature 489:242. https://doi.org/10.1038/nature11552
67. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 504(7480):446
68. Plaisancie P, Dumoulin V, Chayvialle J, Cuber J (1996) Luminal peptide YY-releasing factors in the isolated vascularly perfused rat colon. J Endocrinol 151(3):421–429
69. Zaibi MS, Stocker CJ, O'Dowd J, Davies A, Bellahcene M, Cawthorne MA, Brown AJ, Smith DM, Arch JR (2010) Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. FEBS Lett 584(11):2381–2386
70. Canfora EE, Jocken JW, Blaak EE (2015) Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 11(10):577–591
71. De Vadder F, Kovatcheva-Datchary P, Zitoun C, Duchampt A, Bäckhed F, Mithieux G (2016) Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. Cell Metab 24(1):151–157

72. Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev 70:567
73. Besten G, Lange K, Havinga R, Dijk TH, Gerding A, Eunen K, Muller M, Groen AK, Hooiveld GJ, Bakker BM, Reijngoud DJ (2013) Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. Am J Physiol Gastrointest Liver Physiol 305: G900. https://doi.org/10.1152/ajpgi.00265.2013
74. Boets E, Deroover L, Houben E, Vermeulen K, Gomand SV, Delcour JA, Verbeke K (2015) Quantification of in vivo colonic short chain fatty acid production from inulin. Forum Nutr 7(11):8916–8929
75. Besten G, Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 54:2325. https://doi.org/10.1194/jlr.R036012
76. Inoue D, Tsujimoto G, Kimura I (2014) Regulation of energy homeostasis by GPR41. Front Endocrinol (Lausanne) 5:81
77. Kimura I, Inoue D, Hirano K, Tsujimoto G (2014) The SCFA receptor GPR43 and energy metabolism. Front Endocrinol (Lausanne) 5:85
78. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashihara D, Hirano K, Tani T (2013) The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nat Commun 4:1829
79. Cani PD (2014) Metabolism in 2013: the gut microbiota manages host metabolism. Nat Rev Endocrinol 10:74. https://doi.org/10.1038/nrendo.2013.240
80. Ohira H, Tsutsui W, Fujioka Y (2017) Are short chain fatty acids in gut microbiota defensive players for inflammation and atherosclerosis? J Atheroscler Thromb. https://doi.org/10.5551/jat.RV17006
81. Kahouli I, Malhotra M, Tomaro-Duchesneau C, Saha S, Marinescu D, Rodes L, Alaoui-Jamali M, Prakash S (2015) Screening and in-vitro analysis of *Lactobacillus reuteri* strains for short chain fatty acids production, stability and therapeutic potentials in colorectal cancer. J Bioequivalence Bioavailab 7(1):39
82. Miquel S, Martin R, Rossi O, Bermudez-Humaran LG, Chatel JM, Sokol H, Thomas M, Wells JM, Langella P (2013) Faecalibacterium prausnitzii and human intestinal health. Curr Opin Microbiol 16:255. https://doi.org/10.1016/j.mib.2013.06.003
83. Serpa J, Caiado F, Carvalho T, Torre C, Goncalves LG, Casalou C, Lamosa P, Rodrigues M, Zhu Z, Lam EW, Dias S (2010) Butyrate-rich colonic microenvironment is a relevant selection factor for metabolically adapted tumor cells. J Biol Chem 285:39211. https://doi.org/10.1074/jbc.M110.156026
84. Morris G, Berk M, Carvalho A, Caso JR, Sanz Y, Walder K, Maes M (2017) The role of the microbial metabolites including tryptophan catabolites and short chain fatty acids in the pathophysiology of immune-inflammatory and neuroimmune disease. Mol Neurobiol 54(6): 4432–4451. https://doi.org/10.1007/s12035-016-0004-2
85. Tang Y, Chen Y, Jiang H, Robbins GT, Nie D (2011) G-protein-coupled receptor for short-chain fatty acids suppresses colon cancer. Int J Cancer 128(4):847–856
86. Eeckhaut V, Machiels K, Perrier C, Romero C, Maes S, Flahou B, Steppe M, Haesebrouck F, Sas B, Ducatelle R (2012) Butyricicoccus pullicaecorum in inflammatory bowel disease. Gut. https://doi.org/10.1136/gutjnl-2012-303611
87. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL (2014) Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med 20(2):159–166
88. Tan J, McKenzie C, Vuillermin PJ, Goverse G, Vinuesa CG, Mebius RE, Macia L, Mackay CR (2016) Dietary fiber and bacterial SCFA enhance oral tolerance and protect against food allergy through diverse cellular pathways. Cell Rep 15(12):2809–2824
89. Scheppach W, Sommer H, Kirchner T, Paganelli G-M, Bartram P, Christl S, Richter F, Dusel G, Kasper H (1992) Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 103(1):51–56
90. Havenaar R (2011) Intestinal health functions of colonic microbial metabolites: a review. Benef Microbes 2:103. https://doi.org/10.3920/bm2011.0003

91. Wong JM, De Souza R, Kendall CW, Emam A, Jenkins DJ (2006) Colonic health: fermentation and short chain fatty acids. J Clin Gastroenterol 40(3):235–243
92. Tarini J, Wolever TM (2010) The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. Appl Physiol Nutr Metab 35(1):9–16
93. Van Hoek MJ, Merks RM (2017) Emergence of microbial diversity due to cross-feeding interactions in a spatial model of gut microbial metabolism. BMC Syst Biol 11(1):56
94. Moens F, Verce M, De Vuyst L (2017) Lactate-and acetate-based cross-feeding interactions between selected strains of lactobacilli, bifidobacteria and colon bacteria in the presence of inulin-type fructans. Int J Food Microbiol 241:225–236
95. Argenzio R, Meuten D (1991) Short-chain fatty acids induce reversible injury of porcine colon. Dig Dis Sci 36(10):1459–1468
96. Rodenburg W, Keijer J, Kramer E, Vink C, van der Meer R, Bovee-Oudenhoven IM (2008) Impaired barrier function by dietary fructo-oligosaccharides (FOS) in rats is accompanied by increased colonic mitochondrial gene expression. BMC Genomics 9(1):144
97. Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Hennekens CH, Hennekens CH, Willett WC, Stampfer MJ, Colditz GA, Willett WC, Sampson LA, Rosner BA (1993) Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 341(8845):581–585. https://doi.org/10.1016/0140-6736(93)90350-P
98. Aldai N, de Renobales M, Barron LJR, Kramer JKG (2013) What are the trans fatty acids issues in foods after discontinuation of industrially produced trans fats? Ruminant products, vegetable oils, and synthetic supplements. Eur J Lipid Sci Technol 115(12):1378–1401. https://doi.org/10.1002/ejlt.201300072
99. Bassett CMC, Edel AL, Patenaude AF, McCullough RS, Blackwood DP, Chouinard PY, Paquin P, Lamarche B, Pierce GN (2010) Dietary vaccenic acid has Antiatherogenic effects in LDLr$^{-/-}$ mice. J Nutr 140(1):18–24. https://doi.org/10.3945/jn.109.105163
100. Jensen RG (2002) The composition of bovine milk lipids: January 1995 to December 2000. J Dairy Sci 85(2):295–350
101. Ferlay A, Bernard L, Meynadier A, Malpuech-Brugère C (2017) Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: a review. Biochimie. https://doi.org/10.1016/j.biochi.2017.08.006
102. Precht D, Molkentin J (1995) Trans fatty acids: implications for health, analytical methods, incidence in edible fats and intake. Mol Nutr Food Res 39(5–6):343–374
103. Vahmani P, Meadus W, Turner T, Duff P, Rolland D, Mapiye C, Dugan M (2015) Individual trans 18: 1 isomers are metabolised differently and have distinct effects on lipogenesis in 3T3-L1 adipocytes. Lipids 50(2):195–204
104. Vahmani P, Meadus WJ, Duff P, Rolland DC, Dugan ME (2017) Comparing the lipogenic and cholesterolgenic effects of individual trans-18: 1 isomers in liver cells. Eur J Lipid Sci Technol 119(3). https://doi.org/10.1002/ejlt.201600162
105. Bendsen NT, Christensen R, Bartels EM, Astrup A (2011) Consumption of industrial and ruminant trans fatty acids and risk of coronary heart disease: a systematic review and meta-analysis of cohort studies. Eur J Clin Nutr 65(7):773
106. de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, Uleryk E, Budylowski P, Schünemann H, Beyene J, Anand SS (2015) Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. BMJ 351:h3978. https://doi.org/10.1136/bmj.h3978
107. Brouwer IA, World Health Organization (2016) Effect of trans-fatty acid intake on blood lipids and lipoproteins: a systematic review and meta-regression analysis. World Health Organization, Geneva
108. Ferlay A, Bernard L, Meynadier A, Malpuech-Brugère C (2017) Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: a review. Biochimie 141:107
109. Dilzer A, Park Y (2012) Implication of conjugated linoleic acid (CLA) in human health. Crit Rev Food Sci Nutr 52:488. https://doi.org/10.1080/10408398.2010.501409

110. McCrorie TA, Keaveney EM, Wallace JM, Binns N, Livingstone MBE (2011) Human health effects of conjugated linoleic acid from milk and supplements. Nutr Res Rev 24 (2):206–227
111. Park Y (2014) Conjugated linoleic acid in human health effects on weight control. In Watson RR (ed) Nutrition in the prevention and treatment of abdominal obesity, Elsevier, London, pp 429–446
112. Bauman DE, Corl BA, Peterson DG (2003) The biology of conjugated linoleic acids in ruminants. Adv Conjug Linoleic Acid Res 2:146–173
113. Kim JH, Kim Y, Kim YJ, Park Y (2016) Conjugated linoleic acid: potential health benefits as a functional food ingredient. Annu Rev Food Sci Technol 7(1):221–244. https://doi.org/10.1146/annurev-food-041715-033028
114. Viladomiu M, Hontecillas R, Bassaganya-Riera J (2016) Modulation of inflammation and immunity by dietary conjugated linoleic acid. Eur J Pharmacol 785:87–95
115. Terpstra AHM, Javadi M, Beynen AC, Kocsis S, Lankhorst AE, Lemmens AG, Mohede ICM (2003) Dietary conjugated linoleic acids as free fatty acids and triacylglycerols similarly affect body composition and energy balance in mice. J Nutr 133(10):3181–3186
116. Park Y, Albright KJ, Storkson JM, Liu W, Cook ME, Pariza MW (1999) Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. Lipids 34(3):243–248. https://doi.org/10.1007/s11745-999-0359-7
117. Malpuech-Brugère C, Verboeket-van de Venne WPHG, Mensink RP, Arnal M-A, Morio B, Brandolini M, Saebo A, Lassel TS, Chardigny JM, Sébédio JL, Beaufrère B (2004) Effects of two conjugated linoleic acid isomers on body fat mass in overweight humans. Obes Res 12(4):591–598. https://doi.org/10.1038/oby.2004.68
118. Risérus U, Arner P, Brismar K, Vessby B (2002) Treatment with dietary *trans*10*cis*12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care 25(9):1516–1521. https://doi.org/10.2337/diacare.25.9.1516
119. Onakpoya IJ, Posadzki PP, Watson LK, Davies LA, Ernst E (2012) The efficacy of long-term conjugated linoleic acid (CLA) supplementation on body composition in overweight and obese individuals: a systematic review and meta-analysis of randomized clinical trials. Eur J Nutr 51(2):127–134
120. Yang J, Wang H-P, Zhou L-M, Zhou L, Chen T, Qin L-Q (2015) Effect of conjugated linoleic acid on blood pressure: a meta-analysis of randomized, double-blind placebo-controlled trials. Lipids Health Dis 14(1):11. https://doi.org/10.1186/s12944-015-0010-9
121. Ip C, Chin SF, Scimeca JA, Pariza MW (1991) Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res 51(22):6118–6124
122. Pariza MW, Park Y, Cook ME (1999) Conjugated linoleic acid and the control of cancer and obesity. Toxicol Sci 52(Suppl 1):107–110
123. Ip C, Dong Y, Ip MM, Banni S, Carta G, Angioni E, Murru E, Spada S, Melis MP, Saebo A (2002) Conjugated linoleic acid isomers and mammary cancer prevention. Nutr Cancer 43(1):52–58
124. Arab A, Akbarian S, Ghiyasvand R, Miraghajani M (2016) The effects of conjugated linoleic acids on breast cancer: a systematic review. Adv Biomed Res 5(1):115–115. https://doi.org/10.4103/2277-9175.185573
125. Hennessy AA, Ross RP, Devery R, Stanton C (2011) The health promoting properties of the conjugated isomers of α-Linolenic acid. Lipids 46(2):105–119. https://doi.org/10.1007/s11745-010-3501-5
126. Białek A, Teryks M, Tokarz A (2014) Conjugated linolenic acids (CLnA, super CLA)–natural sources and biological activity. Postepy hig med doswiadczalnej (Online) 68:1238–1250
127. Harzallah A, Hammami M, Kępczyńska MA, Hislop DC, Arch JRS, Cawthorne MA, Zaibi MS (2016) Comparison of potential preventive effects of pomegranate flower, peel and seed oil on insulin resistance and inflammation in high-fat and high-sucrose diet-induced obesity mice model. Arch Physiol Biochem 122(2):75–87. https://doi.org/10.3109/13813455.2016.1148053

128. Boussetta T, Raad H, Lettéron P, Gougerot-Pocidalo M-A, Marie J-C, Driss F, El-Benna J (2009) Punicic acid a conjugated linolenic acid inhibits TNFα-induced neutrophil hyperactivation and protects from experimental colon inflammation in rats. PLoS One 4(7):e6458
129. Schubert SY, Neeman I, Resnick N (2002) A novel mechanism for the inhibition of NF-κB activation in vascular endothelial cells by natural antioxidants. FASEB J 16(14):1931–1933
130. Kohno H, Suzuki R, Yasui Y, Hosokawa M, Miyashita K, Tanaka T (2004) Pomegranate seed oil rich in conjugated linolenic acid suppresses chemically induced colon carcinogenesis in rats. Cancer Sci 95(6):481–486
131. Toi M, Bando H, Ramachandran C, Melnick SJ, Imai A, Fife RS, Carr RE, Oikawa T, Lansky EP (2003) Preliminary studies on the anti-angiogenic potential of pomegranate fractions in vitro and in vivo. Angiogenesis 6(2):121–128. https://doi.org/10.1023/B:AGEN.0000011802.81320.e4
132. Shahidi F, Moonikh K (2017) Effects of pomegranate seed oil followed by resistance exercise on insulin resistance and lipid profile in non-athletic men. Feyz J Kashan Univ Med Sci 21(3):224–231
133. Massart-Leen A, De Pooter H, Decloedt M, Schamp N (1981) Composition and variability of the branched-chain fatty acid fraction in the milk of goats and cows. Lipids 16(5):286–292
134. Favre HA, Powell WH (2013) Nomenclature of organic chemistry: IUPAC recommendations and preferred names 2013. Royal Society of Chemistry, Cambridge
135. Duncan W, Garton G (1978) Differences in the proportions of branched-chain fatty acids in subcutaneous triacylglycerols of barley-fed ruminants. Br J Nutr 40(1):29–33
136. Chilliard Y, Martin C, Rouel J, Doreau M (2009) Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. J Dairy Sci 92(10):5199–5211
137. Ran-Ressler RR, Bae S, Lawrence P, Wang DH, Brenna JT (2014) Branched-chain fatty acid content of foods and estimated intake in the USA. Br J Nutr 112(4):565–572
138. Ran-Ressler RR, Khailova L, Arganbright KM, Adkins-Rieck CK, Jouni ZE, Koren O, Ley RE, Brenna JT, Dvorak B (2011) Branched chain fatty acids reduce the incidence of necrotizing enterocolitis and alter gastrointestinal microbial ecology in a neonatal rat model. PLoS One 6(12):e29032. https://doi.org/10.1371/journal.pone.0029032
139. Downing D (1964) Branched-chain fatty acids in lipids of the newly born lamb. J Lipid Res 5(2):210–215
140. Wang DH, Jackson JR, Twining C, Rudstam LG, Zollweg-Horan E, Kraft C, Lawrence P, Kothapalli K, Wang Z, Brenna JT (2016) Saturated branched chain, normal odd-carbon-numbered, and n-3 (omega-3) polyunsaturated fatty acids in freshwater fish in the Northeastern United States. J Agric Food Chem 64(40):7512–7519. https://doi.org/10.1021/acs.jafc.6b03491
141. Egge H, Murawski U, Ryhage R, György P, Chatranon W, Zilliken F (1972) Minor constituents of human milk IV: analysis of the branched chain fatty acids. Chem Phys Lipids 8(1):42–55
142. Gibson RA, Kneebone GM (1981) Fatty acid composition of human colostrum and mature breast milk. Am J Clin Nutr 34(2):252–257
143. Ran-Ressler RR, Devapatla S, Lawrence P, Brenna JT (2008) Branched chain fatty acids are constituents of the normal healthy newborn gastrointestinal tract. Pediatr Res 64(6):605
144. Veerkamp J (1971) Fatty acid composition of bifidobacterium and lactobacillus strains. J Bacteriol 108(2):861–867
145. Kaneda T (1991) Iso-and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol Rev 55(2):288–302
146. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE (2011) Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci 108(Suppl 1):4578–4585
147. Yang P, Collin P, Madden T, Chan D, Sweeney-Gotsch B, McConkey D, Newman RA (2003) Inhibition of proliferation of PC3 cells by the branched-chain fatty acid, 12-methyltetradecanoic acid, is associated with inhibition of 5-lipoxygenase. Prostate 55(4):281–291

148. Wongtangtintharn S, Oku H, Iwasaki H, Inafuku M, Toda T, Yanagita T (2005) Incorporation of branched-chain fatty acid into cellular lipids and caspase-independent apoptosis in human breast cancer cell line, SKBR-3. Lipids Health Dis 4(1):29
149. Lin T, Yin X, Cai Q, Fan X, Xu K, Huang L, Luo J, Zheng J, Huang J (2012) 13-Methyltetradecanoic acid induces mitochondrial-mediated apoptosis in human bladder cancer cells. Urol Oncol 30(3):339–345. Elsevier
150. Prescha A, Grajzer M, Dedyk M, Grajeta H (2014) The antioxidant activity and oxidative stability of cold-pressed oils. J Am Oil Chem Soc 91(8):1291–1301. https://doi.org/10.1007/s11746-014-2479-1
151. Blekas G, Boskou D (2006) Antioxidant phenols in vegetable oils. Natural Antioxidant Phenols. Research Signpost, Kerala, pp 15–27
152. Boskou D (2017) Edible cold pressed oils and their biologically active components. J Exp Food Chem 3:e108
153. Rueda A, Seiquer I, Olalla M, Giménez R, Lara L, Cabrera-Vique C (2014) Characterization of fatty acid profile of argan oil and other edible vegetable oils by gas chromatography and discriminant analysis. J Chem 2014 https://doi.org/10.1155/2014/843908
154. Gunstone F (2011) Vegetable oils in food technology: composition, properties and uses. Wiley, New York
155. Pieszka M, Migdał W, Gąsior R, Rudzińska M, Bederska-Łojewska D, Pieszka M, Szczurek P (2015) Native oils from apple, blackcurrant, raspberry, and strawberry seeds as a source of polyenoic fatty acids, tocochromanols, and phytosterols: a health implication. J Chem 2015 https://doi.org/10.1155/2015/659541
156. Parry J, Su L, Luther M, Zhou K, Yurawecz MP, Whittaker P, Yu L (2005) Fatty acid composition and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils. J Agric Food Chem 53(3):566–573. https://doi.org/10.1021/jf048615t
157. Castro-Gomez P, Garcia-Serrano A, Visioli F, Fontecha J (2015) Relevance of dietary glycerophospholipids and sphingolipids to human health. Prostaglandins, Leukot Essent Fatty Acids (PLEFA) 101:41–51
158. Vance DE (2002) Phospholipid biosynthesis in eukaryotes. New Compr Biochem 36:205–232
159. Park S-J, Im D-S (2015) G protein-coupled receptors for lysophosphatidylethanolamine. Recept Clin Invest 2(4) https://doi.org/10.14800/rci.999
160. Yamashita A, Oka S, Tanikawa T, Hayashi Y, Nemoto-Sasaki Y, Sugiura T (2013) The actions and metabolism of lysophosphatidylinositol, an endogenous agonist for GPR55. Prostaglandins Other Lipid Mediat 107:103–116. https://doi.org/10.1016/j.prostaglandins.2013.05.004
161. Frasch SC, Bratton DL (2012) Emerging roles for lysophosphatidylserine in resolution of inflammation. Prog Lipid Res 51(3):199–207. https://doi.org/10.1016/j.plipres.2012.03.001
162. Sevastou I, Kaffe E, Mouratis M-A, Aidinis V (2013) Lysoglycerophospholipids in chronic inflammatory disorders: the PLA2/LPC and ATX/LPA axes. Biochim Biophys Acta 1831(1):42–60. https://doi.org/10.1016/j.bbalip.2012.07.019
163. Arouri A, Mouritsen OG (2013) Membrane-perturbing effect of fatty acids and lysolipids. Prog Lipid Res 52(1):130–140. https://doi.org/10.1016/j.plipres.2012.09.002
164. Farooqui AA, Horrocks LA (2001) Book review: Plasmalogens: workhorse lipids of membranes in normal and injured neurons and glia. Neuroscientist 7(3):232–245. https://doi.org/10.1177/107385840100700308
165. Lessig J, Fuchs B (2009) Plasmalogens in biological systems: their role in oxidative processes in biological membranes, their contribution to pathological processes and aging and plasmalogen analysis. Curr Med Chem 16(16):2021–2041
166. Wallner S, Schmitz G (2011) Plasmalogens the neglected regulatory and scavenging lipid species. Chem Phys Lipids 164(6):573–589. https://doi.org/10.1016/j.chemphyslip.2011.06.008
167. Schlame M, Rua D, Greenberg ML (2000) The biosynthesis and functional role of cardiolipin. Prog Lipid Res 39(3):257–288. https://doi.org/10.1016/S0163-7827(00)00005-9

168. Adibhatla RM, Hatcher JF (2009) Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal 12(1):125–169. https://doi.org/10.1089/ars.2009.2668
169. Catalá A (2012) Lipid peroxidation modifies the picture of membranes from the "Fluid mosaic model" to the "Lipid whisker model". Biochimie 94(1):101–109. https://doi.org/10.1016/j.biochi.2011.09.025
170. Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97(6):1634–1658. https://doi.org/10.1111/j.1471-4159.2006.03907.x
171. Adam-Vizi V, Chinopoulos C (2006) Bioenergetics and the formation of mitochondrial reactive oxygen species. Trends Pharmacol Sci 27(12):639–645. https://doi.org/10.1016/j.tips.2006.10.005
172. Chicco AJ, Sparagna GC (2007) Role of cardiolipin alterations in mitochondrial dysfunction and disease. Am J Physiol Cell Physiol 292(1):C33–C44. https://doi.org/10.1152/ajpcell.00243.2006
173. Mignotte B, Vayssiere JL (1998) Mitochondria and apoptosis. Eur J Biochem 252(1):1–15. https://doi.org/10.1046/j.1432-1327.1998.2520001.x
174. Al-Gubory KH (2012) Mitochondria: omega-3 in the route of mitochondrial reactive oxygen species. Int J Biochem Cell Biol 44(9):1569–1573. https://doi.org/10.1016/j.biocel.2012.06.003
175. Spector AA, Yorek MA (1985) Membrane lipid composition and cellular function. J Lipid Res 26(9):1015–1035
176. Lundbæk JA, Collingwood SA, Ingólfsson HI, Kapoor R, Andersen OS (2010) Lipid bilayer regulation of membrane protein function: gramicidin channels as molecular force probes. J R Soc Interface 7(44):373–395
177. Escribá PV, Wedegaertner PB, Goñi FM, Vögler O (2007) Lipid–protein interactions in GPCR-associated signaling. Biochim Biophys Acta Biomembr 1768(4):836–852
178. Hilgemann DW, Feng S, Nasuhoglu C (2001) The complex and intriguing lives of PIP2 with ion channels and transporters. Sci STKE 2001(111):re19-re19. https://doi.org/10.1126/stke.2001.111.re19
179. McLaughlin S, Murray D (2005) Plasma membrane phosphoinositide organization by protein electrostatics. Nature 438(7068):605
180. Suh B-C, Hille B (2008) PIP2 is a necessary cofactor for ion channel function: how and why? Annu Rev Biophys 37(1):175–195. https://doi.org/10.1146/annurev.biophys.37.032807.125859
181. Lee A (2003) Lipid–protein interactions in biological membranes: a structural perspective. Biochim Biophys Acta Biomembr 1612(1):1–40
182. Schmitz G, Ecker J (2008) The opposing effects of n− 3 and n− 6 fatty acids. Prog Lipid Res 47(2):147–155
183. Mouritsen OG, Kinnunen PK (1996) Role of lipid organization and dynamics for membrane functionality. In: Biological membranes. Springer, Birkhäuser, Boston, pp 463–502
184. Janmey PA, Kinnunen PKJ (2006) Biophysical properties of lipids and dynamic membranes. Trends Cell Biol 16(10):538–546. https://doi.org/10.1016/j.tcb.2006.08.009
185. Lee AG (2005) How lipids and proteins interact in a membrane: a molecular approach. Mol BioSyst 1(3):203–212
186. Lundbæk JA (2006) Regulation of membrane protein function by lipid bilayer elasticity – a single molecule technology to measure the bilayer properties experienced by an embedded protein. J Phys Condens Matter 18(28):S1305
187. Andersen OS, Koeppe RE (2007) Bilayer thickness and membrane protein function: an energetic perspective. Annu Rev Biophys Biomol Struct 36:107
188. Marsh D (2008) Protein modulation of lipids, and vice-versa, in membranes. Biochim Biophys Acta Biomembr 1778(7–8):1545–1575. https://doi.org/10.1016/j.bbamem.2008.01.015
189. Sackmann E (1995) Physical basis of self-organization and function of membranes: physics of vesicles. In: Handbook of biological physics, vol 1. Elsevier, North Holland, pp 213–304

190. Hamanaka S, Suzuki A, Hara M, Nishio H, Otsuka F, Uchida Y (2002) Human epidermal glucosylceramides are major precursors of stratum corneum ceramides. J Investig Dermatol 119(2):416–423
191. Merrill AH (2011) Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. Chem Rev 111(10):6387–6422. https://doi.org/10.1021/cr2002917
192. Aureli M, Loberto N, Chigorno V, Prinetti A, Sonnino S (2011) Remodeling of sphingolipids by plasma membrane associated enzymes. Neurochem Res 36(9):1636–1644. https://doi.org/10.1007/s11064-010-0360-7
193. Christie W (2014) Ceramides. Structure, composition, function and analysis. The lipid library AOCS 2710 S. Boulder, Urbana, IL 61802–6996 U.S.A.
194. Grassmé H, Jekle A, Riehle A, Schwarz H, Berger J, Sandhoff K, Kolesnick R, Gulbins E (2001) CD95 signaling via ceramide-rich membrane rafts. J Biol Chem 276(23):20589–20596
195. Grassmé H, Schwarz H, Gulbins E (2001) Molecular mechanisms of ceramide-mediated CD95 clustering. Biochem Biophys Res Commun 284(4):1016–1030
196. Goñi FM, Contreras F-X, Montes L-R, Sot J, Alonso A (2005) Biophysics (and sociology) of ceramides. In: Biochemical society symposia. Portland Press Limited, pp 177–188. London
197. Cremesti AE, Goni FM, Kolesnick R (2002) Role of sphingomyelinase and ceramide in modulating rafts: do biophysical properties determine biologic outcome? FEBS Lett 531(1): 47–53
198. Krönke M (1999) Biophysics of ceramide signaling: interaction with proteins and phase transition of membranes. Chem Phys Lipids 101(1):109–121
199. Ruvolo PP (2003) Intracellular signal transduction pathways activated by ceramide and its metabolites. Pharmacol Res 47(5):383–392
200. Hannun YA, Obeid LM (2002) The ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. J Biol Chem 277(29):25847–25850
201. Ogretmen B, Hannun YA (2004) Biologically active sphingolipids in cancer pathogenesis and treatment. Nat Rev Cancer 4(8):604
202. Saddoughi SA, Ogretmen B (2012) Diverse functions of ceramide in cancer cell death and proliferation. Adv Cancer Res 117:37
203. Sugiura M, Kono K, Liu H, Shimizugawa T, Minekura H, Spiegel S, Kohama T (2002) Ceramide kinase, a novel lipid kinase molecular cloning and functional characterization. J Biol Chem 277(26):23294–23300
204. Tafesse FG, Ternes P, Holthuis JC (2006) The multigenic sphingomyelin synthase family. J Biol Chem 281(40):29421–29425
205. Ichikawa S, Hirabayashi Y (1998) Glucosylceramide synthase and glycosphingolipid synthesis. Trends Cell Biol 8(5):198–202
206. Wijesinghe DS, Massiello A, Subramanian P, Szulc Z, Bielawska A, Chalfant CE (2005) Substrate specificity of human ceramide kinase. J Lipid Res 46(12):2706–2716
207. El Bawab S, Mao C, Obeid LM, Hannun YA (2004) Ceramidases in the regulation of ceramide levels and function. In: Phospholipid metabolism in apoptosis. Springer, Boston, pp 187–205
208. Mao C, Xu R, Szulc ZM, Bielawska A, Galadari SH, Obeid LM (2001) Cloning and characterization of a novel human alkaline ceramidase A mammalian enzyme that hydrolyzes phytoceramide. J Biol Chem 276(28):26577–26588
209. Strelow A, Bernardo K, Adam-Klages S, Linke T, Sandhoff K, Krönke M, Adam D (2000) Overexpression of acid ceramidase protects from tumor necrosis factor–induced cell death. J Exp Med 192(5):601–612
210. Smith E, Merrill AH, Obeid LM, Hannun YA (2000) Effects of sphingosine and other sphingolipids on protein kinase C. Methods Enzymol 312:361–373
211. Cowart LA, Hannun YA (2007) Selective substrate supply in the regulation of yeast de novo sphingolipid synthesis. J Biol Chem 282(16):12330–12340
212. Leidl K, Liebisch G, Richter D, Schmitz G (2008) Mass spectrometric analysis of lipid species of human circulating blood cells. Biochim Biophys Acta 1781(10):655–664

213. Cowart LA (2009) Sphingolipids: players in the pathology of metabolic disease. Trends Endocrinol Metab 20(1):34–42
214. Schneider M, Levant B, Reichel M, Gulbins E, Kornhuber J, Müller CP (2017) Lipids in psychiatric disorders and preventive medicine. Neurosci Biobehav Rev 76(Part B):336–362. https://doi.org/10.1016/j.neubiorev.2016.06.002
215. Haimovitz-Friedman A, Cordon-Cardo C, Bayoumy S, Garzotto M, McLoughlin M, Gallily R, Edwards CK, Schuchman EH, Fuks Z, Kolesnick R (1997) Lipopolysaccharide induces disseminated endothelial apoptosis requiring ceramide generation. J Exp Med 186(11):1831–1841
216. Pfeiffer A, Böttcher A, Orsó E, Kapinsky M, Nagy P, Bodnár A, Spreitzer I, Liebisch G, Drobnik W, Gempel K (2001) Lipopolysaccharide and ceramide docking to CD14 provokes ligand-specific receptor clustering in rafts. Eur J Immunol 31(11):3153–3164
217. Stancevic B, Kolesnick R (2010) Ceramide-rich platforms in transmembrane signaling. FEBS Lett 584(9):1728–1740
218. Kolesnick R (2002) The therapeutic potential of modulating the ceramide/sphingomyelin pathway. J Clin Invest 110(1):3
219. Grassmé H, Gulbins E, Brenner B, Ferlinz K, Sandhoff K, Harzer K, Lang F, Meyer TF (1997) Acidic sphingomyelinase mediates entry of N. gonorrhoeae into nonphagocytic cells. Cell 91(5):605–615
220. Grassme H, Jendrossek V, Riehle A, Von Kürthy G, Berger J, Schwarz H, Weller M, Kolesnick R, Gulbins E (2003) Host defense against *Pseudomonas aeruginosa* requires ceramide-rich membrane rafts. Nat Med 9(3):322
221. Esen M, Schreiner B, Jendrossek V, Lang F, Fassbender K, Grassme H, Gulbins E (2001) Mechanisms of *Staphylococcus aureus* induced apoptosis of human endothelial cells. Apoptosis 6(6):431–439
222. Hanada K, Mitamura T, Fukasawa M, Magistrado PA, Horii T, Nishijima M (2000) Neutral sphingomyelinase activity dependent on Mg^{2+} and anionic phospholipids in the intraerythrocytic malaria parasite *Plasmodium falciparum*. Biochem J 346(3):671–677
223. Finnegan CM, Rawat SS, Puri A, Wang JM, Ruscetti FW, Blumenthal R (2004) Ceramide, a target for antiretroviral therapy. Proc Natl Acad Sci USA 101(43):15452–15457
224. Bielawski J, Szulc ZM, Hannun YA, Bielawska A (2006) Simultaneous quantitative analysis of bioactive sphingolipids by high-performance liquid chromatography-tandem mass spectrometry. Methods 39(2):82–91
225. Merrill AH, Sullards MC, Allegood JC, Kelly S, Wang E (2005) Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry. Methods 36(2):207–224
226. Romiti E, Vasta V, Meacci E, Farnararo M, Linke T, Ferlinz K, Sandhoff K, Bruni P (2000) Characterization of sphingomyelinase activity released by thrombin-stimulated platelets. Mol Cell Biochem 205(1):75–81
227. Delon C, Manifava M, Wood E, Thompson D, Krugmann S, Pyne S, Ktistakis NT (2004) Sphingosine kinase 1 is an intracellular effector of phosphatidic acid. J Biol Chem 279(43): 44763–44774
228. Venable ME, Lee JY, Smyth MJ, Bielawska A, Obeid LM (1995) Role of ceramide in cellular senescence. J Biol Chem 270(51):30701–30708
229. Hannun YA (1996) Functions of ceramide in coordinating cellular responses to stress. Science 274(5294):1855
230. Becker KP, Kitatani K, Idkowiak-Baldys J, Bielawski J, Hannun YA (2005) Selective inhibition of juxtanuclear translocation of protein kinase C βII by a negative feedback mechanism involving ceramide formed from the salvage pathway. J Biol Chem 280(4):2606–2612
231. Jenkins GM, Cowart LA, Signorelli P, Pettus BJ, Chalfant CE, Hannun YA (2002) Acute activation of de novo sphingolipid biosynthesis upon heat shock causes an accumulation of ceramide and subsequent dephosphorylation of SR proteins. J Biol Chem 277(45):42572–42578

232. Liu Y-Y, Han T-Y, Giuliano AE, Cabot MC (2001) Ceramide glycosylation potentiates cellular multidrug resistance. FASEB J 15(3):719–730
233. Goetzl EJ (2001) Pleiotypic mechanisms of cellular responses to biologically active lysophospholipids. Prostaglandins Other Lipid Mediat 64(1):11–20
234. Hla T (2004) Physiological and pathological actions of sphingosine 1-phosphate. In: Seminars in cell & developmental biology, vol 5. Elsevier, UK, pp 513–520
235. Pettus BJ, Bielawska A, Subramanian P, Wijesinghe DS, Maceyka M, Leslie CC, Evans JH, Freiberg J, Roddy P, Hannun YA (2004) Ceramide 1-phosphate is a direct activator of cytosolic phospholipase A2. J Biol Chem 279(12):11320–11326
236. Mitsutake S, Igarashi Y (2005) Calmodulin is involved in the Ca2+−dependent activation of ceramide kinase as a calcium sensor. J Biol Chem 280(49):40436–40441
237. Hinkovska-Galcheva V, Boxer LA, Kindzelskii A, Hiraoka M, Abe A, Goparju S, Spiegel S, Petty HR, Shayman JA (2005) Ceramide 1-phosphate, a mediator of phagocytosis. J Biol Chem 280(28):26612–26621
238. Mitsutake S, Kim T-J, Inagaki Y, Kato M, Yamashita T, Igarashi Y (2004) Ceramide kinase is a mediator of calcium-dependent degranulation in mast cells. J Biol Chem 279 (17):17570–17577
239. Gómez-Muñoz A (2006) Ceramide 1-phosphate/ceramide, a switch between life and death. Biochim Biophys Acta Biomembr 1758(12):2049–2056
240. Schulz A, Mousallem T, Venkataramani M, Persaud-Sawin D-A, Zucker A, Luberto C, Bielawska A, Bielawski J, Holthuis JC, Jazwinski SM (2006) The CLN9 protein, a regulator of dihydroceramide synthase. J Biol Chem 281(5):2784–2794
241. Kraveka JM, Li L, Szulc ZM, Bielawski J, Ogretmen B, Hannun YA, Obeid LM, Bielawska A (2007) Involvement of dihydroceramide desaturase in cell cycle progression in human neuroblastoma cells. J Biol Chem 282(23):16718–16728
242. Zheng W, Kollmeyer J, Symolon H, Momin A, Munter E, Wang E, Kelly S, Allegood JC, Liu Y, Peng Q (2006) Ceramides and other bioactive sphingolipid backbones in health and disease: lipidomic analysis, metabolism and roles in membrane structure, dynamics, signaling and autophagy. Biochim Biophys Acta Biomembr 1758(12):1864–1884
243. Ignatov A, Lintzel J, Hermans-Borgmeyer I, Kreienkamp H-J, Joost P, Thomsen S, Methner A, Schaller HC (2003) Role of the G-protein-coupled receptor GPR12 as high-affinity receptor for sphingosylphosphorylcholine and its expression and function in brain development. J Neurosci 23(3):907–914
244. Gouaze-Andersson V, Cabot MC (2006) Glycosphingolipids and drug resistance. Biochim Biophys Acta Biomembr 1758(12):2096–2103
245. Raggers RJ, van Helvoort A, Evers R, van Meer G (1999) The human multidrug resistance protein MRP1 translocates sphingolipid analogs across the plasma membrane. J Cell Sci 112(3):415–422
246. Vesper H, Schmelz E-M, Nikolova-Karakashian MN, Dillehay DL, Lynch DV, Merrill AH (1999) Sphingolipids in food and the emerging importance of sphingolipids to nutrition. J Nutr 129(7):1239–1250
247. Ofek I, Hasty DL, Sharon N (2003) Anti-adhesion therapy of bacterial diseases: prospects and problems. FEMS Immunol Med Microbiol 38(3):181–191
248. Hoyo Pérez J, Guaus Guerrero E, Torrent Burgués J (2016) Monogalactosyldiacylglycerol and digalactosyldiacylglycerol role, physical states, applications and biomimetic monolayer films. Eur Phys J E Soft Matter 39(3):39
249. Maeda N, Kokai Y, Ohtani S, Sahara H, Kumamoto-Yonezawa Y, Kuriyama I, Hada T, Sato N, Yoshida H, Mizushina Y (2008) Anti-tumor effect of orally administered spinach glycolipid fraction on implanted cancer cells, colon-26, in mice. Lipids 43(8):741. https://doi.org/10.1007/s11745-008-3202-5
250. Maeda N, Kokai Y, Ohtani S, Sahara H, Hada T, Ishimaru C, Kuriyama I, Yonezawa Y, Iijima H, Yoshida H, Sato N, Mizushina Y (2007) Anti-tumor effects of the glycolipids fraction from spinach which inhibited DNA polymerase activity. Nutr Cancer 57(2):216–223. https://doi.org/10.1080/01635580701277908

251. Bruno A, Rossi C, Marcolongo G, Di Lena A, Venzo A, Berrie CP, Corda D (2005) Selective in vivo anti-inflammatory action of the galactolipid monogalactosyldiacylglycerol. Eur J Pharmacol 524(1):159–168. https://doi.org/10.1016/j.ejphar.2005.09.023
252. Chu B-S, Gunning AP, Rich GT, Ridout MJ, Faulks RM, Wickham MSJ, Morris VJ, Wilde PJ (2010) Adsorption of bile salts and pancreatic colipase and lipase onto digalactosyldiacylglycerol and dipalmitoylphosphatidylcholine monolayers. Langmuir 26(12):9782–9793. https://doi.org/10.1021/la1000446
253. de Jesus C-SA, Hernández-Sánchez H, Jaramillo-Flores ME (2013) Biological activity of glycolipids produced by microorganisms: new trends and possible therapeutic alternatives. Microbiol Res 168(1):22–32
254. Al-Araji L, Rahman RNZRA, Basri M, Salleh AB (2007) Microbial surfactant. Asia Pac J Mol Biol Biotechnol 15(3):99–105
255. Nitschke M, Costa S (2007) Biosurfactants in food industry. Trends Food Sci Technol 18(5):252–259
256. Küllenberg D, Taylor LA, Schneider M, Massing U (2012) Health effects of dietary phospholipids. Lipids Health Dis 11(1):3
257. Nicolson GL, Ash ME (2014) Lipid replacement therapy: a natural medicine approach to replacing damaged lipids in cellular membranes and organelles and restoring function. Biochim Biophys Acta Biomembr 1838(6):1657–1679. https://doi.org/10.1016/j.bbamem.2013.11.010
258. Mouritsen OG, Bloom M (1984) Mattress model of lipid-protein interactions in membranes. Biophys J 46(2):141–153. https://doi.org/10.1016/S0006-3495(84)84007-2
259. Drobnies AE, Davies SMA, Kraayenhof R, Epand RF, Epand RM, Cornell RB (2002) CTP: phosphocholine cytidylyltransferase and protein kinase C recognize different physical features of membranes: differential responses to an oxidized phosphatidylcholine. Biochim Biophys Acta Biomembr 1564(1):82–90. https://doi.org/10.1016/S0005-2736(02)00404-2
260. Bagatolli LA, Ipsen JH, Simonsen AC, Mouritsen OG (2010) An outlook on organization of lipids in membranes: searching for a realistic connection with the organization of biological membranes. Prog Lipid Res 49(4):378–389. https://doi.org/10.1016/j.plipres.2010.05.001
261. Zimmerberg J, Gawrisch K (2006) The physical chemistry of biological membranes. Nat Chem Biol 2(11):564–567
262. Dumas F, Sperotto MM, Lebrun MC, Tocanne JF, Mouritsen OG (1997) Molecular sorting of lipids by bacteriorhodopsin in dilauroylphosphatidylcholine/distearoylphosphatidylcholine lipid bilayers. Biophys J 73(4):1940–1953. https://doi.org/10.1016/S0006-3495(97)78225-0
263. Vereyken IJ, Chupin V, Demel RA, Smeekens SCM, De Kruijff B (2001) Fructans insert between the headgroups of phospholipids. Biochim Biophys Acta Biomembr 1510(1–2):307–320. https://doi.org/10.1016/S0005-2736(00)00363-1
264. Nicolson GL, Ash ME (2017) Membrane lipid replacement for chronic illnesses, aging and cancer using oral glycerophospholipid formulations with fructooligosaccharides to restore phospholipid function in cellular membranes, organelles, cells and tissues. Biochim Biophys Acta Biomembr 1859(9, Part B):1704–1724. https://doi.org/10.1016/j.bbamem.2017.04.013
265. Ehehalt R, Braun A, Karner M, Füllekrug J, Stremmel W (2010) Phosphatidylcholine as a constituent in the colonic mucosal barrier – physiological and clinical relevance. Biochim Biophys Acta 1801(9):983–993. https://doi.org/10.1016/j.bbalip.2010.05.014
266. Nicolson GL, Conklin KA (2008) Reversing mitochondrial dysfunction, fatigue and the adverse effects of chemotherapy of metastatic disease by molecular replacement therapy. Clin Exp Metastasis 25(2):161–169. https://doi.org/10.1007/s10585-007-9129-z
267. Nicolson GL (2005) Lipid replacement/antioxidant therapy as an adjunct supplement to reduce the adverse effects of cancer therapy and restore mitochondrial function. Pathol Oncol Res 11(3):139. https://doi.org/10.1007/bf02893390
268. Settineri RA, Palmer JF (2012) Lipid supplements for maintaining health and the treatment of acute and chronic disorders. Google Patents

269. Ellithorpe RR, Settineri R, Jacques B, Mitchell CA, Nicolson GL (2012) Lipid replacement therapy functional food formulation with NT factor for reducing weight, girth, body mass, appetite and fatigue while improving blood lipid profiles. Funct Foods Health Dis 2(1):11–24
270. Schrauwen P, Hesselink MK (2004) Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. Diabetes 53(6):1412–1417
271. Zierenberg O, Assmann G, Schmitz G, Rosseneu M (1981) Effect of polyenephosphatidylcholine on cholesterol uptake by human high density lipoprotein. Atherosclerosis 39(4):527–542. https://doi.org/10.1016/0021-9150(81)90010-1
272. Magnusson CD, Haraldsson GG (2011) Ether lipids. Chem Phys Lipids 164(5):315–340
273. Pedrono F, Martin B, Leduc C, Le Lan J, Saiag B, Legrand P, Moulinoux J-P, Legrand AB (2004) Natural alkylglycerols restrain growth and metastasis of grafted tumors in mice. Nutr Cancer 48(1):64–69. https://doi.org/10.1207/s15327914nc4801_9
274. Skopinska-Rozewska E, Krotkiewski M, Sommer E, Rogala E, Filewska M, Bialas-Chromiec B, Pastewka K, Skurzak H (1999) Inhibitory effect of shark liver oil on cutaneous angiogenesis induced in Balb/c mice by syngeneic sarcoma L-1, human urinary bladder and human kidney tumour cells. Oncol Rep 6:1341
275. Pédrono F, Saïag B, Moulinoux J-P, Legrand AB (2007) 1-O-Alkylglycerols reduce the stimulating effects of bFGF on endothelial cell proliferation in vitro. Cancer Lett 251(2):317–322. https://doi.org/10.1016/j.canlet.2006.11.028
276. Krotkiewski M, Przybyszewska M, Janik P (2003) Cytostatic and cytotoxic effects of alkylglycerols (Ecomer). Med Sci Monit 9(11):Pi131–Pi135
277. Reynolds S, Cederberg H, Chakrabarty S (2000) Inhibitory effect of 1-O (2 methoxy) hexadecyl glycerol and phenylbutyrate on the malignant properties of human prostate cancer cells. Clin Exp Metastasis 18(4):309. https://doi.org/10.1023/a:1011071907047
278. Wang H, Rajagopal S, Reynolds S, Cederberg H, Chakrabarty S (1999) Differentiation-promoting effect of 1-O (2 methoxy) hexadecyl glycerol in human colon cancer cells. J Cell Physiol 178(2):173–178. https://doi.org/10.1002/(SICI)1097-4652(199902)178:2<173::AID-JCP6>3.0.CO;2-Q
279. Iagher F, de Brito Belo SR, Souza WM, Nunes JR, Naliwaiko K, Sassaki GL, Bonatto SJR, de Oliveira HHP, Brito GAP, de Lima C (2013) Antitumor and anti-cachectic effects of shark liver oil and fish oil: comparison between independent or associative chronic supplementation in Walker 256 tumor-bearing rats. Lipids Health Dis 12(1):146
280. Iagher F, de Brito Belo SR, Naliwaiko K, Franzói AM, de Brito GAP, Yamazaki RK, Muritiba AL, Muehlmann LA, Steffani JA, Fernandes LC (2011) Chronic supplementation with shark liver oil for reducing tumor growth and cachexia in Walker 256 tumor-bearing rats. Nutr Cancer 63(8):1307–1315. https://doi.org/10.1080/01635581.2011.607540
281. Hallgren B (ed) (1983) Therapeutic effects of ether lipids. In: Ether lipids: biochemical and biomedical aspects. Academic, New York
282. Joelsson I (1988) Effect of alkylglycerols on the frequency of fistulas following radiation therapy. Lipidforum, Lund, pp 1–7
283. Berdel WE, Bausert WR, Weltzien HU, Modolell ML, Widmann KH, Munder PG (1980) The influence of alkyl-lysophospholipids and lysophospholipid-activated macrophages on the development of metastasis of 3-Lewis lung carcinoma. Eur J Cancer (1965) 16(9):1199–1204. https://doi.org/10.1016/0014-2964(80)90179-6
284. Acevedo R, Gil D, Campo JD, Bracho G, Valdes Y, Perez O (2006) The adjuvant potential of synthetic alkylglycerols. Vaccine 24(Suppl 2):S32–S33
285. Kantah M-K, Wakasugi H, Kumari A (2012) Intestinal immune-potentiation by a purified alkylglycerols compound. Acta Biomed 83(1):36–43
286. Qian L, Zhang M, Wu S, Zhong Y, Van Tol E, Cai W (2014) Alkylglycerols modulate the proliferation and differentiation of non-specific agonist and specific antigen-stimulated splenic lymphocytes. PLoS One 9(4):e96207
287. Tchórzewski H, Banasik M, Głowacka E, Lewkowicz P (2002) Modification of innate immunity in humans by active components of shark liver oil. Pol Merkur Lekarski 13(76):329–332

288. Palmieri B, Pennelli A, Di Cerbo A (2014) Jurassic surgery and immunity enhancement by alkyglycerols of shark liver oil. Lipids Health Dis 13(1):178. https://doi.org/10.1186/1476-511x-13-178
289. Mitre R, Etienne M, Martinais S, Salmon H, Allaume P, Legrand P, Legrand AB (2007) Humoral defence improvement and haematopoiesis stimulation in sows and offspring by oral supply of shark-liver oil to mothers during gestation and lactation. Br J Nutr 94(5):753–762. https://doi.org/10.1079/BJN20051569
290. Oh SY, Jadhav LS (1994) Effects of dietary alkylglycerols in lactating rats on immune responses in pups. Pediatr Res 36(3):300–305
291. Erdlenbruch B, Jendrossek V, Eibl H, Lakomek M (2000) Transient and controllable opening of the blood-brain barrier to cytostatic and antibiotic agents by alkylglycerols in rats. Exp Brain Res 135(3):417–422. https://doi.org/10.1007/s002210000553
292. Erdlenbruch B, Alipour M, Fricker G, Miller DS, Kugler W, Eibl H, Lakomek M (2003) Alkylglycerol opening of the blood–brain barrier to small and large fluorescence markers in normal and C6 glioma-bearing rats and isolated rat brain capillaries. Br J Pharmacol 140(7):1201–1210
293. Erdlenbruch B, Schinkhof C, Kugler W, Heinemann DE, Herms J, Eibl H, Lakomek M (2003) Intracarotid administration of short-chain alkylglycerols for increased delivery of methotrexate to the rat brain. Br J Pharmacol 139(4):685–694
294. Hülper P, Dullin C, Kugler W, Lakomek M, Erdlenbruch B (2011) Monitoring proteins using in vivo near-infrared time-domain optical imaging after 2-*O*-hexyldiglycerol-mediated transfer to the brain. Mol Imaging Biol 13(2):275–283
295. Hülper P, Veszelka S, Walter F, Wolburg H, Fallier-Becker P, Piontek J, Blasig I, Lakomek M, Kugler W, Deli M (2013) Acute effects of short-chain alkylglycerols on blood-brain barrier properties of cultured brain endothelial cells. Br J Pharmacol 169(7):1561–1573
296. Zhang M, Sun S, Tang N, Cai W, Qian L (2013) Oral administration of alkylglycerols differentially modulates high-fat diet-induced obesity and insulin resistance in mice. Evid Based Complement Alternat Med 2013:834027
297. Parri A, Fitó M, Torres C, Muñoz-Aguayo D, Schröder H, Cano J, Vázquez L, Reglero G, Covas M-I (2016) Alkylglycerols reduce serum complement and plasma vascular endothelial growth factor in obese individuals. Inflammopharmacology 24(2–3):127–131
298. Cheminade C, Gautier V, Hichami A, Allaume P, Le Lannou D, Legrand AB (2002) 1-*O*-alkylglycerols improve boar sperm motility and fertility. Biol Reprod 66(2):421–428
299. Mitre R, Cheminade C, Allaume P, Legrand P, Legrand AB (2004) Oral intake of shark liver oil modifies lipid composition and improves motility and velocity of boar sperm. Theriogenology 62(8):1557–1566
300. Haynes M, Buckley HR, Higgins ML, Pieringer RA (1994) Synergism between the antifungal agents amphotericin B and alkyl glycerol ethers. Antimicrob Agents Chemother 38(7):1523–1529
301. Ved H, Gustow E, Mahadevan V, Pieringer R (1984) Dodecylglycerol. A new type of antibacterial agent which stimulates autolysin activity in *Streptococcus faecium* ATCC 9790. J Biol Chem 259(13):8115–8121
302. Wong J, Rios-Solis L, Keasling JD (2017) Microbial production of isoprenoids. In: Lee SY (ed) Consequences of microbial interactions with hydrocarbons, oils, and lipids: production of fuels and chemicals. Springer International Publishing, Cham, pp 1–24. https://doi.org/10.1007/978-3-319-31421-1_219-2
303. Beller HR, Lee TS, Katz L (2015) Natural products as biofuels and bio-based chemicals: fatty acids and isoprenoids. Nat Prod Rep 32(10):1508–1526
304. McCaskill D, Croteau R (1997) Prospects for the bioengineering of isoprenoid biosynthesis. In: Berger RG, Babel W, Blanch HW et al (eds) Biotechnology of aroma compounds. Springer, Berlin/Heidelberg, pp 107–146. https://doi.org/10.1007/BFb0102064
305. Croteau R, Ketchum REB, Long RM, Kaspera R, Wildung MR (2006) Taxol biosynthesis and molecular genetics. Phytochem Rev 5(1):75–97. https://doi.org/10.1007/s11101-005-3748-2

306. Fraga BM (2012) Natural sesquiterpenoids. Nat Prod Rep 29(11):1334–1366. https://doi.org/10.1039/c2np20074k
307. McGarvey DJ, Croteau R (1995) Terpenoid metabolism. Plant Cell 7(7):1015–1026
308. Wang H, Zou Z, Wang S, Gong M (2013) Global analysis of transcriptome responses and gene expression profiles to cold stress of Jatropha curcas L. PLoS One 8(12):e82817. https://doi.org/10.1371/journal.pone.0082817
309. Jennewein S, Croteau R (2001) Taxol: biosynthesis, molecular genetics, and biotechnological applications. Appl Microbiol Biotechnol 57(1):13–19
310. Skeel RT, Khleif SN (2011) Handbook of cancer chemotherapy. Lippincott Williams & Wilkins, Philadelphia
311. Vasas A, Hohmann J (2014) Euphorbia diterpenes: isolation, structure, biological activity, and synthesis (2008–2012). Chem Rev 114(17):8579–8612
312. Kirby J, Nishimoto M, Park JG, Withers ST, Nowroozi F, Behrendt D, Rutledge EJG, Fortman JL, Johnson HE, Anderson JV, Keasling JD (2010) Cloning of casbene and neocembrene synthases from Euphorbiaceae plants and expression in *Saccharomyces cerevisiae*. Phytochemistry 71(13):1466–1473. https://doi.org/10.1016/j.phytochem.2010.06.001
313. Blumberg PM (1988) Protein kinase C as the receptor for the Phorbol Ester tumor promoters: sixth Rhoads memorial award lecture. Cancer Res 48(1):1–8
314. Halaweish FT, Kronberg S, Hubert MB, Rice JA (2002) Toxic and aversive diterpenes of *Euphorbia esula*. J Chem Ecol 28(8):1599–1611
315. Jiao W, Dong W, Li Z, Deng M, Lu R (2009) Lathyrane diterpenes from *Euphorbia lathyris* as modulators of multidrug resistance and their crystal structures. Bioorg Med Chem 17(13):4786–4792. https://doi.org/10.1016/j.bmc.2009.04.041
316. Srivalli KMR, Lakshmi P (2012) Overview of P-glycoprotein inhibitors: a rational outlook. Braz J Pharm Sci 48(3):353–367
317. Gelb MH, Tamanoi F, Yokoyama K, Ghomashchi F, Esson K, Gould MN (1995) The inhibition of protein prenyltransferases by oxygenated metabolites of limonene and perillyl alcohol. Cancer Lett 91(2):169–175
318. Gould MN (1997) Cancer chemoprevention and therapy by monoterpenes. Environ Health Perspect 105(Suppl 4):977
319. Hohl RJ (1996) Monoterpenes as regulators of malignant cell proliferation. In: Dietary phytochemicals in cancer prevention and treatment. Springer, New York, pp 137–146
320. Sen CK, Khanna S, Roy S (2007) Tocotrienols in health and disease: the other half of the natural vitamin E family. Mol Asp Med 28(5):692–728. https://doi.org/10.1016/j.mam.2007. 03.001
321. Traber MG, Burton GW, Hamilton RL (2004) Vitamin E trafficking. Ann N Y Acad Sci 1031(1):1–12. https://doi.org/10.1196/annals.1331.001
322. Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H, Inoue K (1997) Affinity for α-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. FEBS Lett 409(1):105–108. https://doi.org/10.1016/s0014-5793(97)00499-7
323. Khanna S, Patel V, Rink C, Roy S, Sen CK (2005) Delivery of orally supplemented α-tocotrienol to vital organs of rats and tocopherol-transport protein deficient mice. Free Radic Biol Med 39(10):1310–1319. https://doi.org/10.1016/j.freeradbiomed.2005.06.013
324. Araya H, Tomita M, Hayashi M (2006) The novel formulation design of self-emulsifying drug delivery systems (SEDDS) type O/W microemulsion III: the permeation mechanism of a poorly water soluble drug entrapped O/W microemulsion in rat isolated intestinal membrane by the using chamber method. Drug Metab Pharmacokinet 21(1):45–53. https://doi.org/10.2133/dmpk.21.45
325. Gao P, Morozowich W (2006) Development of supersaturatable self-emulsifying drug delivery system formulations for improving the oral absorption of poorly soluble drugs. Expert Opin Drug Deliv 3(1):97–110. https://doi.org/10.1517/17425247.3.1.97
326. Hong J-Y, Kim J-K, Song Y-K, Park J-S, Kim C-K (2006) A new self-emulsifying formulation of itraconazole with improved dissolution and oral absorption. J Control Release 110(2):332–338. https://doi.org/10.1016/j.jconrel.2005.10.002

327. Khosla P, Patel V, Whinter JM, Khanna S, Rakhkovskaya M, Roy S, Sen CK (2006) Postprandial levels of the natural vitamin E Tocotrienol in human circulation. Antioxid Redox Signal 8(5–6):1059–1068. https://doi.org/10.1089/ars.2006.8.1059
328. O'Byrne D, Grundy S, Packer L, Devaraj S, Baldenius K, Hoppe PP, Kraemer K, Jialal I, Traber MG (2000) Studies of LDL oxidation following α-, γ-, or δ-tocotrienyl acetate supplementation of hypercholesterolemic humans. Free Radic Biol Med 29(9):834–845. https://doi.org/10.1016/S0891-5849(00)00371-3
329. Yap SP, Yuen KH, Wong JW (2001) Pharmacokinetics and bioavailability of α-, γ- and δ-tocotrienols under different food status. J Pharm Pharmacol 53(1):67–71. https://doi.org/10.1211/0022357011775208
330. Hensley K, Benaksas EJ, Bolli R, Comp P, Grammas P, Hamdheydari L, Mou S, Pye QN, Stoddard MF, Wallis G, Williamson KS, West M, Wechter WJ, Floyd RA (2004) New perspectives on vitamin E: γ-tocopherol and carboxyethylhydroxychroman metabolites in biology and medicine. Free Radic Biol Med 36(1):1–15. https://doi.org/10.1016/j.freeradbiomed.2003.10.009
331. Ghosh S, Hauer-Jensen M, Sree Kumar K (2008) Chemistry of tocotrienols. In: Tocotrienols. CRC Press, Boca Raton, FL, pp 85–96. doi:https://doi.org/10.1201/9781420080391.ch7
332. Sen CK, Khanna S, Roy S (2006) Tocotrienols: vitamin E beyond tocopherols. Life Sci 78(18):2088–2098. https://doi.org/10.1016/j.lfs.2005.12.001
333. Pearce BC, Parker RA, Deason ME, Qureshi AA, Wright JJK (1992) Hypocholesterolemic activity of synthetic and natural tocotrienols. J Med Chem 35(20):3595–3606. https://doi.org/10.1021/jm00098a002
334. Pearce BC, Parker RA, Deason ME, Dischino DD, Gillespie E, Qureshi AA, Wright JJK, Volk K (1994) Inhibitors of cholesterol biosynthesis. 2. Hypocholesterolemic and antioxidant activities of benzopyran and tetrahydronaphthalene analogs of the tocotrienols. J Med Chem 37(4):526–541. https://doi.org/10.1021/jm00030a012
335. Qureshi AA, Sami SA, Salser WA, Khan FA (2002) Dose-dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF25) of rice bran in hypercholesterolemic humans. Atherosclerosis 161(1):199–207. https://doi.org/10.1016/S0021-9150(01)00619-0
336. Adachi H, Ishii N (2000) Effects of tocotrienols on life span and protein carbonylation in *Caenorhabditis elegans*. J Gerontol Ser A 55(6):B280–B285. https://doi.org/10.1093/gerona/55.6.B280
337. Mo H, Elson CE (1999) Apoptosis and cell-cycle arrest in human and murine tumor cells are initiated by isoprenoids. J Nutr 129(4):804–813
338. Khanna S, Roy S, Parinandi NL, Maurer M, Sen CK (2006) Characterization of the potent neuroprotective properties of the natural vitamin E α-tocotrienol. J Neurochem 98(5):1474–1486. https://doi.org/10.1111/j.1471-4159.2006.04000.x
339. Khanna S, Roy S, Slivka A, Craft TKS, Chaki S, Rink C, Notestine MA, DeVries AC, Parinandi NL, Sen CK (2005) Neuroprotective properties of the natural vitamin E α-Tocotrienol. Stroke 36(10):2258–2264. https://doi.org/10.1161/01.str.0000181082.70763.22
340. Sen CK, Khanna S, Roy S (2004) Tocotrienol: the natural vitamin E to defend the nervous system? Ann N Y Acad Sci 1031(1):127–142. https://doi.org/10.1196/annals.1331.013
341. Valitova JN, Sulkarnayeva AG, Minibayeva FV (2016) Plant sterols: diversity, biosynthesis, and physiological functions. Biochem Mosc 81(8):819–834. https://doi.org/10.1134/s0006297916080046
342. Benveniste P (2004) Biosynthesis and accumulation of sterols. Annu Rev Plant Biol 55:429–457
343. Brown RE (1998) Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. J Cell Sci 111(1):1–9
344. Magarkar A, Dhawan V, Kallinteri P, Viitala T, Elmowafy M, Róg T, Bunker A (2014) Cholesterol level affects surface charge of lipid membranes in saline solution. Sci Rep 4:5005. https://doi.org/10.1038/srep05005. https://www.nature.com/articles/srep05005#supplementary-information

345. Kamat VS, Beckman JE, Russell JA (1995) Enzyme activity in supercritical fluids, vol 15, no 1. Informa Healthcare, London, ROYAUME-UNI
346. Schuler I, Duportail G, Glasser N, Benveniste P, Hartmann M-A (1990) Soybean phosphatidylcholine vesicles containing plant sterols: a fluorescence anisotropy study. Biochim Biophys Acta Biomembr 1028(1):82–88
347. Lindsey K, Pullen ML, Topping JF (2003) Importance of plant sterols in pattern formation and hormone signalling. Trends Plant Sci 8(11):521–525. https://doi.org/10.1016/j.tplants.2003.09.012
348. Wang Z-Y, Wang Q, Chong K, Wang F, Wang L, Bai M, Jia C (2006) The brassinosteroid signal transduction pathway. Cell Res 16(5):427–434
349. Mongrand S, Morel J, Laroche J, Claverol S, Carde J-P, Hartmann M-A, Bonneu M, Simon-Plas F, Lessire R, Bessoule J-J (2004) Lipid rafts in higher plant cells: purification and characterization of triton X-100-insoluble microdomains from tobacco plasma membrane. J Biol Chem 279(35):36277–36286. https://doi.org/10.1074/jbc.M403440200
350. Laloi M, Perret A-M, Chatre L, Melser S, Cantrel C, Vaultier M-N, Zachowski A, Bathany K, Schmitter J-M, Vallet M, Lessire R, Hartmann M-A, Moreau P (2007) Insights into the role of specific lipids in the formation and delivery of lipid microdomains to the plasma membrane of plant cells. Plant Physiol 143(1):461–472. https://doi.org/10.1104/pp.106.091496
351. Silvestro D, Andersen TG, Schaller H, Jensen PE (2013) Plant sterol metabolism. Δ7-sterol-C5-desaturase (STE1/DWARF7), Δ5,7-sterol-Δ7-reductase (DWARF5) and Δ24-sterol-Δ24-reductase (DIMINUTO/DWARF1) show multiple subcellular localizations in *Arabidopsis thaliana* (Heynh) L. PLoS One 8(2):e56429. https://doi.org/10.1371/journal.pone.0056429
352. Normén L, Dutta P, Lia Å, Andersson H (2000) Soy sterol esters and β-sitostanol ester as inhibitors of cholesterol absorption in human small bowel. Am J Clin Nutr 71(4):908–913
353. Ikeda I, Tanaka K, Sugano M, Vahouny G, Gallo L (1988) Inhibition of cholesterol absorption in rats by plant sterols. J Lipid Res 29(12):1573–1582
354. Sharmila R, Sindhu G (2016) Modulation of angiogenesis, proliferative response and apoptosis by β-sitosterol in rat model of renal carcinogenesis. Indian J Clin Biochem 32(2):142–152
355. Loizou S, Lekakis I, Chrousos GP, Moutsatsou P (2010) β-Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. Mol Nutr Food Res 54(4):551–558. https://doi.org/10.1002/mnfr.200900012
356. Gupta M, Nath R, Srivastava N, Shanker K, Kishor K, Bhargava K (1980) Anti-inflammatory and antipyretic activities of β-sitosterol. Planta Med 39(06):157–163
357. Awad A, Von Holtz R, Cone J, Fink C, Chen Y (1998) beta-sitosterol inhibits growth of HT-29 human colon cancer cells by activating the sphingomyelin cycle. Anticancer Res 18(1A):471–473
358. von Holtz RL, Fink CS, Awad AB (1998) β-sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells. Nutr Cancer 32:8
359. Park C, Moon D-O, Rhu C-H, Choi BT, Lee WH, Kim G-Y, Choi YH (2007) β-Sitosterol induces anti-proliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio. Biol Pharm Bull 30(7):1317–1323
360. Zhao Y, Chang SK, Qu G, Li T, Cui H (2009) β-Sitosterol inhibits cell growth and induces apoptosis in SGC-7901 human stomach cancer cells. J Agric Food Chem 57(12):5211–5218
361. Vundru SS, Kale RK, Singh RP (2013) β-sitosterol induces G1 arrest and causes depolarization of mitochondrial membrane potential in breast carcinoma MDA-MB-231 cells. BMC Complement Altern Med 13(1):280. https://doi.org/10.1186/1472-6882-13-280
362. Shuang R, Rui X, Wenfang L (2016) Phytosterols and dementia. Plant Foods Hum Nutr 71(4):347–354. https://doi.org/10.1007/s11130-016-0574-1
363. Burg VK, Grimm HS, Rothhaar TL, Grösgen S, Hundsdörfer B, Haupenthal VJ, Zimmer VC, Mett J, Weingärtner O, Laufs U (2013) Plant sterols the better cholesterol in Alzheimer's disease? A mechanistical study. J Neurosci 33(41):16072–16087
364. Ostlund RE, Spilburg CA, Stenson WF (1999) Sitostanol administered in lecithin micelles potently reduces cholesterol absorption in humans. Am J Clin Nutr 70(5):826–831

365. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R, Participants SW (2003) Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc, Elsevier, Rochester, MN, Vol 78, pp 965–978
366. Moreau RA, Whitaker BD, Hicks KB (2002) Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Prog Lipid Res 41(6):457–500
367. Jonker D, Van der Hoek G, Glatz J, Homan C, Posthumus M, Katan M (1985) Combined determination of free, esterified and glycosilated plant sterols in foods. Nutr Rep Int 32:943–952
368. Lin X, Ma L, Racette SB, Spearie CLA, Ostlund RE (2009) Phytosterol glycosides reduce cholesterol absorption in humans. Am J Physiol-Gastrointest Liver Physiol 296(4): G931–G935
369. Baumgartner S, Ras RT, Trautwein EA, Mensink RP, Plat J (2017) Plasma fat-soluble vitamin and carotenoid concentrations after plant sterol and plant stanol consumption: a meta-analysis of randomized controlled trials. Eur J Nutr 56(3):909–923. https://doi.org/10.1007/s00394-016-1289-7
370. Scholz B, Guth S, Engel KH, Steinberg P (2015) Phytosterol oxidation products in enriched foods: occurrence, exposure, and biological effects. Mol Nutr Food Res 59 (7):1339–1352
371. Kozubek A, Tyman JHP (2005) Bioactive phenolic lipids. In: Atta-ur-Rahman (ed) Studies in natural products chemistry: bioactive natural products (Part K), 1st edn. Elsevier, Amsterdam, pp 111–190
372. Hadacek F (2017) Phenolic lipids in plants: functional diversity of. https://doi.org/10.1007/978-94-007-7864-1_136-1
373. Stasiuk M, Kozubek A (2010) Biological activity of phenolic lipids. Cell Mol Life Sci 67(6):841–860. https://doi.org/10.1007/s00018-009-0193-1
374. Barbini L, Lopez P, Ruffa J, Martino V, Ferraro G, Campos R, Cavallaro L (2006) Induction of apoptosis on human hepatocarcinoma cell lines by an alkyl resorcinol isolated from Lithraea molleoides. World J Gastroenterol 12(37):5959–5963. https://doi.org/10.3748/wjg.v12.i37.5959
375. Gąsiorowski K, Brokos B, Kozubek A, Oszmiański J (2000) The antimutagenic activity of two plant-derived compounds. A comparative cytogenetic study. Cell Mol Biol Lett 5(2):171–190
376. Gąsiorowski K, Szyba K, Brokos B, Kozubek A (1996) Antimutagenic activity of alkylresorcinols from cereal grains. Cancer Lett 106(1):109–115. https://doi.org/10.1016/0304-3835(96)04294-2
377. Begum P, Hashidoko Y, Tofazzal Islam M, Ogawa Y, Tahara S (2002) Zoosporicidal activities of anacardic acids against *Aphanomyces cochlioides*. Z Naturforsch C 57:874. https://doi.org/10.1515/znc-2002-9-1020
378. Chitra M, Shyamala Devi CS, Sukumar E (2003) Antibacterial activity of embelin. Fitoterapia 74(4):401–403. https://doi.org/10.1016/S0367-326X(03)00066-2
379. Kubo J, Lee JR, Kubo I (1999) Anti-*Helicobacter pylori* agents from the cashew apple. J Agr Food Chem 47(2):533–537. https://doi.org/10.1021/jf9808980
380. Narasimhan B, Panghal A, Singh N, Dhake AS (2008) Efficiency of anacardic acid as preservative in tomato products. J Food Process Preserv 32(4):600–609. https://doi.org/10.1111/j.1745-4549.2008.00201.x
381. Lanfranchi D-A, Laouer H, El Kolli M, Prado S, Maulay-Bailly C, Baldovini N (2010) Bioactive phenylpropanoids from daucus crinitus Desf. from Algeria. J Agr Food Chem 58(4):2174–2179. https://doi.org/10.1021/jf903760b
382. McGovern TW, Barkley TM (1998) Botanical dermatology. Int J Dermatol 37(5):321–334. https://doi.org/10.1046/j.1365-4362.1998.00385.x
383. Torres de Pinedo A, Peñalver P, Pérez-Victoria I, Rondón D, Morales JC (2007) Synthesis of new phenolic fatty acid esters and their evaluation as lipophilic antioxidants in an oil matrix. Food Chem 105(2):657–665. https://doi.org/10.1016/j.foodchem.2007.04.029

384. Moo-Puc R, Caamal-Fuentes E, Peraza-Sánchez SR, Slusarz A, Jackson G, Drenkhahn SK, Lubahn DB (2015) Antiproliferative and antiestrogenic activities of bonediol an alkyl catechol from *Bonellia macrocarpa*. Biomed Res Int 2015:6. https://doi.org/10.1155/2015/847457
385. Kubo I, Muroi H, Himejima M (1993) Structure-antibacterial activity relationships of anacardic acids. J Agric Food Chem 41:1016–1019
386. Carvalho ALN, Annoni R, Torres LHL, Durao ACCS, Shimada ALB, Almeida FM, Hebeda CB, Lopes FDTQS, Dolhnikoff M, Martins MA, Silva LFF, Farsky SHP, Saldiva PHN, Ulrich CM, Owen RW, Marcourakis T, Trevisan MTS, Mauad T (2013) Anacardic acids from cashew nuts ameliorate lung damage induced by exposure to diesel exhaust particles in mice. J Evid Based Complement Alternat Med 2013:549879. https://doi.org/10.1155/2013/549879
387. Hamad F, Mubofu E (2015) Potential biological applications of bio-based anacardic acids and their derivatives. Int J Mol Sci 16(4):8569
388. Baerson SR, Schröder J, Cook D, Rimando AM, Pan Z, Dayan FE, Noonan BP, Duke SO (2010) Alkylresorcinol biosynthesis in plants. Plant Signal Behav 5(10):1286–1289. https://doi.org/10.4161/psb.5.10.13062
389. Kahveci D, Laguerre M, Villeneuve P (2015) Phenolipids as new antioxidants: production, activity and potential applications. In: Ahmad MU, Xu X (eds) Polar lipids: biology, chemistry and technology. AOCS Press, Urbana
390. Ross AB (2012) Present status and perspectives on the use of alkylresorcinols as biomarkers of wholegrain wheat and rye intake. J Nutr Metab 2012:462967
391. Kruk J, Aboul-Enein B, Bernstein J, Marchlewicz M (2017) Dietary alkylresorcinols and cancer prevention: a systematic review. Eur Food Res Technol 243(10):1693–1710. https://doi.org/10.1007/s00217-017-2890-6
392. Chang H-S, Lin Y-J, Lee S-J, Yang C-W, Lin W-Y, Tsai I-L, Chen I-S (2009) Cytotoxic alkyl benzoquinones and alkyl phenols from *Ardisia virens*. Phytochemistry 70(17):2064–2071. https://doi.org/10.1016/j.phytochem.2009.09.006
393. Dayan FE, Rimando AM, Pan Z, Baerson SR, Gimsing AL, Duke SO (2010) Sorgoleone. Phytochemistry 71(10):1032–1039. https://doi.org/10.1016/j.phytochem.2010.03.011
394. Figueroa-Espinoza M-C, Villeneuve P (2005) Phenolic acids enzymatic lipophilization. J Agr Food Chem 53(8):2779–2787
395. Viskupicova J, Maliar T (2017) Rutin fatty acid esters: from synthesis to biological health effects and application. J Food Nutr Res 56(3):232–243
396. Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agr Food Chem 49(11):5165–5170. https://doi.org/10.1021/jf010697n
397. Reddy KK, Shanker KS, Ravinder T, Prasad RBN, Kanjilal S (2010) Chemo-enzymatic synthesis and evaluation of novel structured phenolic lipids as potential lipophilic antioxidants. Eur J Lipid Sci Technol 112(5):600–608. https://doi.org/10.1002/ejlt.200900200
398. Shahidi F, Zhong Y (2011) Revisiting the polar paradox theory: a critical overview. J Agr Food Chem 59(8):3499–3504
399. Figueroa-Espinoza M, Villeneuve P (2005) Phenolic acids enzymatic lipophilization. J Agr Food Chem 53(8):2779–2787
400. Laguerre M, Bayrasy C, Lecomte J, Chabi B, Decker EA, Wrutniak-Cabello C, Cabello G, Villeneuve P (2013) How to boost antioxidants by lipophilization? Biochimie 95(1):20–26. https://doi.org/10.1016/j.biochi.2012.07.018
401. Wang J, Shahidi F (2014) Acidolysis of p-coumaric acid with omega-3 oils and antioxidant activity of phenolipid products in in vitro and biological model systems. J Agr Food Chem 62(2):454–461
402. Laguerre M, López Giraldo LJ, Lecomte J, Figueroa-Espinoza MC, Baréa B, Weiss J, Decker EA, Villeneuve P (2009) Chain length affects antioxidant properties of chlorogenate esters in emulsion: the cutoff theory behind the polar paradox. J Agr Food Chem 57(23):11335–11342
403. Laguerre M, López Giraldo LJ, Jérôme Lecomte J, Figueroa-Espinoza MC, Baréa B, Weiss J, Decker EA, Villeneuve P (2010) Relationship between hydrophobicity and antioxidant ability

of "Phenolipids" in emulsion: a parabolic effect of the chain length of rosmarinate esters. J Agr Food Chem 58(5):2869–2876
404. Kubo I, Fujita K-i, Nihei K-i, Nihei A (2004) Antibacterial activity of akyl gallates against *Bacillus subtilis*. J Agr Food Chem 52(5):1072–1076. https://doi.org/10.1021/jf0347741
405. Katsoura MH, Polydera AC, Tsironis LD, Petraki MP, Rajačić SK, Tselepis AD, Stamatis H (2009) Efficient enzymatic preparation of hydroxycinnamates in ionic liquids enhances their antioxidant effect on lipoproteins oxidative modification. New Biotechnol 26(1):83–91. https://doi.org/10.1016/j.nbt.2009.02.004
406. Danihelová M, Viskupičová J, Šturdík E (2012) Lipophilization of flavonoids for their food, therapeutic and cosmetic applications. Acta Chim Slovaca 5:59. https://doi.org/10.2478/v10188-012-0010-6
407. Figueroa-Espinoza MC, Laguerre M, Villeneuve P, Lecomte J (2013) From phenolics to phenolipids: optimizing antioxidants in lipid dispersions. Lipid Technol 25(6):131–134. https://doi.org/10.1002/lite.201300277
408. Ardhaoui M, Falcimaigne A, Engasser J-M, Moussou P, Pauly G, Ghoul M (2004) Acylation of natural flavonoids using lipase of candida antarctica as biocatalyst. J Mol Catal B-Enzym 29(1):63–67. https://doi.org/10.1016/j.molcatb.2004.02.013
409. Bok SH, Jeong TS, Lee SK, Kim JR, Moon SS, Choi MS (2001) Flavanone derivatives and composition for preventing or treating blood lipid level-related diseases comprising same. Patent US 20010006978A1
410. Chebil L, Humeau C, Falcimaigne A, Engasser J-M, Ghoul M (2006) Enzymatic acylation of flavonoids. Process Biochem 41(11):2237–2251. https://doi.org/10.1016/j.procbio.2006.05.027
411. Otto RT, Scheib H, Bornscheuer UT, Pleiss J, Syldatk C, Schmid RD (2000) Substrate specificity of lipase B from *Candida antarctica* in the synthesis of arylaliphatic glycolipids. J Mol Catal B-Enzym 8(4):201–211. https://doi.org/10.1016/S1381-1177(99)00058-2
412. Katsoura MH, Polydera AC, Tsironis L, Tselepis AD, Stamatis H (2006) Use of ionic liquids as media for the biocatalytic preparation of flavonoid derivatives with antioxidant potency. J Biotechnol 123(4):491–503. https://doi.org/10.1016/j.jbiotec.2005.12.022
413. Cao S-L, Deng X, Xu P, Huang Z-X, Zhou J, Li X-H, Zong M-H, Lou W-Y (2017) Highly efficient enzymatic acylation of dihydromyricetin by the immobilized lipase with deep eutectic solvents as cosolvent. J Agr Food Chem 65(10):2084–2088. https://doi.org/10.1021/acs.jafc.7b00011
414. Youn SH, Kim HJ, Kim TH, Shin CS (2007) Lipase-catalyzed acylation of naringin with palmitic acid in highly concentrated homogeneous solutions. J Mol Catal B-Enzym 46(1):26–31. https://doi.org/10.1016/j.molcatb.2007.02.002
415. Humeau C, Girardin M, Rovel B, Miclo A (1998) Enzymatic synthesis of fatty acid ascorbyl esters. J Mol Catal B-Enzym 5(1):19–23. https://doi.org/10.1016/S1381-1177(98)00090-3
416. Humeau C, Girardin M, Coulon D, Miclo A (1995) Synthesis of 6-*O*-palmitoyl L-ascorbic acid catalyzed by *Candida antartica* lipase. Biotechnol Lett 17(10):1091–1094. https://doi.org/10.1007/bf00143107
417. Watanabe Y, Minemoto Y, Adachi S, Nakanishi K, Shimada Y, Matsuno R (2000) Lipase-catalyzed synthesis of 6-*O*-eicosapentaenoyl L-ascorbate in acetone and its autoxidation. Biotechnol Lett 22(8):637–640. https://doi.org/10.1023/a:1005675111042
418. Lee K-T, Akoh CC, Dawe DL (1999) Effects of structured lipid containing omega-3 and medium chain fatty acids on serum lipids and immunological variables in mice. J Food Biochem 23(2):197–208. https://doi.org/10.1111/j.1745-4514.1999.tb00014.x
419. Karboune S, St-Louis R, Kermasha S (2008) Enzymatic synthesis of structured phenolic lipids by acidolysis of flaxseed oil with selected phenolic acids. J Mol Catal B-Enzym 52:96–105. https://doi.org/10.1016/j.molcatb.2007.10.015
420. Akoh CC, Lee KT, Fomuso LB (1998) Synthesis of positional isomers of structured lipids with lipases as biocatalyst. In: Christophe AB (ed) Structural modified food fats: synthesis, biochemistry, and use. AOCS Press, Champaign, pp 46–72

421. Sorour N, Karboune S, Saint-Louis R, Kermasha S (2012) Enzymatic synthesis of phenolic lipids in solvent-free medium using flaxseed oil and 3,4-dihydroxyphenyl acetic acid. Process Biochem 47(12):1813–1819. https://doi.org/10.1016/j.procbio.2012.06.020
422. Namal Senanayake SPJ, Shahidi F (2002) Enzyme-catalyzed synthesis of structured lipids via acidolysis of seal (*Phoca groenlandica*) blubber oil with capric acid. Food Res Int 35(8):745–752. https://doi.org/10.1016/S0963-9969(02)00070-4
423. Sun S, Zhu S, Bi Y (2014) Solvent-free enzymatic synthesis of feruloylated structured lipids by the transesterification of ethyl ferulate with castor oil. Food Chem 158:292–295. https://doi.org/10.1016/j.foodchem.2014.02.146
424. Sabally K, Karboune S, St-Louis R, Kermasha S (2006) Lipase-catalyzed transesterification of dihydrocaffeic acid with flaxseed oil for the synthesis of phenolic lipids. J Biotechnol 127(1):167–176
425. Sabally K, Karboune S, St-Louis R, Kermasha S (2006) Lipase-catalyzed transesterification of trilinolein or trilinolenin with selected phenolic acids. J Am Oil Chem Soc 83(2):101–107. https://doi.org/10.1007/s11746-006-1181-3
426. Karboune S, Safari M, Lue B-M, Yeboah FK, Kermasha S (2005) Lipase-catalyzed biosynthesis of cinnamoylated lipids in a selected organic solvent medium. J Biotechnol 119(3):281–290. https://doi.org/10.1016/j.jbiotec.2005.03.012
427. Aziz S, Dutilleul P, Kermasha S (2012) Lipase-catalyzed transesterification of krill oil and 3,4-dihydroxyphenyl acetic acid in solvent-free medium using response surface methodology. J Mol Catal B-Enzym 84:189–197. https://doi.org/10.1016/j.molcatb.2012.05.011
428. Sorour N, Karboune S, Saint-Louis R, Kermasha S (2012) Lipase-catalyzed synthesis of structured phenolic lipids in solvent-free system using flaxseed oil and selected phenolic acids as substrates. J Biotechnol 158(3):128–136. https://doi.org/10.1016/j.jbiotec.2011.12.002
429. Ciftci D, Saldaña MDA (2012) Enzymatic synthesis of phenolic lipids using flaxseed oil and ferulic acid in supercritical carbon dioxide media. J Supercrit Fluids 72:255–262. https://doi.org/10.1016/j.supflu.2012.09.007
430. Wildman RE (2007) Nutraceuticals and functional foods. In: Wildman RE (ed) Handbook of nutraceuticals and functional foods. CRC press, Boca Raton, pp 37–65
431. McClements DJ, Decker EA, Park Y, Weiss J (2009) Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. Crit Rev Food Sci Nutr 49(6):577–606
432. Sagalowicz L, Leser ME (2010) Delivery systems for liquid food products. Curr Opin Colloid Interface Sci 15(1):61–72
433. Velikov KP, Pelan E (2008) Colloidal delivery systems for micronutrients and nutraceuticals. Soft Matter 4(10):1964–1980
434. McClements DJ (2010) Design of nano-laminated coatings to control bioavailability of lipophilic food components. J Food Sci 75(1):R30
435. McClements DJ (2010) Emulsion design to improve the delivery of functional lipophilic components. Annu Rev Food Sci Technol 1:241–269
436. McClements DJ (2015) Active ingredients. In: McClements DJ (ed) Nanoparticle-and microparticle-based delivery systems: encapsulation, protection and release of active compounds. CRC Press, Boca Raton, pp 1–22
437. Lauridsen C, Hedemann MS, Jensen SK (2001) Hydrolysis of tocopheryl and retinyl esters by porcine carboxyl ester hydrolase is affected by their carboxylate moiety and bile acids. J Nutr Biochem 12(4):219–224
438. McClements DJ, Decker EA, Park Y (2008) Controlling lipid bioavailability through physicochemical and structural approaches. Crit Rev Food Sci Nutr 49(1):48–67
439. Mouhid L, Corzo-Martínez M, Torres C, Vázquez L, Reglero G, Fornari T, Ramírez de Molina A (2017) Improving in vivo efficacy of bioactive molecules: an overview of potentially antitumor phytochemicals and currently available lipid-based delivery systems. J Oncol 2017:7351976
440. Kalepu S, Manthina M, Padavala V (2013) Oral lipid-based drug delivery systems–an overview. Acta Pharm Sin B 3(6):361–372

441. Shrestha H, Bala R, Arora S (2014) Lipid-based drug delivery systems. J Pharm 2014 https://doi.org/10.1155/2014/801820
442. Kakkar V, Mishra AK, Chuttani K, Kaur IP (2013) Proof of concept studies to confirm the delivery of curcumin loaded solid lipid nanoparticles (C-SLNs) to brain. Int J Pharm 448(2):354–359
443. Kreuter J (2001) Nanoparticulate systems for brain delivery of drugs. Adv Drug Deliv Rev 47(1):65–81
444. Gabizon A, Papahadjopoulos D (1988) Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. Proc Natl Acad Sci 85(18):6949–6953
445. Kunjachan S, Rychlik B, Storm G, Kiessling F, Lammers T (2013) Multidrug resistance: physiological principles and nanomedical solutions. Adv Drug Deliv Rev 65(13):1852–1865. https://doi.org/10.1016/j.addr.2013.09.018
446. Barui S, Saha S, Mondal G, Haseena S, Chaudhuri A (2014) Simultaneous delivery of doxorubicin and curcumin encapsulated in liposomes of pegylated RGDK-lipopeptide to tumor vasculature. Biomaterials 35(5):1643–1656
447. Humberstone AJ, Charman WN (1997) Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Deliv Rev 25(1):103–128
448. Liu Y, Feng N (2015) Nanocarriers for the delivery of active ingredients and fractions extracted from natural products used in traditional Chinese medicine (TCM). Adv Colloid Interf Sci 221:60–76
449. Liu J, Liu J, Xu H, Zhang Y, Chu L, Liu Q, Song N, Yang C (2014) Novel tumor-targeting, self-assembling peptide nanofiber as a carrier for effective curcumin delivery. Int J Nanomedicine 9:197
450. Wang X-X, Li Y-B, Yao H-J, Ju R-J, Zhang Y, Li R-J, Yu Y, Zhang L, Lu W-L (2011) The use of mitochondrial targeting resveratrol liposomes modified with a dequalinium polyethylene glycol-distearoylphosphatidyl ethanolamine conjugate to induce apoptosis in resistant lung cancer cells. Biomaterials 32(24):5673–5687
451. Chen C-C, Hsieh D-S, Huang K-J, Chan Y-L, Hong P-D, Yeh M-K, Wu C-J (2014) Improving anticancer efficacy of (−)-epigallocatechin-3-gallate gold nanoparticles in murine B16F10 melanoma cells. Drug Des Devel Ther 8:459
452. Manju S, Sreenivasan K (2012) Gold nanoparticles generated and stabilized by water soluble curcumin–polymer conjugate: blood compatibility evaluation and targeted drug delivery onto cancer cells. J Colloid Interface Sci 368(1):144–151
453. Moorthi C, Kathiresan K (2013) Curcumin–Piperine/Curcumin–Quercetin/Curcumin–Silibinin dual drug-loaded nanoparticulate combination therapy: a novel approach to target and treat multidrug-resistant cancers. J Med Hypotheses Ideas 7(1):15–20. https://doi.org/10.1016/j.jmhi.2012.10.005
454. Torres CF, Vázquez L, Señoráns FJ, Reglero G (2009) Enzymatic synthesis of short-chain diacylated alkylglycerols: a kinetic study. Process Biochem 44(9):1025–1031
455. Madrona A, Pereira-Caro G, Mateos R, Rodríguez G, Trujillo M, Fernández-Bolaños J, Espartero JL (2009) Synthesis of hydroxytyrosyl alkyl ethers from olive oil waste waters. Molecules 14(5):1762–1772
456. Corzo-Martínez M, Vázquez L, Arranz-Martínez P, Menéndez N, Reglero G, Torres CF (2016) Production of a bioactive lipid-based delivery system from ratfish liver oil by enzymatic glycerolysis. Food Bioprod Process 100:311–322
457. Lawrence T, Willoughby D A, Gilroy D W (2002) Anti-inflammatory lipid mediators and insights into the resolution of inflammation. Nat Rev Immunol 2(10):787–795

Oxidative Stability of Edible Plant Oils

18

Terrence Madhujith and Subajiny Sivakanthan

Contents

T. Madhujith (✉)
Faculty of Agriculture, Department of Food Science and Technology, University of Peradeniya, Peradeniya, Sri Lanka
e-mail: madujith@yahoo.com; tmadhujith@gmail.com

S. Sivakanthan
Faculty of Agriculture, Department of Agricultural Chemistry, University of Jaffna, Jaffna, Sri Lanka
e-mail: suba106@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_94

Abstract

Edible plant oils play a vital role in daily diets of people worldwide. Stability against oxidation is the major factor limiting the application of most edible plant oils for cooking and processing. Most native plant oils vary greatly in their stability to oxidation depending on their composition. Oxidative stability of edible plant oils has been extensively studied to find out the ways of improving their stability against oxidation to widen their application. Synthetic antioxidants are effective to improve the oxidative stability of these oils, however, recently, following the evidences on possible toxicities of synthetic antioxidants, the use of natural plant sources as antioxidant is gaining interest. In addition, modification of composition of the oils through genetic modification is another successful means to improve the oxidative stability of these oils. This chapter focuses on the mechanism and factors of oxidation and ways of improving oxidative stability of oils.

Keywords

Antioxidants · Autoxidation · Hydroperoxides · Photooxidation · Radicals · Refining

1 Introduction

Edible oils from various sources are important components of the daily diet of people from around the world. Plant oils account for more than 75% of edible oil consumption worldwide [1]. According to the US Department of Agriculture, 189.11 million metric tons of vegetable oils have been produced globally during the season 2016/2017. Further, the world vegetable oil production is increasing continuously, especially the production of palm, soybean and sunflower oil. Palm oil found to be the major plant oil ranked highest (more than 60%) in the world production followed by soybean oil [2]. In the recent past, the use of blended oils is becoming popular in several countries such as Europe, China, Thailand, and Japan. Two or more vegetable oils are mixed at different proportion to get blended oils for different nutritional and processing purpose [3]. Vegetable oils are the healthier choice relative to animal fats in view of their fatty acid contents and cholesterol-free nature [4].

Studies on edible oils remain one of the prime areas of research, and nowadays people are much health-conscious. Diet-related noncommunicable diseases,

especially coronary heart diseases, are the major cause of death in developed as well as developing countries. In this context, edible oils play a crucial role in maintaining human well-being. Edible oils differ in their characteristics based on their composition. Oils are used as cooking oils and ingredients in variety of foods. Thus, the oils undergo various processing, mainly, heat treatment. Therefore, the stability of these oils for processing as well as storage conditions is the major property of oils. Major deteriorative reaction occurring in most of the edible oils is the oxidation. Lipid oxidation primarily depends on the fatty acid composition and the presence of minor components. Lipid oxidation leads to the production of primary and secondary oxidative compounds which are harmful to health. This chapter emphasizes the mechanism and factors of oxidation of oils, effect of processing conditions and antioxidants in oxidation, oxidative stability of some major edible oils, and measurement methods.

2 Mechanism of Lipid Oxidation

Lipid oxidation is the major cause of deterioration of the quality of edible oils. Oxidative stability of oils is defined as the resistance to oxidation during processing and storage [5]. Lipid oxidation breaks down the fatty acids, thus, causes the loss of nutritional quality, and produces undesirable color, flavor, and toxic components making the food unacceptable or less acceptable by consumers; thus it is an indicator to determine the quality and shelf life [6]. Different mechanisms have been proposed as responsible for the oxidation of edible oils during processing and storage such as autoxidation and photosensitized oxidation. The kind of mechanism depends on the kind of oxygen available. Triplet oxygen ($^3O_2$) and singlet oxygen ($^1O_2$) can react with oxygen to cause autoxidation and photosensitized oxidation, respectively [7].

2.1 Lipid Autoxidation

Unsaturated fatty acids are prone to autoxidation which proceeds via free-radical chain mechanism. Rate of oxidation depends on the degree of unsaturation and increases with increase in the number of double bond [8]. Free-radical mechanism of lipid autoxidation involves the attack of oxygen at the allylic position leading to the formation of hydroperoxides. These primary oxidation products further decompose into secondary oxidation products [9].

Lipid autoxidation involves three steps: initiation phase (induction period), propagation phase (exponential phase), and termination stage [10]. In the initiation step, when lipid (LH) is exposed to an initiator (heat, light or metal ions), hydrogen atom of double bond is abstracted, and alkyl radical (L•) is formed [11]. This free radical abstracts hydrogen from other lipid molecules and reacts with the hydrogen to form hydroperoxide (LOOH) (primary oxidation product) and another alkyl radical. Alkyl radical also reacts with molecular oxygen ($^3O_2$) to form peroxy radical (LOO•), which abstracts hydrogen from other lipid molecules and reacts with hydrogen to

form hydroperoxide and another alkyl radical. These radicals catalyze the oxidation reaction, and autoxidation is called the free-radical chain reaction [7]. The rate of formation of peroxy radical and hydroperoxide depends only on oxygen availability and temperature [12]. Radicals react with each other to form non-radical species, and the reaction is terminated [7]. The most labile hydrogen atom in monounsaturated acids is on the carbon atoms adjacent to the double bond, whereas, in polyunsaturated acids, the most labile hydrogens are on methylene groups between two double bonds. The abstraction of hydrogen is followed by electron rearrangement to form conjugated dienes since the radicals formed are unstable [13].

Hydroperoxides are nonvolatile, odorless, and very unstable and further break down via several steps into secondary oxidation products called carbonyl compounds such as aldehyde, ketones, acids, alcohols, acids, esters, and short-chain hydrocarbons via monomolecular and bimolecular reactions [7, 13–15]. Hydroperoxides begin to decompose as soon as they are formed. During the first stages of autoxidation, their rate of formation exceeds their rate of decomposition, and the reverse is true at later stages of autoxidation [16]. Most of the secondary oxidative products are responsible for the off-flavor in the oxidized edible oil. Aliphatic carbonyl compounds (alkanals, 2-alkenals, and *trans*, *trans*-2,4-alkadienals) have more influence on the oxidized oil flavor because of their low threshold values [7]. Breakdown of hydroperoxides also produces nonvolatile monomeric compounds, including di- and tri-oxygenated esters derived from the corresponding keto-, hydroxy-, hydroperoxy-, and epoxide esters. During induction period, only very little changes occur in lipids, followed by fast deterioration of lipids, and off-flavors become noticeable and the lipid is no longer edible [13].

Autoxidation of oleate (C18:1, n−9) produces four allylic hydroperoxides containing OOH groups on carbon 8,11 (*cis*-8-OOH, *cis*-11-OOH) and 9,10, (*trans*-9-OOH, *trans*10-OOH). Autoxidation of linoleate (C18:2, n−6) produces a mixture of *cis*, *trans* and *trans*, *trans* (9-OOH and 13-OOH) conjugated diene hydroperoxides. Autoxidation of linolenate (C18:3, n−3) produces a mixture of *cis*, *trans*, *trans*, *trans*, and *cis* (9-OOH, 12-OOH, 13-OOH and 16-OOH) conjugated diene hydroperoxides [13, 16].

Initiation:

$$LH \rightarrow L\cdot + H\cdot$$

Propagation:

$$L\cdot + O2 \rightarrow LOO\cdot$$

$$LOO\cdot + LH \rightarrow LOOH + L\cdot$$

Termination:

$$LOO\cdot + LOO\cdot \rightarrow \text{non-radical product}$$

$$LOO\cdot + L\cdot \rightarrow \text{non-radical product}$$

$$L\cdot + L\cdot \rightarrow \text{non-radical product}$$

Hydroperoxides initiate new chain reaction after reacting with free radicals [17].

2.2 Photooxidation

Oxidation of edible oils is accelerated by light, especially in the presence of sensitizers [7]. In the presence of light, photosensitizers such as chlorophyll and porphyrin convert triplet oxygen into singlet oxygen, which is a highly reactive non-radical molecule [15]. If wavelength of solar light is less than 220 nm, unsaturated fatty acids cannot absorb light; however photosensitizers absorb light energy and convert triplet state sensitizer to singlet-state sensitizer. Ground-state singlet sensitizers absorb light energy very rapidly and become excited singlet sensitizer which can return to their ground state via emission of light, internal conversion, or intersystem crossing. Fluorescence, heat, and excited triplet state of sensitizers are produced by emission of light, internal conversion, and intersystem crossing, respectively [7]. Photooxidation can proceed via two types of mechanism. An electron or a hydrogen atom transfers between an excited triplet sensitizer and a substrate, producing free radicals or radical ions (Type I). Excited triplet sensitizers react with triplet oxygen to produce superoxide anion by electron transfer. Superoxide anion produces hydrogen peroxide which reacts with superoxides and forms singlet oxygen in the presence of transition metals (iron or copper). Excitation energy of triplet sensitizers can be transferred to triplet oxygen by triplet–triplet annihilation to produce singlet oxygen, which reacts with the double bond of unsaturated fatty acids, producing an allylic hydroperoxide (Type II). The rate of type I and type II process may differ depending on the kinds of sensitizers, substrates, and concentrations of substrates and oxygen [7, 13, 15, 18–20]. Hydroperoxides formed by photooxidation are decomposed by the same mechanisms for the hydroperoxides formed by autoxidation. The mechanism of photooxidation is explained in detail by Choe and Min [7].

2.3 Lipoxygenase Catalyzed Oxidation

Lipoxygenase is an enzyme found in plants. It is another reason for the deterioration of edible plant oils, especially during oil extraction. Lipoxygenase reacts with oils containing1,4-*cis*, *cis*-pentadiene system producing corresponding hydroperoxy derivatives and *cis-trans* conjugated hydroperoxide [21]. Reaction of this enzyme produces hydroperoxides, which decompose to form secondary oxidation products with strong undesirable flavors. Lipoxygenase produces similar volatile compounds to those produced by autoxidation [22]. Lipoxygenase contains a ferrous atom as inactive Fe (II) and is oxidized to active Fe (III) by fatty acid hydroperoxides or hydrogen peroxide. The active enzyme abstracts a hydrogen atom from the methylene group of a polyunsaturated fatty acid with the iron being reduced to Fe (II) producing conjugated dienes followed by reaction with oxygen generating peroxyl radical and hydroperoxide [13, 20]. Rice bran and soybean are the two major plant sources containing lipoxygenase; thus the oils extracted from these sources can easily undergo lipoxygenase-catalyzed oxidation during processing.

3 Harmful Effects of Oxidation of Edible Oils

Lipid oxidation reduces the shelf life and nutritive value of food by limiting the content of essential polyunsaturated fatty acids [23]. Lipid oxidation products are considered to be harmful for health as they consist of compounds with mutagenic, carcinogenic, and cytotoxic properties [24]. These compounds cause health problems such as growth of tumor cells through lipid peroxidation as hydroxides of fatty acids are cytotoxic. Oxidation of long chain fatty acids causes neuromyopathic disease both in infant and adults [15, 25].

Consumption of lipids containing excessive free radicals may cause alterations in the redox state of human body, leading to lipid peroxidation [4]. Unstable free radicals tend to stabilize themselves by abstracting electrons from membrane lipids to start a self-propagating chain reaction causing structural rearrangement of the lipids. The rate of bond cleavage increases until the molecule gets stabilized. Oxidative damage to lipid structures eventually leads to disorganization and dysfunction of, as well as damage to membranes, enzymes and proteins [4, 26].

4 Factors Affecting the Oxidative Stability of Edible Plant Oils

The oxidative stability of oil is influenced by the fatty acid composition of the oil; processing, heat or light; the concentration and type of oxygen; free fatty acids, mono-, and diacylglycerol concentration; transition metals; peroxides; thermally oxidized compounds; pigments; and antioxidants. These factors interactively affect the oxidation of oil, and it is not easy to differentiate the individual effect of the factors [7].

4.1 Fatty Acid Composition

The natural fatty acid composition of oil and the positions of the fatty acids in the glycerol backbone determine the susceptibility and resistance to oxidation [27]. Oils containing more unsaturated fatty acids are oxidized more quickly than the oils containing less unsaturated fatty acids [28]. As the degree of unsaturation increases, the rate of oxidation increases [7] producing more complex mixtures of hydroperoxides. In addition, as the degree of unsaturation of fatty acids increases, the rate of formation and the amount of primary oxidation products are accumulated [29]. The relative rate of autoxidation of oleate/linoleate/linolenate was reported to be in the order of 1:40–50:100 on the basis of the oxygen uptake and 1:12:25 on the basis of peroxide development [13]. Soybean, sunflower, corn and rapeseed oils are often considered unsuitable for continuous frying due to their high content of polyunsaturated fatty acids [30]. Xu and others [31] studied the stability of camellia oil after frying potatoes by comparing with palm oil and peanut oil and reported that camellia oil possesses highest oxidative stabilities followed by palm oil, while peanut oil

showed the least stability. They have concluded that the fatty acid composition and tocopherol contents of oils are the important factors of oxidative stability of the oils. Camellia oil contains higher proportions of monounsaturated fatty acid compared to saturated and polyunsaturated fatty acid than other two oils, and the peanut oil contains higher proportion of polyunsaturated fatty acid. The monounsaturated fatty acid is relatively stable toward oxidation, while polyunsaturated fatty acids are highly susceptible to oxidation [32]. Even though the fatty acid composition of the oils is the major factor determining the oxidative stability of the oil, oxidative stability of oils is a function of several other factors [30].

4.2 Oil Processing

The oil processing affects the oxidative stability of an oil. All crude oils after extraction from their sources contain nontriglyceride components such as free fatty acids, partial acylglycerols, sterols, tocopherols, hydrocarbons, pigments (gossypol, chlorophyll), vitamins, phosphatides, protein fragments, trace metals, and resinous and mucilaginous materials. The objectionable nontriglycerides cause unwanted effects during processing such as darkening and formation of foam, smoke, and precipitate and develop off-flavors. Thus, crude oils are refined to remove the objectionable constituents [33]. Typical refining process consists of neutralization, bleaching, winterization, and deodorization steps. Even though the objective of the refining is to remove impurities with minimum damage to the oil, unfortunately, these processing steps may result in losses in naturally occurring bioactives such as tocopherols, tocotrienols, sterols, and phenols, and the extent of loss depends on the processing parameters and nature of the oil [33, 34]. Thus, the refined oils may have less oxidative stability than their unrefined counterparts [30].

4.3 Temperature and Light

Autoxidation of oils and the decomposition of hydroperoxides increase as the temperature increases. As temperature increases, the solubility of oxygen decreases drastically although all the oxidation reactions are accelerated [35]. At temperatures above 150 °C, hydroperoxides are reduced to very low level or become absent, and the formation of new compounds is very rapid, indicating that the rate of peroxide decomposition is higher than that of their formation [36].

The formation of autoxidation products during the induction period of autoxidation is slow at low temperature, and the content of polymerized compounds increases significantly at the end of the induction period. Light of shorter wavelengths has more detrimental effects on the oils than longer wavelengths, and the effect of light on oil oxidation becomes less as temperature increases [7, 12]. However, as temperature increases, the influence of light on oxidation becomes less important [35]. The effect of temperature on photooxidation is less prominent than on autoxidation. Light has more influence than temperature in photooxidation [7].

The packaging of oils is very important to minimize the photooxidation. Transparent plastic bottles can increase oil oxidation. The incorporation of UV absorbers such as Tinuvin 234 (2-(2-hydroxy-3,5-di (1,1-dimethylbenzyl)phenyl) benzotriazole) or Tinuvin 326 (2-(3′-*tert*-butyl-2′-hydroxy-5′-methylphenyl)-5-chlorobenzo-triazole) into the transparent plastic bottles can be effective to improve the oxidative and sensory stability of edible plant oils stored under light [7, 37, 38].

4.3.1 Frying

Frying is one of the commonly used culinary methods. In recent years, frying oils became an important component of daily diet due to rising demand for deep-fried products. However, much concern has been raised on the biological effects of consumption of fried foods containing oxidized lipids. During the frying process, oil is exposed to an extremely high temperature in the presence of air and moisture. It may result in a high rate of production and decomposition of peroxides. Deep-fat frying decreases the unsaturated fatty acids and increases polar material [39]. Thus, the quality of the frying oil is of paramount importance.

The chemistry of oxidation during frying is very complex since both thermal and oxidative reactions take place during frying at high temperatures. Reaction mechanism of thermal oxidation is principally the same as the autoxidation mechanism; however, the thermal oxidation takes place at faster rate than the rate of autoxidation [39–41]. These oxidative reactions are mainly influenced by the phenolic compounds, tocopherols, and fatty acid composition of the oil and frying temperature [4, 16, 42, 43]. Repeated frying accelerates the oxidation of oil leading to formation of primary and secondary oxidative products which are absorbed by the fried food and eventually get into the gastrointestinal tract and the circulation system after ingestion leading to health problems such as high blood pressure [4].

Juárez and others [44] investigated the discontinuous deep frying of churros in soybean oil, sunflower oil, and partially hydrogenated fats and found that, after 80.5 h of deep frying, all the oils exceeded 25% of total polar compounds except partially hydrogenated fat and losses of tocopherols during frying reached 76.0%. Marinova and others [39] investigated the oxidative stabilities of refined sunflower, grape seed, soybean, corn, and olive oils at frying temperature. Their results demonstrated that olive oil possesses better stability against thermal oxidation when compared to polyunsaturated oils. Further, it is shown that corn and soybean oils are most resistant to oxidation at frying temperature among unsaturated oils.

4.4 Oxygen Concentration

The oxidation of oil takes place when oxygen and catalysts are in contact with the oil. Both concentration and type of oxygen affect oxidation of oils. The oxygen concentration in the oil depends on the oxygen partial pressure in the headspace of the oil [45]. If the partial pressure of oxygen in the headspace is high, invariably high amount of oxygen becomes dissolved in the oil. The amount of dissolved oxygen is positively associated with enhanced oxidation of the oil. Prooxidants such as

transition metals and light accelerate the effect of oxygen concentration on the oxidation of oil [7, 45]. For example, addition of 70 ppm copper to rapeseed oil exposed to air for 35 days resulted in 70 times higher hexanal concentration compared to the sample devoid of copper [45]. If the oxygen concentration is sufficiently high, the rate of oxidation of oil is independent on oxygen concentrations and vice versa. Ratio of surface to volume of the oil also has influence on the oxidative stability. When the surface to volume ratio is increasing, oil can react more efficiently with oxygen; thus, the relative rate of oxidation is less oxygen-dependent with a low oxygen content [7, 45, 46].

4.5 Prooxidants

Crude oils contain nontriglycerides which can act as prooxidants such as free fatty acids, mono- and diacylglycerols, metals, phospholipids, peroxides, and chlorophylls. Trace amounts of metals such as copper, iron, manganese, and nickel can be absorbed by plants during the growth and during fat and oil processing. These metals substantially reduce the oxidative stability of oils [47]. Transition metal ions are involved in redox reaction, which leads to hydroperoxide decomposition [13]. These metals' effects can be diminished by the use of chelating agents such as citric and phosphoric acids [47].

Free radicals produced during hydroperoxide decomposition act as initiators of autoxidation. Radicals generated from food contaminants may also participate in oxidation process acting as catalysts. Some molecules absorb UV light energy and are converted into an excited singlet state. Pigments such as riboflavin and porphyrins (chlorophyll, hemoglobin, myoglobin) and some synthetic dyes can act as initiators of lipid oxidation [13].

4.6 Antioxidants

Antioxidants are the compounds which inhibit the oxidation of fats and oils. They extend the induction period or slow down the rate of oxidation [7]. The presence of antioxidants (naturally present or intentionally added) is one of the most important factors determining the stability of oils against oxidation.

Tocopherols, tocotrienols, carotenoids, phenolic compounds, and sterols are the naturally occurring antioxidants in plant oils. Among these, tocopherols are the most important antioxidants, especially in soybean, canola, sesame, sunflower, and corn oils. Palm oil contains a high amount of tocotrienols. β-Carotene is one of the important natural antioxidants present in unrefined plant oils. β-Carotene acts as antioxidant by light filtering, singlet oxygen quenching, sensitizer inactivation, and free-radical scavenging [7]. In addition to tocopherols, lignans are important antioxidants in sesame oils, which is well known to possess high stability against oxidation, even though it contains high level of unsaturation [46].

Non-refined oils have a better stability at elevated temperature than refined oils [30] as the refining can lead to reduction in the natural antioxidants. Szydłowska-Czerniak and Łaszewska [48] reported that the refining process of rapeseed oils decreased the antioxidant capacity by about 60% and total phenolic content by above 80%.

5 Oxidative Stability of Important Edible Plant Oils

Edible plant oils can be categorized into three groups based on their fatty acid composition such as oils containing high levels of saturated fatty acids (lauric acid, myristic acid, palmitic acid, and stearic acid), oils with high levels of monounsaturated fatty acids (oleic acid), and oils containing high amount of polyunsaturated fatty acids (linoleic and linolenic acid). Examples for first category include coconut oil and palm kernel oil. Examples for oils with high levels of monounsaturated fatty acid include olive, canola, rapeseed, peanut, hazelnut, avocado, and sesame oils. Corn, soybean, sunflower, cottonseed, grape seed, and safflower are examples for the third category [49]. Table 1 shows the range of fatty acid found in important plant oils.

5.1 Coconut Oil

Coconut (*Cocos nucifera*) oil remains an important edible oil for the food industry for many years. It contains more than 90% of saturated fatty acids [51]. Coconut oil being a highly saturated oil is extremely stable against oxidation, therefore suitable for frying [52]. Lauric acid is the major saturate fatty acid present in coconut oil. The generation of *trans*-fatty acids is also very minimal during frying operations.

5.2 Palm Oil and Palm Kernel Oil

Palm oil and palm kernel oil are obtained from oil palm (*Elaeis guineensis*). In addition to palm oil and palm kernel oil, their fractions are also produced globally to be used for edible purposes. Palm oil is edible oil derived from the fleshy mesocarp of the oil palm fruit, and palm kernel oil is derived from the kernel of the fruit of the oil palm. Palm oil contains almost equal portions of saturated and unsaturated fatty acids, mainly as palmitic acid (42–47%) and oleic acid (37–41%). Palm oil is highly stable against oxidation [33, 53]. Palm kernel oil contains high amount of lauric acid (45–55%), thus known as lauric oil. Fractionation of palm oil into palm stearin and palm olein and palm kernel oil into palm kernel stearin and palm kernel olein further enhances their applications in foods with different stabilities [54].

Table 1 Fatty acid composition of important edible plant oils

Fatty acid	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3
Coconut oil	ND-0.7	4.6–10.0	5.0–8.0	45.1–53.2	16.8–21.0	7.5–10.2	ND	ND	ND	2.0–4.0	5.0–10.0	1.0–2.5	ND-0.2
Palm oil	ND	ND	ND	ND-0.5	0.5–2.0	39.3–47.5	ND-0.6	ND-0.2	ND	3.5–6.0	36.0–44.0	9.0–12.0	ND-0.5
Palm kernel oil	ND-0.8	2.4–6.2	2.6–5.0	45.0–55.0	14.0–18.0	6.5–10.0	ND-0.2	ND	ND	1.0–3.0	12.0–19.0	1.0–3.5	ND-0.2
Palm olein	ND	ND	ND	0.1–0.5	0.5–1.5	38.0–43.5	ND-0.6	ND-0.2	ND-0.1	3.5–5.0	39.8–46.0	10.0–13.5	ND-0.6
Palm kernel olein	ND-0.7	2.9–6.3	2.7–4.5	39.7–47.0	11.5–15.5	6.2–10.6	ND-0.1	ND	ND	1.7–3.0	14.4–24.6	2.4–4.3	ND-0.3
Palm kernel stearin	ND-0.2	1.3–3.0	2.4–3.3	52.0–59.7	20.0–25.0	6.7–10.0	ND	ND	ND	1.0–3.0	4.1–8.0	0.5–1.5	ND-0.1
Palm stearin	ND	ND	ND	0.1–0.5	1.0–2.0	48.0–74.0	ND-0.2	ND-0.2	ND-0.1	3.9–6.0	15.5–36.0	3.0–10.0	ND-0.5
Palm superolein	ND	ND	ND	0.1–0.5	0.5–1.5	30.0–39.0	ND-0.5	ND-0.1	ND	2.8–4.5	43.0–49.5	10.5–15.0	0.2–1.0
Rapeseed oil	ND	ND	ND	ND	ND-0.2	1.5–6.0	ND-3.0	ND-0.1	ND-0.1	0.5–3.1	8.0–60.0	11.0–23.0	5.0–13.0
Rapeseed oil (low erucic acid)	ND	ND	ND	ND	ND-0.2	2.5–7.0	ND-0.6	ND-0.3	ND-0.3	0.8–3.0	51.0–70.0	15.0–30.0	5.0–14.0
Safflower seed oil	ND	ND	ND	ND	ND-0.2	5.3–8.0	ND-0.2	ND-0.1	ND-0.1	1.9–2.9	8.4–21.3	67.8–83.2	ND-0.1
Safflower seed oil (high-oleic acid)	ND	ND	ND	ND-0.2	ND-0.2	3.6–6.0	ND-0.2	ND-0.1	ND-0.1	1.5–2.4	70.0–83.7	9.0–19.9	ND-1.2
Sesame seed oil	ND	ND	ND	ND	ND-0.1	7.9–12.0	ND-0.2	ND-0.2	ND-0.1	4.5–6.7	34.4–45.5	36.9–47.9	0.2–1.0
Soybean oil	ND	ND	ND	ND-0.1	ND-0.2	8.0–13.5	ND-0.2	ND-0.1	ND-0.1	2.0–5.4	17–30	48.0–59.0	4.5–11.0
Sunflower seed oil	ND	ND	ND	ND-0.1	ND-0.2	5.0–7.6	ND-0.3	ND-0.2	ND-0.1	2.7–6.5	14.0–39.4	48.3–74.0	ND-0.3
Sunflower seed oil (high-oleic acid)	ND	ND	ND	ND	ND-0.1	2.6–5.0	ND-0.1	ND-0.1	ND-0.1	2.9–6.2	75–90.7	2.1–17	ND-0.3
Sunflower seed oil (mid-oleic acid)	ND	ND	ND	ND	ND-1	4.0–5.5	ND-0.05	ND-0.05	ND-0.06	2.1–5.0	43.1–71.8	18.7–45.3	ND-0.5

Source: Codex standard for named vegetable oils (CODEX-STAN 210 – 1999) [50]
ND Non-detectable

5.3 Olive Oil

Olive (*Olea europaea*) oil is best suited for food applications involving high temperatures such as frying. Fatty acid composition of the oils determines the suitability of oils for various applications. In this context, the fatty acid composition of the olive oil complies with the criteria of the stable healthy frying oils, that is, olive oil contains high ratio of monounsaturated-to-polyunsaturated fatty acid, low in saturated and polyunsaturated fatty acids, and most importantly very low in linolenic acid [35, 55].

Further, olive oil is considered to be a good-quality frying oil owing to its relatively low melting point; thus, the oil can drain easily from the fried food resulting in low content of trapped oil in the fried food [56].

Virgin olive oil has a higher resistance to oxidative deterioration compared to refined oils because of the presence of phenolic antioxidants such as polyphenols and tocopherols and low polyunsaturation. Polyphenols are eliminated or reduced drastically during the refining process [35].

5.4 Canola (Rapeseed) Oil

The edible canola/rapeseed oil is obtained from low erucic and low glucosinolate species such as *Brassica campestris*, *Brassica napus*, and *Brassica juncea*. Canola oil carries excellent nutritional value because of its unique fatty acid composition with a high amount of oleic acid (50–66%) and also contains linoleic acid (18–30%) and linolenic acid (8–12%) [33, 57]. However, canola oil has less oxidative stability because of the high amount of polyunsaturated fatty acids. Thus, canola oil is subjected to various modifications to improve their oxidative stability such as hydrogenation, fractionation, interesterification, and physical blending [57].

5.5 Soybean Oil

Soybean (*Glycine max* L.) oil is an important plant oil in terms of nutritional quality attributed to its high content of polyunsaturated fatty acids (n−6 and n−3) and tocopherols; however, it is highly susceptible to oxidation [58]. Soybean oil is mainly composed of polyunsaturated fatty acids such as linoleic acid (n−6) (56%) and α-linolenic acid (n−3) (8%) which are considered essential fatty acids as they are necessary for growth and development of the human body. They act as precursors of prostaglandins and hormones which play an important role in the regulation of some physiological and biochemical functions of the human body [58]. However the high amount of linolenic acid reduces oxidative stability of the oil, leading to decreased shelf life [59].

Soybean oil contains relatively high amount of tocopherol compared to other major polyunsaturated oils such as rapeseed, corn, and sunflower [60]. Research

studies have shown that refined soybean oil is highly susceptible to oxidation because of its high content of unsaturated fatty acids and the absence or less amount of natural minor compounds with antioxidant effect [58].

5.6 Sesame Oil

Sesame (*Sesamum indicum*) oil contains mainly monounsaturated fatty acids (45–49%) and polyunsaturated fatty acid (37–41%). It contains thermally stable natural antioxidants as lignans; however, its high content of polyunsaturated fatty acids makes sesame oil highly prone to autoxidation [51].

5.7 Sunflower Oil

Sunflower (*Helianthus annuus* L.) oil contains linoleic (48–74%) and oleic acid (14–39%) as the major fatty acids [33]. High content of unsaturation makes the sunflower oil highly susceptible for autoxidation and less suitable for deep frying. As explained later in this chapter, new phenotypes with modified fatty acid composition, mainly with increased oleic acid content, have been developed through genetic and breeding techniques in order to make the sunflower oil suitable for thermal applications such as deep frying.

6 Improving Oxidative Stability of Oils

6.1 The Use of Antioxidants

Antioxidants are substances that when introduced into substrate at low concentration compared to that of an oxidizable substrate significantly inhibit oxidation of that substrate by inhibiting formation of free radicals or by interrupting propagation of the free radical [61, 62]. Addition of antioxidant during oil processing is one of the most effective means to retard fat oxidation. Antioxidants are of two types based on mechanism of action: primary antioxidants and secondary antioxidants. This can be further classified into natural and synthetic.

6.1.1 Primary Antioxidants

Primary antioxidants are chain-breaking antioxidants, that is, they are capable of neutralizing lipid free radicals by stopping their radical state by donating hydrogen. Butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylhydroquinone (TBHQ), tocopherols, and flavonoids are examples for primary antioxidants [62, 63].

The reaction mechanism can be explained schematically as follows (antioxidant molecule is denoted by AH).

$ROO\bullet + AH \rightarrow ROOH + A\bullet$
$R\bullet + AH \rightarrow RH + A\bullet$
$RO\bullet + AH \rightarrow ROH + A\bullet$
$ROO\bullet + A\bullet \rightarrow ROOA$
$RO\bullet + A\bullet \rightarrow ROA$
$A\bullet + A\bullet \rightarrow A - A$

Some primary antioxidants such as propyl gallate, proanthocyanidins, and ascorbic acid act as antioxidants via more than one mechanisms such as free-radical scavenging, oxygen sequestering, metal chelation, and light energy absorption [64].

6.1.2 Secondary Antioxidants

The secondary antioxidants (preventive antioxidant) retard the rate of oxidation through the removal of the substrate or singlet oxygen quenching mechanism [65–67]. Mechanisms of secondary antioxidants include metal chelating, singlet oxygen quenching and inactivation of photosensitizers and lipoxygenase [7]. Metals act as prooxidants by reducing the activation energy of the oxidation, especially in the initiation step, to accelerate oil oxidation. Metal chelators form insoluble metal complexes or provide steric hindrance between metals and food components. Examples include citric acid, EDTA, polyphenols, lignans, and ascorbic acid [7, 45, 64].

The mechanism of action of singlet oxygen quenchers involves deactivation of singlet oxygen to the ground-state triplet oxygen or getting oxidized themselves by singlet oxygen [7, 68]. Tocopherols, carotenoids, phenolics, and ascorbic acid retard oxidation of lipids through quenching singlet oxygen [69]. Photosensitized compounds such as chlorophyll and riboflavin transfer the energy to triplet oxygen to form singlet oxygen, or transfer an electron to the triplet oxygen to form a superoxide anion radical, which react with lipid to produce free radicals [7]. Energy of the photosensitizers is transferred to the singlet state of antioxidant to become a triplet state of antioxidant, which is changed to singlet state by transferring the energy to the surrounding or emitting phosphorescence. Carotenoids retard lipid oxidation through inactivating photosensitizers [70].

Synergism

Addition of combinations of primary and secondary antioxidants is often reported to have synergistic effect, that is, combined action is more effective to retard lipid oxidation than the sum of their single effect and increases the length of induction period [71, 72].

6.1.3 Natural and Synthetic Antioxidants

Natural antioxidants are found in several plant sources such as grain, seeds, cereals, nuts, fruits, vegetables, and spices [73]. Plant extracts contain various antioxidants such as flavonoids (quercetin, kaempferol, myricetin), catechins or phenols (carnosol, rosmanol, rosamaridiphenol), and phenolic acids (carnosic acid, rosmarinic acid) [74–76]. Tocols are the natural antioxidants found in plant-based oils, which include four tocopherol and four tocotrienol isomers, each designated as

alpha, beta, gamma, and delta on the basis of the chromanol ring. The α-tocopherol has been reported to be the most active isomer biologically, whereas the γ-tocopherol is perceived as the best antioxidant [47]. The vitamin E, most importantly α-tocopherol, is the well-known antioxidant found in vegetable oil. They are known to give protection against peroxidation of polyunsaturated fatty acids [77]. Among all types of tocopherols, α-tocopherol is the most unstable, thus easily destroyed at high temperatures [31]. Juárez and others [44] reported that there were significant losses of α-tocopherol during frying because α-tocopherol degrades more quickly at high temperatures than at room temperature [78].

Vegetable oils containing high amounts of unsaturated fatty acids such as soybean, sunflower, sesame, corn, and peanut oils are very unstable for continuous frying due to their content of polyunsaturated fatty acids. However, the presence of natural substances such as tocopherols, oryzanol, sterol fraction, squalene, among others enhances their stability at higher temperatures [30].

Several studies attempted to study the effect of addition of synthetic antioxidants to the edible plant oils. Azeez and others [67] investigated the effect of direct incorporation of TBHQ, BHT, and mixed (TBHQ and BHT) on the oxidative stability of palm olein, soybean oil, and linseed oil at room temperature and 70 °C for 168 h and reported that TBHQ had significant effect on the oxidative stability of palm olein at 70 °C, while TBHQ and BHT had synergetic effect on stability of soybean oil at room temperature and Linseed oil at 70 °C.

Gertz and others [30] investigated the efficacy of some antioxidants on stability of refined sunflower and rapeseed oils and reported that α-tocopherol, tocopherol esters, and BHA exhibit low antioxidant effects at frying temperature, while ascorbic acid 6-palmitate and some phytosterol fractions were found to possess the greatest antioxidant activity.

Synthetic antioxidants, such as BHT, BHA, and TBHQ, are very effective in edible plant oils to protect against oxidation and, thus, widely used in many oils. However, recently, their use has been discouraged following findings related to possible toxicity and carcinogenicity of these synthetic antioxidants as evidenced by animal studies [79]. For example, BHA and BHT have been shown to possess carcinogenic activity in rodents [80–82]. This has drawn considerable attention to the use of natural antioxidants from plant sources as replacement for synthetic antioxidants for improving the oxidative stability of oils [58, 74].

During the past two decades, research studies have been focused extensively toward the use of natural plant extracts as sources of antioxidants to replace the synthetic antioxidants [79]. In recent years, tendency toward the use of agro-industrial by-products as sources of antioxidant is increasing [73, 74, 83, 84]. Compared to synthetic antioxidants, natural plant extracts have been shown to exhibit higher antioxidant activity and thermal stability which are the most important criteria for an antioxidant to be used for fats and oils [62, 79]. Some examples for such sources include α-tocopherol, pomegranate peel [85, 86], green tea [87], olive waste [88], sesame cake [74], sesame seed [89], rosemary (*Rosmarinus officinalis* L.) [85, 90], *Eucalyptus citriodora* leaf [91], celery [92], oregano (*Origanum vulgare*) [85], cinnamon, and other spices and herbs [62]. In recent years, ascorbyl palmitate is

gaining popularity to be used in edible oils. Ascorbyl palmitate is "generally recognized as safe" (GRAS) according to the Food Drug Administration without limitation on levels to be used in food [70].

Antioxidative effect of α-tocopherol (vitamin E), fat-soluble carotenoid, has been extensively studied [62]. Hraš et al. [93] studied the antioxidative activities of four natural antioxidants such as rosemary extract, α-tocopherol, ascorbyl palmitate, and citric acid in sunflower oil stored at 60 °C. Among them, rosemary extract had the best antioxidative activity. Rosemary extract exhibited additive antioxidative effect when combined with citric acid and ascorbyl palmitate. Further they have reported that α-tocopherol exhibited prooxidative effect. Choe and Min [7] explained that at high concentration, tocopherol radical may abstract hydrogen from lipids with very low concentration of peroxy radical and produces tocopherol and lipid radical, which may increase the lipid oxidation; thus, tocopherol act as prooxidant instead of antioxidant. Thus, the natural antioxidants may not be always preventive against oxidation [94].

Abdelazim and others [74] found that sesame cake extract possesses stronger antioxidant activity than BHT and BHA, however less than that of TBHQ. Hassanien and Abdel-Razek [95] have found that addition of roasted sesame seed as a source of natural antioxidant can improve the stability of edible oils. Sesame seeds contain a myriad of natural antioxidant components including lignans such as sesamin, sesamolin, and sesaminol. In addition to enhancing the stability of oils against oxidation, these compounds play a vital role in maintaining good health. Green tea extracts at higher concentrations than 200 ppm showed excellent antioxidant activity in both oils, and its efficacy was higher than that of BHA, BHT, and α-tocopherol but less than that of TBHQ. Sayyad et al. [90] studied the effect of rosemary extract on thermoxidative stability of soybean oil and reported the higher stability of the soybean oil added with rosemary extract (3000 ppm) than the oil added with TBHQ (50 ppm).

Gertz et al. [30] reported that natural substances such as squalene, sterol fraction, quercetin, oryzanol, and ferulic acid are more efficient than synthetic antioxidants to enhance the stability of vegetable oils at higher temperatures. Further, ascorbyl palmitate is more efficient than BHA or α-tocopherols to increase the stability of vegetable oils during frying at high temperature [30]. Alavi and Golmakani [66] reported that spirulina can improve the oxidative stability of olive oil.

Recently, a study has been reported on the antioxidant activity of encapsulated olive leaf extract in soybean oil. And their results indicated that nano-encapsulation of olive leaf could be a suitable novel technique to improve the antioxidant activity of natural sources [96].

6.2 Modification of the Fatty Acid Composition

Modification of fatty acid composition through natural plant breeding or genetic modification is another way of improving the oxidative stability of vegetable oils [49]. Refined edible oils such as soybean, rapeseed, sunflower, or peanut oils have

high contents of polyunsaturated fatty acids, linoleic, and linolenic acids; thus, they are not suitable for repeated deep fat frying. Even though palm kernel and coconut oils are more stable, their high content of saturated fatty acid limits their applications [97]. Genetic and breeding techniques are employed mainly to alter the composition of saturates, oleic acid, and linolenic acid. High and mid-oleic acid content can increase the oxidative stability at high cooking temperatures [98], especially during deep frying. In recent years, high and mid-oleic acid oil crops developed through breeding techniques by private companies are commercially available [49, 99]. Some examples are Nexera™ (Omega-9 canola and Omega-9 sunflower oils), Plenish™ high-oleic soybeans by E. I. du Pont de Nemours (Wilmington, DE, USA), and Vistive-Gold™ low-saturated high-oleic soybeans by Monsanto Co. (St. Louis, MO, USA) [49, 100, 101]. Some examples for oil crops developed by genetic means to contain higher oleic acid levels than normal include soybean (from 24% to 84%), palm (from 36% to 59%), canola (from 57% to 89%), sunflower (from 29% to 84%), peanut (from 55% to 76%), cottonseed (from 13% to 78%), and safflower (from 10% to 81%) [49]. Soybean phenotypes with linolenic acid less than 4% (low linolenic) and less than 2% (ultralow linolenic acid) have been developed through mutation [59].

High content of linolenic acid is the important factor responsible for the poor oxidative stability of some oils such as soybean oil. Partial hydrogenation of highly unsaturated oils can increase the oxidative stability significantly. However, the use of partially hydrogenated oils is discouraged because partial hydrogenation leads to formation of *trans* fats. Therefore, modification of fatty acid composition by breeding and genetic methods is effective [49]. New phenotypes of soybean, sunflower, corn, safflower, and rapeseed have been produced to have improved oxidative stability as well as nutritional value. High-oleic peanut oil developed through breeding has much greater autoxidation stability as compared to normal oleic peanut oil [102].

6.3 Blending

Blended oils are prepared by blending of different oils together. Blending is a way of modifying the fatty acid composition, physicochemical properties, and functional properties of edible oils without changing their chemical composition [89].

Okogeri [103] studied the frying stability of peanut oil blended with palm kernel oil at different ratios (90:10, 80:20, 70:30 and 60:40) and reported that all blends after used for frying contained less polar compounds than control.

6.4 Modifications in Oil Processing

The heat applied during the conventional oil processing technique accounts for the major loss of natural antioxidants present in edible oils. Cold-pressing oils may help retain higher levels of natural antioxidants to have acceptable shelf life

without added synthetic antioxidants [95]. Oils obtained by cold pressing are known as virgin oils, which are very popular due to their typical color, taste, and flavor. Since there is no heat treatment, most of the natural components are present in the oil [104]. It is reported that cold-pressed olive oil has a stronger antioxidant activity attributed to the presence of natural phenolic compounds [89]. Wroniak et al. [105] have reported that oil flushing with nitrogen was a very effective way to reduce the changes caused by oxidation in cold-pressed rapeseed and sunflower oil.

7 Conclusion

Autoxidation and photosensitized oxidation are the main deteriorative processes that occur in edible plant oils during processing and storage. The oxidation of edible oil leads to the production of off-flavor and toxic compounds and diminishes the oil quality and shelf life. Oxidative stability of edible plant oils is thus the determining factor of the selection of suitable oil for different processing and storage methods. Oxidative stability of oils differs depending on fatty acid composition, the presence of minor components (antioxidants or prooxidants), and the processing or storage conditions, mainly, temperature, light, and oxygen. Oxidative stability of the oils can be improved by modifying the processing conditions such as the use of low temperature, exclusion of light and oxygen, removal of prooxidants, and the use of antioxidants.

References

1. Fasina OO, Craig-Schmidt M, Colley Z, Hallman H (2008) Predicting melting characteristics of vegetable oils from fatty acid composition. LWT Food Sci Technol 41(8):1501–1505. https://doi.org/10.1016/j.lwt.2007.09.012
2. USDA (2018) Oil seeds: world markets and trade. United States Department of Agriculture. https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf. Accessed 9 Mar 2018
3. Syed A (2016) Oxidative stability and shelf life of vegetable oils. In: Hu M, Jacobsen C (eds) Oxidative stability and shelf life of foods containing oils and fats. AOCS Press, pp 187–207. https://doi.org/10.1016/B978-1-63067-056-6.00004-5
4. Leong XF, Ng CY, Jaarin K, Mustafa MR (2015) Effects of repeated heating of cooking oils on antioxidant content and endothelial function. Austin J Pharmacol Ther 3(2):1068
5. Guillen MD, Cabo N (2002) Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. Food Chem 77(4):503–510
6. Hamilton RJ (1994) The chemistry of rancidity in foods. In: Allen JC, Hamilton RJ (eds) Rancidity in foods, 3rd edn. Blackie Academic & Professional, London, pp 1–21
7. Choe E, Min DB (2006) Mechanisms and factors for edible oil oxidation. Compr Rev Food Sci Food Saf 5:169–186
8. Adrian LK, Ronald BP, Anwesha S, Brian DC (2015) Update on the methods for monitoring UFA oxidation in food products. Eur J Lipid Sci Technol 117(1):1–14
9. Bruheim I (2009) Solid-phase microextraction (SPME) in the fish oil industry. LC GC Europe 22(3):126–130

10. Ghnimi S, Budilarto E, Kamal-Eldin A (2017) The new paradigm for lipid oxidation and insights to microencapsulation of Omega-3 fatty acids. Compr Rev Food Sci Food Saf 16:1206–1218. https://doi.org/10.1111/1541-4337.12300
11. Lee J, Koo N, Min DB (2004) Reactive oxygen species, aging, and antioxidative nutraceuticals. Compr Rev Food Sci Food Saf 3(1):21–33
12. Velasco J, Andersen ML, Skibsted LH (2003) Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the Rancimat method and differential scanning calorimetry. Food Chem 77:623–632
13. W¹sowicz E, Gramza A, Hêœ M, Jeleñ HH, Korczak J, Maecka M, Mildner-Szkudlarz S, Rudziñska M, Samotyja U, Zawirska-Wojtasiak R (2004) Oxidation of lipids in foods. Pol J Food Nutr Sci 13(54):87–100
14. Tirosh O, Shpaizer A, Kanner J (2015) Lipid peroxidation in a stomach medium is affected by dietary oils (olive/fish) and antioxidants: the Mediterranean versus Western diet. J Agric Food Chem 63(31):7016–7023
15. Ahmed M, Pickova J, Ahmad T, Liaquat M, Farid A, Jahangir M (2016) Oxidation of lipids in foods. Sarhad J Agric 32(3):230–238
16. Fennema OR (1996) Food chemistry, 3rd edn. Marcel Decker, Inc., New York
17. Porter NA (2013) A perspective on free radical autoxidation: the physical organic chemistry of polyunsaturated fatty acid and sterol peroxidation. J Organomet Chem 78(8):3511–3524
18. Song W, Bardowell S, O'Shea K (2007) Mechanistic study and the influence of oxygen on the photosensitized transformations of microcystins (cyanotoxins). Environ Sci Technol 41(15):5336–5341
19. Galano J-M, Lee YY, Durand T, Lee JC-Y (2015) Special issue on "analytical methods for oxidized biomolecules and antioxidants" the use of isoprostanoids as biomarkers of oxidative damage, and their role in human dietary intervention studies. Free Radic Res 49(5):583–598
20. Gordon MH (2001) The development of oxidative rancidity. In: Pokorny J, Yanishlieva N, Gordon M (eds) Antioxidants in food – practical applications. CRC Press, Washington, pp 7–22
21. Tayeb AH, Sadeghifar H, Hubbe MA, Rojas OJ (2017) Lipoxygenase-mediated peroxidation of model plant extractives. Ind Crop Prod 104:253–262
22. Wang T, Hammond EG (2010) Lipoxygenase and lipid oxidation in foods. In: Decker EA, Elias RJ, McClements DJ (eds) Oxidation in foods and beverages and antioxidant applications. Woodhead Publishing Limited, pp 105–121. https://doi.org/10.1533/9780857090447.1.105
23. Böttcher S, Steinhäuser U, Drusch S (2015) Off-flavour masking of secondary lipid oxidation products by pea dextrin. Food Chem 169(1):492–498
24. Julia K, Caroline C, Corinne B, Marizel AG, Ana-Paulina B, Michel R, dSSD M, Robert S, Anne NS, Françoise G (2015) Antiatherogenic and antitumoral properties of Opuntia cladodes: inhibition of low density lipoprotein oxidation by vascular cells, and protection against the cytotoxicity of lipid oxidation product 4-hydroxynonenal in a colorectal cancer cellular model. J Physiol Biochem 71(3):557–587
25. Olpin SE (2005) Fatty acid oxidation defect as a cause of neuromypathic disease in infants and adults. Clin Lab 51(5–6):289–306
26. McIntyre TM, Hazen SL (2010) Lipid oxidation and cardiovascular disease: introduction to a review series. Circ Res 107:1167–1169
27. Li H, Fan Y-W, Jing L, Tang L, Hu J-N, Deng Z (2013) Evaluating and predicting the oxidative stability of vegetable oils with different fatty acid compositions. J Food Sci 78(4):H633–H641. https://doi.org/10.1111/1750-3841.12089
28. Parker TD, Adams DA, Zhou K, Harris M, Yu L (2003) Fatty acid composition and oxidative stability of cold-pressed edible seed oils. J Food Sci 68:1240–1243
29. Martín-Polvillo M, Márquez-Ruiz G, Dobarganes M (2004) Oxidative stability of sunflower oils differing in unsaturation degree during long-term storage at RT. J Am Oil Chem Soc 81(6):577–583. https://doi.org/10.1007/s11746-006-0944-1

30. Gertz C, Klostermann S, Kochhar SP (2000) Testing and comparing oxidative stability of vegetable oils and fats at frying temperature. Eur J Lipid Sci Technol 102(8–9):543–551
31. Xu T, Li J, Fan Y, Zheng T, Deng Z (2015) Comparison of oxidative stability among edible oils under continuous frying conditions. Int J Food Prop 18(7):1478–1490. https://doi.org/10.1080/10942912.2014.913181
32. Wahrburg U (2004) What are the health effects of fat? Eur J Nutr 43:i6–i11
33. O'Brien RD (2004) Fats and oils, 2nd edn. CRC Press, Boca Raton
34. Ayyildiz HF, Topkafa M, Kara H, Sherazi STH (2015) Evaluation of fatty acid composition, Tocols profile, and oxidative stability of some fully refined edible oils. Int J Food Prop 18 (9):2064–2076. https://doi.org/10.1080/10942912.2014.962657
35. Velasco J, Dobarganes C (2002) Oxidative stability of virgin olive oil. Eur J Lipid Sci Technol 104:661–676
36. Dobarganes MC (1998) Formation and analysis of high molecular-weight compounds in frying fats and oil. OCL 5:41–47
37. Azeredo HMC, Faria JAF, Silva MAAP (2003) The efficiency of TBHQ, β-carotene, citiric acid, and tinuvin 234 on the sensory stability of soybean oil packaged in PET bottles. J Food Sci 68:302–306
38. Pascall MA, Harte BR, Giacin JR, Gray JI (1995) Decreasing lipid oxidation in soybean oil by a UV-absorber in the packaging material. J Food Sci 60:1116–1119
39. Marinova EM, Seizova KA, Totseva IR, Panayotova SS, Marekov IN, Momchilova SM (2012) Oxidative changes in some vegetable oils during heating at frying temperature. Bulg Chem Commun 44(1):57–63
40. Marquez-Ruiz G, Dobarganes MC (2007) Nutritional and physiological effects of used frying oil and fats. In: Erickson MD (ed) Deep frying: chemistry, nutrition and practical application. AOCS Press, Urbana, pp 173–203
41. Choe E, Min DB (2007) Chemistry of deep-fat frying oils. J Food Sci 72(5):R77–R86
42. Karakaya S, Simsek S (2011) Changes in total polar compounds, peroxide value, total phenols, and antioxidant activity of various oils used in deep fat frying. J Am Oil Chem Soc 88:1361–1366
43. Casal S, Malheiro R, Sendas A, Oliveira BPP, Pereira JA (2010) Olive oil stability under deep-frying conditions. Food Chem Toxicol 48:2972–2979
44. Juárez MD, Osawa CC, Acuña ME, Sammán N, Gonçalves LAG (2010) Degradation in soybean oil, sunflower oil, and partially hydrogenated fats after food frying, monitored by conventional and unconventional methods. Food Control 22:1920–1927
45. Andersson K, Lingnert H (1998) Influence of oxygen and copper concentration on lipid oxidation in rapeseed oil. J Am Oil Chem Soc 75:1041. https://doi.org/10.1007/s11746-998-0284-4
46. Tan CP, Che Man YB, Selamat J, Yusoff MSA (2002) Comparative studies of oxidative stability of edible oils by differential scanning calorimetry and oxidative stability index methods. Food Chem 76(3):385–389. https://doi.org/10.1016/S0308-8146(01)00272-2
47. O'Brien RD (2009) Fats and oils: formulating and processing for applications, 3rd edn. CRC Press, New York
48. Szydłowska-Czerniak A, Łaszewska A (2015) Effect of refining process on antioxidant capacity, total phenolics and prooxidants contents in rapeseed oils. LWT-Food Sci Technol 64(2):853–859
49. Wilson RF (2012) The role of genomics and biotechnology in achieving global food security for high-oleic vegetable oil. J Oleo Sci 61(7):357–367
50. Alimentarius C (2015) Codex standards for named vegetable oils (CODEX-STAN 210–1999, Amendment: 2005, 2011, 2013 and 2015)
51. Khan MI, Asha MR, Bhat KK, Khatoon S (2011) Studies on chemical and sensory parameters of coconut oil and its olein blends with sesame oil and palmolein during wheat flour-based product frying. J Food Sci Technol 48(2):175–182. https://doi.org/10.1007/s13197-010-0145-7
52. Eyres L, Eyres MF, Chisholm A, Brown RC (2016) Coconut oil consumption and cardiovascular risk factors in humans. Nutr Rev 74(4):267–280. https://doi.org/10.1093/nutrit/nuw002

53. Koushki M, Nahidi M, Cheraghali F (2015) Physico-chemical properties, fatty acid profile and nutrition in palm oil. J Paramed Sci 6(3):117–134
54. Mat Dian NL, Hamid RA, Kanagaratnam S, Isa WRA, Hassim NAMH, NIsmail NH, Omar Z, Sahri MM (2017) Palm oil and palm kernel oil: versatile ingredients for food applications. J Oil Palm Res 29(4):487–511. https://doi.org/10.21894/jopr.2017.00014
55. Kochhar SP (2000) Stable and healthful oils for the 21st century. Inform 11:642–647
56. Rossel JB (2001) Factors affecting the quality of frying oils and fats. In: Rossel JB (ed) Frying: improving quality. Woodhead Publishing Ltd., Cambridge, pp 115–164
57. Aniołowska M, Zahran H, Kita A (2016) The effect of pan frying on thermooxidative stability of refined rapeseed oil and professional blend. J Food Sci Technol 53(1):712–720. https://doi.org/10.1007/s13197-015-2020-z
58. Medina-Juárez LA, Gámez-Meza N (2011) Effect of refining process and use of natural antioxidants on soybean oil. In: Ng T (ed) Soybean – biochemistry, chemistry and physiology. InTech, pp 435–460
59. Clemente TE, Cahoon EB (2009) Soybean oil: genetic approaches for modification of functionality and total content. Plant Physiol 151:1030–1040
60. Kellens M (1997) Current developments in oil refining technology. Technical report, Belgium
61. Halliwell B, Gutteridge JC (1995) The definition and measurement of antioxidants in biological systems. Free Radic Biol Med 18(1):125–126. https://doi.org/10.1016/0891-5849(95)91457-3
62. Brewer MS (2011) Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Compr Rev Food Sci Food Saf 10:221–247. https://doi.org/10.1111/j.1541-4337.2011.00156.x
63. Budilarto-Poulin E, Kamal-Eldin A (2015) The supramolecular chemistry of lipid oxidation and antioxidation in bulk oils. Eur J Lipid Sci Technol 117(8):1095–1137. https://doi.org/10.1002/ejlt.201400200
64. Chaiyasit W, Elias RJ, McClements DJ, Decker EA (2007) Role of physical structures in bulk oils on lipid oxidation. Crit Rev Food Sci Nutr 47(3):299–317. https://doi.org/10.1080/10408390600754248
65. Azizkhani M, Zandi P (2009) Effects of some natural antioxidants mixtures on margarine stability. World Acad Sci Eng Technol 49:93–96
66. Müller WW, Jakob I, Li C, Tatzky-Gerth R (2009) Antioxidant depletion and OIT values of high impact PP strands. Chin J Polym Sci 27(3):435–445
67. Azeez OT, Ejeta KO, Frank EO, Gerald NE (2013) Effects of antioxidants on the oxidative stability of vegetable oil at elevated temperature. Int J Appl Sci Technol 3(5):107–115
68. Halliwell B, Gutteridge JMC (2001) Free radicals in biology and medicine, 3rd edn. Oxford Univ Press Inc, New York
69. Choe E, Min DB (2005) Chemistry and reactions of reactive oxygen species in foods. J Food Sci 70(9):142–159. https://doi.org/10.1111/j.1365-2621.2005.tb08329.x
70. Stahl W, Sies H (1993) Physical quenching of singlet oxygen and cis-trans isomerization of carotenoids. Ann N Y Acad Sci 691:10–19. https://doi.org/10.1111/j.1749-6632.1993.tb26153.x
71. Marinova E, Toneva A, Yanishlieva N (2008) Synergistic antioxidant effect of α-tocopherol and myricetin on the autoxidation of triacylglycerols of sunflower oil. Food Chem 106(2):628–633. https://doi.org/10.1016/j.foodchem.2007.06.022
72. Haj Hamdo H, Khayata W, Al-Assaf Z (2014) Synergistic effect of combined some natural and synthetic antioxidants to increase oxidative stability using DPPH test. Int J ChemTech Res 6(4):2539–2545
73. Ribeiro MDMM, Ming CC, Silvestre IM, Grimaldi R, Gonçalves LG (2017) Comparison between enzymatic and chemical interesterification of high oleic sunflower oil and fully hydrogenated soybean oil. Eur J Lipid Sci Technol 119(2)
74. Abdelazim AA, Mahmoud A, Ramadan-Hassanien MF (2013) Oxidative stability of vegetable oils as affected by sesame extracts during accelerated oxidative storage. J Food Sci Technol 50(5). https://doi.org/10.1007/s13197-011-0419-8

75. Jeong SM, Kim SY, Kim DR, Nam KC, Ahn DU, Lee SC (2004) Effect of seed roasting conditions on the antioxidant activity of defatted sesame meal extracts. Food Chem Toxicol 69(5):C377–C381
76. Yanishlieva VN, Marinova E (2001) Stabilisation of edible oils with natural antioxidants. Eur J Lipid Sci Technol 103(11):752–767. https://doi.org/10.1002/1438-9312(200111)103:11<752::AID-EJLT752>3.0.CO;2-0
77. Afaf KE, Andersson R (1997) A multivariate study of the correlation between tocopherol content and fatty acid composition in vegetable oils. J Am Oil Chem Soc 74:375–380
78. Warner K, Su C, White PJ (2004) Role of antioxidants and polymerization inhibitors in protecting frying oils. In: Warner K, Gupta MK, White PJ (eds) Frying technology and practices, 1st edn. AOCS Press, New York, pp 37–49
79. Taghvaei M, Jafari SM (2013) Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. J Food Sci Technol 52(3):1272–1282. https://doi.org/10.1007/s13197-013-1080-1
80. Saito M, Sakagami H, Fujisawa S (2003) Cytotoxicity and apoptosis induction by butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Anticaner Res 23:4693–4701
81. Sarafian TA, Kouyoumjian S, Tashkin D, Roth MD (2002) Synergistic cytotoxicity of 9-tetrahydrocannabinol and butylated hydroxyanisole. Toxicol Lett 133(2):171–179. https://doi.org/10.1016/S0378-4274(02)00134-0
82. Farag RS, El-Baroty GS, Basuny A (2003) Safety evaluation of olive phenolic compounds as natural antioxidants. Int J Food Sci Nutr 54(3):159–174. https://doi.org/10.1080/09637480310001361306
83. Sachindra NM, Airanthi MKWA, Hosokawa M, Miyashita K (2010) Radical scavenging and singlet oxygen quenching activity of extracts from Indian seaweeds. J Food Sci Technol 47:94–99
84. Shui G, Leong LP (2006) Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. Food Chem 97:277–284
85. Hemachandra TP, Jayathilake RRGDK, Madhujith WMT (2017) The effect of Antioxidative extracts on mitigating autoxidation of selected edible oils during deep frying. Trop Agric Res 28(3):247–255
86. Bopitiya D, Madhujith T (2015) Efficacy of pomegranate (*Punica granatum* L.) peel extracts in suppressing oxidation of white coconut oil used for deep frying. Trop Agric Res 25(3):298–306. https://doi.org/10.4038/tar.v25i3.8040
87. Yin J, Becker EM, Andersen ML, Skibsted LH (2012) Green tea extract as food antioxidant. Synergism and antagonism with α-tocopherol in vegetable oils and their colloidal systems. Food Chem 135(4):2195–2202. https://doi.org/10.1016/j.foodchem.2012.07.025
88. Abd-ElGhany ME, Ammar MS, Hegazy AE (2010) Use of olive waste cake extract as a natural antioxidant for improving the stability of heated sunflower oil. World Appl Sci J 11(1):106–113
89. Abdel-Razek AG, El-Shami SM, El-Mallah MH, Hassanien MMM (2011) Blending of virgin olive oil with less stable edible oils to strengthen their antioxidative potencies. Aust J Basic Appl Sci 5(10):312–318
90. Sayyad R, Jafari S, Ghomi M (2017) Thermoxidative stability of soybean oil by natural extracted antioxidants from rosemary (*Rosmarinus officinalis* L.). Int J Food Prop 20(2):436–446. https://doi.org/10.1080/10942912.2016.1166127
91. Ali S, Chatha SAS, Ali Q, Hussain AI, Hussain SM, Perveen R (2016) Oxidative stability of cooking oil blend stabilized with leaf extract of Eucalyptus citriodora. Int J Food Prop 19(7):1556–1565. https://doi.org/10.1080/10942912.2015.1047514
92. Maleki M, Ariaii P, Fallah H (2015) Effects of celery extracts on the oxidative stability of canola oil under thermal condition. J Food Process Preserv 40(3):531–540. https://doi.org/10.1111/jfpp.12632

93. Hraš AR, Hadolin M, Knez Ž, Bauman D (2000) Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chem 71(2):229–233. https://doi.org/10.1016/S0308-8146(00)00161-8
94. Baştürk A, Boran G, Javidipour I (2017) Effects of ascorbyl palmitate and metal ions on oxidation of sunflower oil under accelerated oxidation conditions. J Anim Plant Sci 27(6):2014–2024
95. Hassanein M, Abdel-razek A (2012) Improving the stability of edible oils by blending with roasted sesame seed oil as a source of natural antioxidants. J Appl Sci Res 8(8):4074–4083
96. Mohammadi A, Jafari SM, Esfanjani AF, Akhavan S (2016) Application of nano-encapsulated olive leaf extract in controlling the oxidative stability of soybean oil. Food Chem 190:513–519. https://doi.org/10.1016/j.foodchem.2015.05.115
97. Sakurai H, Yoshihashi T, Nguyen HTT, Pokorný J (2018) A new generation of frying oils. Czech J Food Sci 21(4):145–151. https://doi.org/10.17221/3491-CJFS
98. Lee J-D, Bilyeu KD, Grover Shannon J (2007) Genetics and breeding for modified fatty acid profile in soybean seed oil. J Crop Sci Biotchnol 10(4):201–210
99. Murphy DJ (2014) Using modern plant breeding to improve the nutritional and technological qualities of oil crops. OCL 21(6):D607. https://doi.org/10.1051/ocl/2014038
100. Bellaloui N, Reddy KN, Mengistu A (2015) Drought and heat stress effects on soybean fatty acid composition and oil stability. In: Processing and impact on active components in food. Elsevier Inc., pp 377–384. https://doi.org/10.1016/B978-0-12-404699-3.00045-7
101. Kaushik I, Grewal RB (2017) Trans fatty acids: replacement technologies in food. Adv Res 9(5):1–14. https://doi.org/10.9734/AIR/2017/33297
102. O'Keefe SF, Wiley VA, Knauft DA (1993) Comparison of oxidative stability of high and normaI-oleic peanut oils. J Am Oil Chem Soc 70(5):489–492
103. Okogeri O (2016) Improving the frying stability of peanut oil through blending with palm kernel oil. J Food Res 5(1):82–87. https://doi.org/10.5539/jfr.v5n1p82
104. Matthäus B (2012) Oil technology. In: Gupta SK (ed) Technological innovations in major world oil crops, vol 2. Springer, pp 23–92. https://doi.org/10.1007/978-1-4614-0827-7_2
105. Wroniak M, Florowska A, Rękas A (2016) Acta scientiarum polonorum. Technol Aliment 15(1):79–87. https://doi.org/10.17306/J.AFS.2016.1.8

The Anti-inflammatory Properties of Food Polar Lipids

19

Ronan Lordan, Constantina Nasopoulou, Alexandros Tsoupras, and Ioannis Zabetakis

Contents

Abstract

Cardiovascular diseases (CVD) are the leading cause of death globally. Inflammation is central to the pathology of CVD and is present throughout the atherosclerotic process. Unresolved inflammation can lead to atherosclerosis and the subsequent development of CVD that can cause a major cardiovascular event. Lifestyle and nutrition are modifiable risk factors for the prevention of CVD.

R. Lordan · A. Tsoupras · I. Zabetakis (✉)
Department of Biological Sciences, University of Limerick, Limerick, Ireland
e-mail: Ronan.lordan@ul.ie; Alexandros.tsoupras@ul.ie; Ioannis.zabetakis@ul.ie

C. Nasopoulou
Department of Food Science and Nutrition, School of the Environment, University of the Aegean, Myrina, Lemnos, Greece
e-mail: knasopoulou@aegean.gr

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_95

Research shows that some dietary patterns, such as the Mediterranean diet, are associated with a decreased risk of CVD. Polar lipids, which are found in abundance in foods of the Mediterranean diet, are lipids that possess potent anti-inflammatory and antithrombotic effects against the actions of platelet-activating factor (PAF). PAF is potent phospholipid mediator of inflammation that plays a significant role in all stages of atherosclerosis. Bioactive lipids present in various foods can inhibit the pro-inflammatory activities of PAF via their effects on the PAF/PAF receptor (PAF-R) signaling but also via modulating PAF metabolism toward homeostasis. This chapter reviews the relevant research pertaining to the anti-inflammatory and cardioprotective properties of polar lipids in various foods.

Keywords
Polar lipids · Inflammation · Platelet-activating factor · Cardiovascular disease · Mediterranean diet

Abbreviations

CHD	Coronary heart disease
CRP	C-Reactive protein
CVD	Cardiovascular disease
FA	Fatty acids
HDL-C	High-density lipoprotein cholesterol
IHD	Ischemic heart disease
IL-6	Interleukin-6
LDL-C	Low-density lipoprotein cholesterol
Lyso-PAF AT	Lyso-PAF acetyltransferases
MFGM	Milk fat globule membrane
MI	Myocardial infarction
PAF	Platelet-activating factor
PAF-AH	PAF acetylhydrolase
PAF-CPT	PAF-cholinephosphotransferase
PAF-R	Platelet-activating factor receptor
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PS	Phosphatidylserine
ROS	Reactive oxygen species
SFA	Saturated fatty acids
SM	Sphingomyelin

1 Introduction

Cardiovascular diseases (CVD) are the leading cause of death in Europe accounting for 45% of all deaths; however, with increased success in lifestyle alteration, developing new treatments, and advancements in medicine, CVD mortality is modestly

falling in most European countries. CVD is a collective term used to describe diseases affecting the heart and/or blood vessels and includes coronary heart disease (CHD) also referred to as ischemic heart disease (IHD), cerebrovascular disease, peripheral artery disease, congenital heart disease, hypertension, heart failure, and stroke [1].

However, CHD is still the leading single cause of mortality accounting for 19% of all deaths among men and 20% of all deaths among women each year. Stroke is the second most common single cause of death in Europe, accounting for 9% in men and 13% of all deaths in women each year [2]. Atherosclerosis is a slow progressive vascular disease, which underpins CVD. Atherosclerotic lesions or plaques develop in large- and medium-sized arteries, through the formation of foam cells that leads to a plaque covering a necrotic core in the vessel wall that can fissure, erode, and rupture, resulting in a major cardiovascular event such as a myocardial infarction (MI) [3, 4]. Systemic and unresolved inflammation not only participates in all stages of atherosclerosis but is also proposed as one of the major causes for the development of this disorder and its subsequent CVD manifestations [5].

Increasing evidence suggests that lifestyle and nutrition play a pivotal role, either in the development or in the prevention of chronic diseases such as CVD [6, 7]. It is well-established that several risk factors for CVD are modifiable through dietary and lifestyle changes. Thus, healthy nutrition plays a key role in the primary prevention of CVD, particularly in relation to the attenuation and prevention of systemic inflammation [8]. Over the last 70 years, evidence suggests that the Mediterranean diet may be a particularly healthy dietary pattern, which if adopted can reduce cardiovascular disease risk.

In the last decade, there has been a surge of reviews and meta-analyses referring to the beneficial outcome of the adoption of Mediterranean diet in a plethora of chronic diseases that are either directly or indirectly related to inflammation and chronic diseases such as heart failure and CVD [5, 9–14]. It is thought that this dietary pattern is full of bioactive nutrients that work synergistically to reduce the inflammatory milieu and prevent the onset of chronic disease.

In particular, platelet-activating factor (PAF) seems to play a key role in inflammatory signaling and manifestations associated with all stages of atherosclerosis development and its subsequent cardiovascular disorders. However, specific micronutrients of the Mediterranean diet, belonging to the food-derived polar lipids family, beneficially affect the activities of this potent inflammatory mediator [5]. This chapter reviews the recent literature surrounding the relationship between PAF, inflammation, atherosclerosis, CVD, and the anti-inflammatory properties of food-derived polar lipids.

2 Inflammation and Platelet-Activating Factor

2.1 Inflammation and Cardiovascular Diseases

The human body encounters a large number of stimuli, many of which lead to injury or infections, damaging cells and tissues. Inflammation is a necessary and protective physiological response of the innate immune system designed to overcome such

damage. Upon an inflammatory response, activated immune cells, such as blood leukocytes, produce various agents including reactive oxygen species (ROS), elastases, cathepsins, proteinases, eicosanoids, and other lipid mediators such as PAF that either promote or suppress inflammation. If the injury or infection is unresolved, the inflammatory response becomes chronic, leading to oxidative damage of plasma lipoproteins that results in the inappropriate recruitment of immune cells to the site of the inflammatory response, thus further exacerbating the inflammatory response [15].

Previously, atherosclerosis and CVD were referred to as simply lipid-related disorders due to dyslipidemia (hypercholesterolemia or hyperlipidemia) in humans and various evidence from animal studies. It has long been believed that atherosclerosis is merely involved in the passive accumulation of cholesterol into the arterial walls as part of the formation of foam cells, which was recognized as the hallmark of atherosclerotic lesions and subsequent CVD. However, recent systematic reviews and meta-analyses have begun to question the validity of the lipid hypothesis since they have revealed that there is lack of an association or an inverse association between low-density lipoprotein cholesterol (LDL-C) and both all-cause and CVD mortality in the elderly [16]. As such, these studies underpin the rationale for more research in relation to the cause (and not only the risk factors) of chronic diseases such as atherosclerosis and CVD, but also it may be time to re-evaluate the guidelines for cardiovascular prevention [5].

Even though atherosclerosis and CVD were previously viewed as lipid storage disorders, thanks to recent research advancements, we now recognize that inflammation plays a key role in the initiation and progression of atherosclerosis [5, 17]; chronic and unresolved inflammatory manifestations seem to be the key causative underlying mechanistic players at the molecular and cellular level that are responsible for the onset and development of subsequent inflammation-related chronic disorders such as atherosclerosis and CVD. In particular, endothelial dysfunction plays a significant role [5].

Evidence from epidemiological studies also supports the hypothesis that inflammation is the underlying cause of atherosclerosis and the development of CVD [18, 19]. For instance, it has been demonstrated that elevated levels of C-reactive protein (CRP) and inflammatory markers in the blood are associated with an increased risk of future CVD events, even when classical risk factors have been taken into account [18–22]. Furthermore, several systematic reviews and randomized trials targeting a number of cytokines suggest that low-grade or systemic inflammation precedes incident cardiovascular events, and as such that also implies that inflammation might cause vascular diseases that lead to major CVD events [19].

Inflammation plays a key role in all stages of the formation of vascular lesions maintained and exacerbated by several risk factors such as an unhealthy diet and lifestyle, smoking, hyperlipidemia/hypercholesterolemia, hypertension, autoimmune diseases, etc. [5]. The consequence of chronic inflammation is endothelial dysfunction. Endothelial dysfunction is usually characterized by an inflammatory milieu acting on leukocytes and endothelial cells, through an interplay with other immune cells such as T lymphocytes, neutrophils, mast cells, dendritic cells, and

platelets, which is orchestrated by the overexpression and increased production of pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor (TNF) and its receptor, CRP, type I interferons (IFN-α, IFN-β), adhesion molecules, chemokines, lipid inflammatory mediators such as PAF and eicosanoids, increased generation of ROS, increased oxidation of LDL-cholesterol, and a reduction of the bioavailability and levels of protective nitric oxide [5]. Inflammation is governed by the crosstalk between all of these molecules and their cellular interactions [5].

Therefore, deciphering the mechanistic pathways implicated in the inflammatory crosstalk in the onset, development, and progression of atherosclerosis is of great importance, in order to unravel possible preventive and therapeutic approaches to CVD, with less side effects [5]. Common junctions in the mechanistic crosstalk of inflammatory mediators, signaling pathways, and cellular interactions that occur during chronic and unresolved inflammation seem to be a promising therapeutic target for the prevention and treatment of inflammation-related chronic diseases. Drug-based therapeutic interventions targeting inflammatory mediators such as cytokines (i.e., by using specific antibodies against pro-inflammatory cytokines and their receptors) and eicosanoids (i.e., by using specific inhibitors of COX-1 and COX-2) have also been proposed, and relevant trials such as CANTOS and CIRT are still in progress. However, such approaches can sometimes lead to undesirable effects and may leave the individual immunocompromised and at greater risk of infections, since disruption of the physiological balance of the immune system seems to be a risky strategy [23, 24]. These observations are clearly due to the multifaceted effects of such mediators in normal physiology.

On the other hand, it is also important to elucidate the mechanistic pathways of inflammation in order to timely and beneficially resolve inflammatory process. Several lipid mediators play specific roles in resolving systemic inflammation [25]. Characteristic examples of such lipid mediators are metabolites derived from omega-3 (ω3) or omega-6 (ω6) polyunsaturated fatty acids (PUFA), including linoleic acid (18:2 ω6), arachidonic acid (20:4 ω6), eicosapentaenoic acid (EPA, 20:5 ω3), and docosahexaenoic acid (DHA, 22:6 ω3). Generally, ω6 PUFA are described as pro-inflammatory, whereas ω3 are described as anti-inflammatory, while the ratio of ω6/ω3 also gives an insight into the correlation between the inflammatory status related to these lipid mediators and several pathologies [26]. Furthermore, Serhan et al. have proposed that the resolution of inflammation is an active process orchestrated by distinct cellular events and endogenous lipid mediators, including lipoxins, resolvins, protectins, and maresins [27–29]. These novel lipid mediators are derived from ω3 and ω6 fatty acids, which may explain some of the mechanistic beneficial effects of dietary PUFA; thus research in this field is ongoing. For instance, ω6 PUFA supplementation from fish oil has been associated with anti-inflammatory and cardioprotective properties, due to their interactions with the eicosanoid pathways [30]. However, recent reviews suggest that the protective properties of ω3 and ω6 may be exaggerated somewhat and have come under scrutiny in recent years. In particular, administration of fish oil supplements high in ω3 may not have any significant beneficial effect against CVD [31]. This will be discussed further in Sect. 3.1.

Since PAF-related inflammatory cascades belong to the most vital joint mechanistic pathways of inflammation-related chronic disorders, these inflammatory pathways have been identified as promising targets for the attenuation of the inflammatory response, with respect to dietary interventions studies, especially to those with foods containing bioactive polar lipids [5, 32]. The inverse effects of the Mediterranean diet on chronic diseases are mostly related to the pleiotropic effects of its food constituents on several of the inflammation-related pathways. For example, following a Mediterranean dietary pattern leads to the reduction of several inflammatory mediators and biomarkers related to the endothelial functionality, such as decreases in plasma CRP, IL-6, and intracellular adhesion molecule-1 (ICAM-1) [33]. Moreover, the Mediterranean diet beneficially affects the PAF pathway and metabolism toward homeostasis. These beneficial effects may be due to the presence of polar lipids with anti-inflammatory potential [5].

2.2 The Role of Platelet-Activating Factor in Inflammation and Atherosclerosis

PAF, 1-*O*-alkyl-2-*sn*-acetyl-glycero-3-phosphocholine [34], is a potent phospholipid mediator, which is characterized by an alkyl ether linkage at the *sn*-1 position, an acetyl group at the *sn*-2 position, and a phosphocholine group at the *sn*-3 position (Fig. 1). These are important structurally distinguishing features that are critical to the biological activity of PAF, which is mediated by stereospecific binding to its specific receptor (PAF-R) [35, 36]. PAF plays a role in several chronic diseases, in particular CVD [5, 37]. The structure of PAF is distinctive due to the position of an ether linkage at the *sn*-1 position; as such moieties are not common in animals. Similarly it is unusual for an acetic acid to be esterified directly to the *sn*-2 position of glycerol. PAF was the first phospholipid known to possess messenger functions by binding to its specific receptor on the cell membrane, rather than by physicochemical effects on the plasma membrane of the target cell [38]. Interestingly, there are several proposed phospholipid structures that are considered part of the "PAF family," a newly coined term to describe these PAF-like molecules that not only share similar

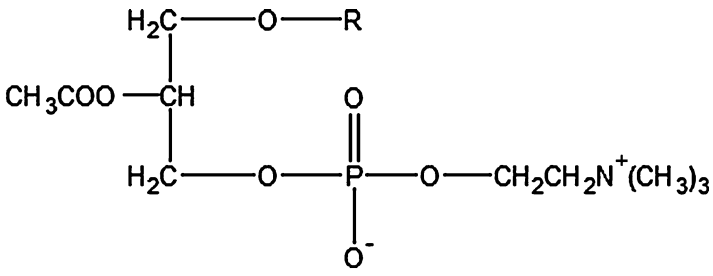


Fig. 1 The structure of the classic platelet-activating factor molecule [34]

structures but can also exhibit similar bioactivities [37]. However, these molecules do not seem to possess the same level of activity as the classical PAF structure, as increasing the chain length beyond three carbons at the *sn*-2 position decreases its biological potency. For instance, there are several compounds synthesized by numerous cells that are similar to PAF but bear a fatty acid instead of a fatty alcohol at *sn*-1 position. These moieties have less than 1% of the potency of PAF. Similarly, modifying the polar group at the *sn*-3 position decreases the potency of the PAF-like molecules [39, 40].

PAF and PAF-like molecules act through their binding to a unique G-protein-coupled seven-transmembrane receptor (PAF-R), which triggers multiple intracellular signaling pathways depending on the target cell and the levels of PAF in the tissue or blood [41]. As a result, PAF is involved in several physiological processes including the modulation of the normal inflammatory response, regulation of blood pressure and the coagulation response, fetal implantation, lung maturation, initiation of parturition, and exocrine gland functions [32]. PAF is most known for its role in anaphylaxis [42] and atherosclerosis [5, 43].

PAF is produced and released in large amounts by inflammatory cells in response to certain stimuli including upstream regulators like IL-1, IL-6, endothelin, TNF-α, and PAF itself [37, 44, 45]. Increased PAF levels at the site of inflammation can activate several cells that can lead to a broad spectrum of effects as a result of PAF production, depending on the type of cell or tissue. This occurs due to the effects of various downstream mediators enhancing production and release of PAF and several other inflammatory mediators, including eicosanoids, TNF-α, IL-1α, IL-6, IL-8, growth factor, and ROS, and the expression of integrins and selectins in the membranes of activated cells [32, 43, 44]. The crosstalk between PAF and various upstream and downstream mediators that affect PAF production seems to be interconnected during inflammatory manifestations [5, 32]. In particular, PAF can induce leukocyte adhesion, leukocyte degranulation, chemotaxis, respiratory burst, and increased vascular permeability [46, 47]. Recently it has been demonstrated that the activation of mast cell PAF-R promotes the immunosuppressive effects of PAF in part through histamine and prostaglandin E_2-dependent mechanisms [48].

The PAF pathways serve as one of the main junctions between many inflammatory cascades that can lead to inflammatory-related disorders such as CVD and cancer [5]. As a result the PAF pathways have been identified as a possible therapeutic target for a number of inflammation-related diseases. Research in this field initially were focused on inhibiting the PAF/PAF-R interactions, thus preventing the initiation of the complex PAF inflammatory pathways [32, 37, 49–52]. Several molecules of synthetic and natural origin [3, 53] have been have been identified, which have the capacity to competitively or noncompetitively displace PAF from its binding sites [54]. Although specific PAF antagonists have exhibited promising results in various studies, the most beneficial effects have been identified in polar lipid extracts of several foods [5]. These food extracts exhibit antithrombotic, antioxidant, and anti-inflammatory activities through inhibiting PAF activities and beneficially modulating PAF levels by altering its metabolism [32]. These molecules are discussed further in Sect. 3.

2.3 PAF Metabolic Enzymes

PAF is biosynthesized at sub-picomolar concentrations [55] and is strictly controlled by two enzymatic pathways known as the remodeling pathway and the de novo pathway [56]. The de novo pathway is catalyzed by a specific dithiothreitol-insensitive CDP-choline, 1-alkyl-2-acetyl-*sn*-glycerol cholinephosphotransferase (PAF-cholinephosphotransferase [PAF-CPT], EC 2.7.8.2) [57], which converts 1-*O*-alkyl-2-acetyl-glycerol to PAF. The remodeling pathway is catalyzed by lyso-PAF acetyltransferases (lyso-PAF ATs, EC 2.3.1.67) [57], which acetylates lyso-PAF. The de novo pathway is known as the minor endogenous PAF synthesis pathway [58], which maintains hemostatic levels of PAF during normal cellular functions [59]. The remodeling pathway is considered the primary enzymatic pathway for PAF synthesis in various inflammatory and allergic disorders [60].

Interestingly, apart from the remodeling pathway which is always activated in both acute and chronic inflammation, the key enzyme of the de novo pathway, PAF-CPT, seems to be more active during chronic inflammatory manifestations, thus contributing to an increase of basal levels of PAF that seem to be related to the continuous activation of inflammatory cascades during the development of inflammation-related chronic disorders [5, 44, 61, 62]. Thus, the regulation of the biosynthetic pathways of PAF seems to be more complicated than was initially thought. In addition, both PAF biosynthetic routes correlate with well-established inflammatory and immunological biomarkers (i.e., several cytokines, viral load, CD40L, etc.) in several chronic diseases [5, 38, 44, 61–66].

Apart from its enzymatic biosynthetic pathways, PAF and PAF-like lipids can also be produced through nonenzymatic synthesis by oxidative transformation of other lipids during oxidative stress or inflammation [55, 67]. When lipids on lipoproteins such as LDL are oxidized, Ox-LDL is produced where bonded PAF-like molecules can mimic the actions of PAF affecting PAF-related inflammation [5]. These pathways are not regulated enzymatically. Vice versa, PAF and PAF-like lipids can also stimulate the production of ROS and nitrogenous species such as reactive nitrogen species (RNS) during oxidative and nitrosative stress in inflammation-induced endothelial dysfunction and atherosclerosis [37].

PAF is catabolized to its biologically inactive form, lyso-PAF, by a specific PAF-acetylhydrolase (PAF-AH, EC 3.1.1.47). The plasma isoform of PAF-AH is known as lipoprotein-associated phospholipase A_2 (Lp-PLA_2) [68], since it circulates in blood in association with plasma lipoprotein particles such as LDL-C and high-density lipoprotein cholesterol (HDL-C), or PLA_2 group 7 [68–71]. PAH-AH also has the capacity to cleave short-chain acyl chains at the *sn*-2 position of oxidized phospholipids, PAF-like phospholipids, and PAF [43].

The amount of PAF present in biological tissue is controlled by the balance of the anabolic and catabolic PAF pathways [72]. Enzymatic biosynthesis of PAF contributes to basal PAF levels and a periodic increase of PAF levels during normal inflammatory responses, while during unresolved and chronic inflammatory manifestations, the enzymatic biosynthesis of PAF is responsible for pathologically increased PAF levels through a continuous induction of the PAF cycle [5]. Oxidative

stress and inflammatory conditions also favor nonenzymatic synthesis of both PAF and PAF-like molecules, such as oxidized phospholipids and oxidized lipids bonded to Ox-LDL. These molecules can amplify the PAF cycle-related inflammatory cascades [5]. On the other hand, PAF catabolism is activated during both acute and chronic inflammatory manifestations and inactivates both PAF and PAF-like molecules, as a homeostatic response against PAF-related inflammatory manifestations. Thus, the PAF metabolic enzymes are implicated in a number of inflammatory manifestations, including heart failure [73, 74], inflammatory bowel disease [72], HIV [62], cancer [44], and atherosclerosis [5].

2.4 Polar Lipids, Inflammation, and Cardioprotection

Polar lipids, such as phospholipids, glycolipids, sphingolipids, etc., are a lipid class that structurally contain hydrophobic hydrocarbon residues and a hydrophilic group such as a carbohydrate group, or a phosphate head group. Phospholipids consist of a glycerol, usually esterified to a saturated long-chain fatty acid at *sn*-1 position, to an unsaturated long-chain fatty acid at *sn*-2 position, and to a phosphorylated head group at *sn*-3 position [8]. Phospholipid head groups are generally cytidine monophosphate, hydroxyl, choline, ethanolamine, serine, or inositols [75]. The general structure of phospholipids is outlined in Fig. 2.

Even though the main function of phospholipids is to support the formation, integrity, and functionality of cell membranes, there are various phospholipid species that possess a plethora of additional functions, in cell signaling, inflammation, and digestion [32]. For example, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are concentrated in the plasma membrane of macrophages [76] and are major phospholipids in human plasma, where 76% and 17% of the total glycerophospholipids present are PC and PE, respectively [77]. PC and PE are also the most abundant phospholipids in erythrocytes [78]. Dietary phospholipids are usually digested in the lumen, while almost 20% of intestinal phospholipids are absorbed passively and without hydrolyzation and preferentially incorporated directly into plasma lipoproteins. These phospholipids travel through the blood stream and may be incorporated into several cell membranes [32]. The same processes occur for phospholipids baring PUFA within their structures, which are mainly of marine origin. Thus, PC and PE are the largest phospholipid reservoirs of dietary ω3 and ω6 PUFA in the cells and biological fluids involved in inflammatory processes [15]. Other less abundant phospholipids can affect inflammatory responses independent of the production of lipid mediators; cardiolipin, which contains two phosphatidic acids esterified to glycerol, is the most abundant phospholipid in the inner membrane of mitochondria [79]; it can mediate apoptosis [80] and compromise cellular respiration [81] under conditions of oxidative stress. Other phospholipids also seem to contribute directly and/or indirectly to several inflammatory cascades and thus are involved in the onset, progression, and often remediation of inflammatory diseases. These phospholipids include plasmalogens, PAF, oxidized phospholipids, and phospholipids that are carriers of fatty acids precursors of eicosanoids [32].

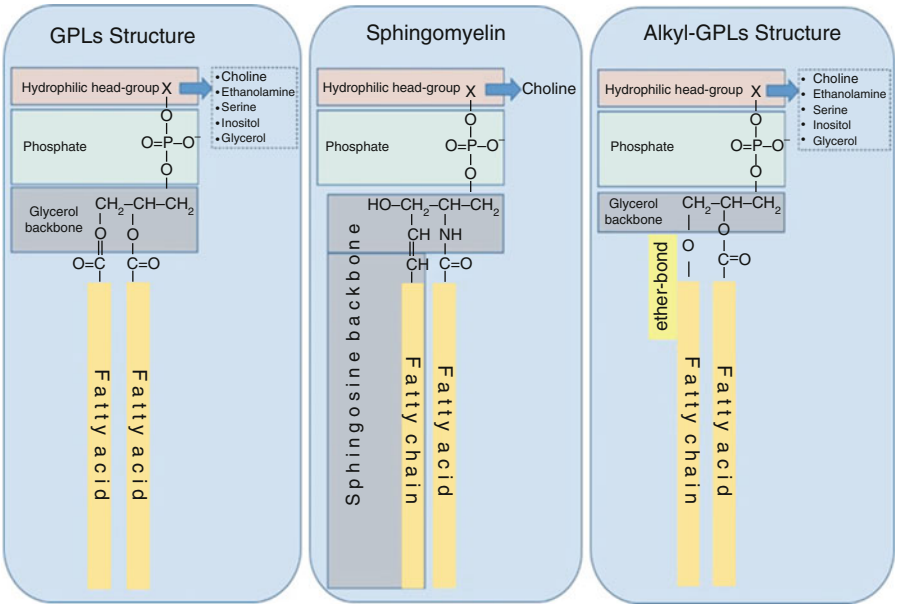


Fig. 2 Phospholipids are generally composed of glycerol, two fatty acids esterified to glycerol at the *sn*-1 and *sn*-2 positions, and a phosphorylated head group esterified at the *sn*-3. The most common structures of various phospholipids are presented. Phospholipids possess a glycerol backbone (GPLs). Sphingomyelin is characterized by a sphingosine backbone (SPLs). Alkyl-phospholipids (Alkyl-GPLs) have a fatty chain linked with an ether bond at the *sn*-1 position of the glycerol backbone. (Reproduced with permission from Lordan et al. [32])

On the other hand, several foods contain bioactive polar lipid compounds that have well-established modes of anti-inflammatory action, whose pleiotropic therapeutic effectiveness and lack of toxicity ensure clinical safety [5, 32]. Dietary polar lipids have been found to exert anti-inflammatory actions in various models of inflammation [3]. Research has shown that there are specific structures of polar lipids that possess antithrombotic and anti-inflammatory effects against PAF [5, 82]. For example, specific PC and PE lipid species of marine origin as well as specific glycolipids in wine and olive oil have exhibited potent bioactivity against PAF-induced inflammation, with beneficial effects against atherosclerosis and CVD [5]. Some of these bioactive structures are presented in Fig. 3. These structures will be further discussed in Sect. 3.

2.5 Diet and Inflammation

Research has established that there is a clear link between an individual's diet and systemic inflammation [84–86]. A maladaptive diet and lifestyle are the dominant underlying causes of low-grade inflammation [87, 88]. Processed and energy-rich

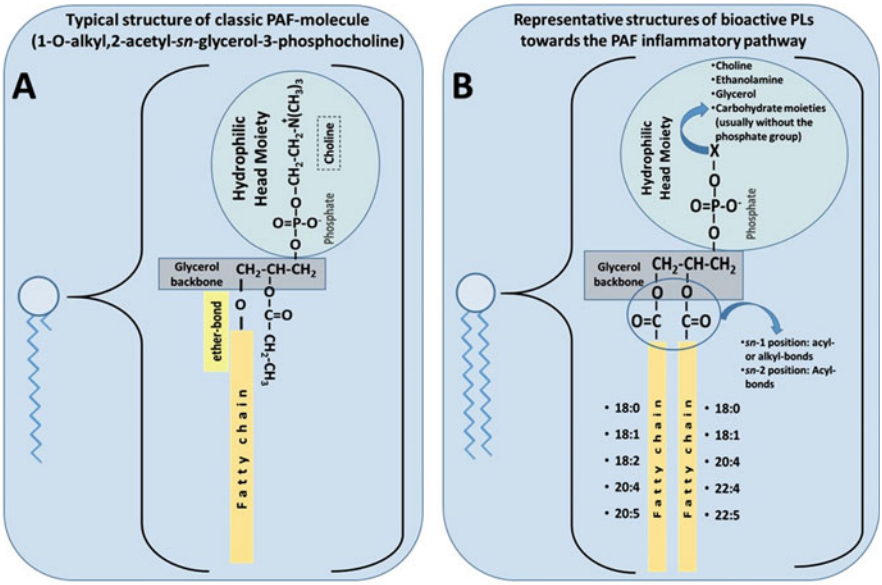


Fig. 3 (**a**) The typical structure of PAF. (**b**) A representative diagram of the bioactive marine phospholipids as elucidated previously by Sioriki et al. and Nasopoulou et al. [82, 83]. Generally, PC and PE derivatives exhibit the greatest biological activity in marine sources. (Reproduced with permission from Tsoupras et al. [5]). *PAF* platelet-activating factor, *PLs* polar lipids

foods and beverages lead to exaggerated postprandial elevations in plasma glucose and triglycerides. As a consequence of our increased intake of these foods in Western societies, postprandial hyperglycemia and hyperlipemia are common [3, 87]. Furthermore, postprandial lipemia is now considered an independent risk factor for CVD, obesity, type 2 diabetes mellitus, and metabolic syndrome [3]. Spikes in glucose and triglycerides can lead to the production of excess plasma ROS that can initiate pro-inflammatory reactions [87–89]. Furthermore, it has been demonstrated that excessive intake of macronutrients such as glucose and saturated fat promotes various inflammatory responses, including an increase in cytokine activity and inflammatory transcription factors [87, 90, 91]. Activated immune cells can either promote or suppress inflammatory processes. It is postulated that prolonged exposure to such inflammatory responses on a daily basis can contribute to the pathogenesis of chronic diseases by establishing a perpetual low-grade inflammatory state [92]; therefore, reducing inflammation to homeostatic levels is essential to avoid long-lasting damage to host tissue [93].

Various dietary patterns can resolve the inflammatory process due to the presence of various bioactive molecules in foods, such as bioactive lipids [3] and peptides [94]. The Mediterranean and Nordic dietary patterns seem to be two such diets that evidence suggests may affect health due to anti-inflammatory properties [95]. Recently, a significant number of studies refer to the beneficial outcomes observed due to the adoption of a Mediterranean dietary pattern in a plethora of several chronic

diseases that are either directly or indirectly related to inflammation [5]. In addition, the Mediterranean diet has also been associated with beneficial outcomes in secondary CVD prevention [12]. When patients suffering from CVD or diabetes adhere to the Mediterranean dietary pattern, the incidence of recurrent myocardial infarction and cerebrovascular events is reduced. The protective effect of the Mediterranean dietary pattern can be maintained for up to 4 years after the first myocardial infarction (Lyon Diet Heart Study) [96]. Moreover, in contrast to the contradictions of lipid hypothesis and mortality in elderly people [16], the HALE project has also shown that individuals aged 70–90 years adhering to the Mediterranean diet and a healthy lifestyle have a 50% lower rate of all-cause and cause-specific mortality [97]. Followers of the Mediterranean diet are also less likely to suffer sudden cardiac death and age-related cognitive decline [14].

The inverse association between Mediterranean diet and all-cause mortality and cardiovascular mortality has been attributed to several of its pleiotropic protective effects. The Mediterranean diet can beneficially influence several risk factors including lowering BMI, blood pressure, and lipid levels (i.e., the ratio of LDL-C/HDL-C), reducing insulin resistance, and improving HDL-C functionality. However, the main beneficial impact of Mediterranean diet seems to be associated with the improvement of endothelial function and a decrease of the inflammatory milieu. It seems that the reduction of inflammation-related mediators and biomarkers such as PAF and several cytokines and of oxidative stress (with lower concentrations of oxidized LDL and improved apolipoprotein profiles) and platelet aggregation and blood coagulation are responsible for the observed beneficial effects of the Mediterranean diet [5, 8, 32].

Even though the beneficial outcomes of healthy dietary patterns such as the Mediterranean diet are profound and well documented, in Westernized and developing countries, there is an increase in the consumption of ultra-processed foods, which is associated with systemic inflammation [87, 98] and may contribute to the rise in noncommunicable diseases. It is suggested that reducing the dietary share of ultra-processed foods by increasing the consumption of unprocessed or minimally processed foods and freshly prepared meals made from these foods can be an effective way to substantially improve the substantial and nutritional quality of Westernized societies. However, given the ubiquity of ultra-processed foods in general, radical whole population strategies are needed to achieve the necessary reductions in ultra-processed food consumption [99].

3 Food Polar Lipids with Anti-inflammatory and Cardioprotective Properties

Polar lipids from various foods, particularly from foods of the Mediterranean diet, seem to exhibit biological activities against the PAF pathway [5]. Some polar lipids act as PAF antagonists that block the binding of PAF with its receptor, and others act as PAF-R agonists but, as aforementioned, possess far less potency than PAF itself. Polar lipids are also thought to directly or indirectly interrupt the binding of PAF to

its receptor by altering the lipid raft microenvironment of the PAF-R in cell membranes and thus its subsequent intercellular pathways [32]. In addition, polar lipids can modulate the activities of the regulatory metabolic enzymes of PAF. Several studies show that these lipid constituents can downregulate the metabolic enzymes that control PAF biosynthesis and upregulate the enzymes responsible for PAF degradation [61, 100]. Thus, dietary patterns based on foods rich in such bioactive polar lipids seem to possess beneficial effects through the modulation of PAF metabolism toward homeostatic PAF levels and activities [5]. Bioactive polar lipids are present in various foods of the Mediterranean diet and seem to exhibit specific structures (Fig. 3). These foods include fish, dairy, wine, olive oil, and various other foods. Interestingly, the anti-inflammatory properties of various types of polar lipids seem to be as a result of a synergistic effect between the different polar lipids [5]. The various sources of polar lipids and their bioactivities are discussed.

3.1 Fish Polar Lipids

Epidemiological studies and clinical trials have demonstrated the protective role of fish and fish oil consumption against CVD [101], while their nutritional benefits have mainly been associated with their omega-3 PUFA content [102, 103]. However, several recent reviews and meta-analyses have concluded that insufficient evidence exists to suggest a beneficial effect of ω3 PUFA supplementation in adults with chronic diseases such as atherosclerosis and CVD [31, 32]. It is now well-established that more complex mechanisms underlie the beneficial effects of fish and fish oil consumption and administration of marine products that go far beyond the ω3 PUFA-/eicosanoid-related mechanisms [32]. Other lipid constituents are also present in fish and fish oils that have different metabolic effects after absorption with distinct biological activities not only limited to their superior incorporation to plasma lipoproteins and cell membranes and bioavailability of their omega fatty acids but also to their reported anti-inflammatory activities through also other mechanisms than the ARA/eicosanoid pathway, such as the inhibition of the PAF pathway and the modulation of PAF metabolism [32].

Fish contains between 1% and 1.5% phospholipid by weight, where PC derivatives are generally the predominant phospholipids followed by PE, phosphatidylinositol (PI), phosphatidylserine (PS), lyso-PC, and sphingomyelin (SM) [32]. Polar lipids of fish exhibit both in vitro antithrombotic [82, 83, 104–109] and in vivo antiatherogenic properties [110]. In 1996, Rementzis et al. [104] reported the presence of polar lipids in *Scomber scombrus* (mackerel) that exhibited inhibitory activity against PAF and thrombin-induced platelet aggregation. This leads to a series of other studies that identified polar lipids in various fish species that exhibited effects against PAF in vitro and in vivo [32].

Notably, Nasopoulou et al. have explored the anti-PAF and antiatherogenic properties of marine polar lipids in a series of studies that examined the polar lipid extracts from wild and cultured sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). These polar lipids seem to exhibit strong agonistic and antagonistic

effects against PAF-induced platelet aggregation [106, 110, 111], and they seem to inhibit the activities of the basic biosynthetic enzymes of PAF [61]. Furthermore, an in vivo study separated 12 healthy male New Zealand rabbits of specific weight and age into 2 even groups: A and B [110]. Group A was given an atherogenic diet for 45 days as was group B; however, the group B diet was also supplemented with marine polar lipid extracts from *Sparus aurata.* After 45 days the HDL-C levels were significantly increased in the rabbits that were supplemented with marine polar lipids in comparison with the control group. In addition, PAF catabolic enzyme activity (plasma PAF-AH) was significantly increased, and platelet aggregation efficiency was reduced in the rabbits fed marine PL in comparison with the control group. Of greater significance was the fact that hypercholesterolemic rabbits supplemented with polar lipids developed early atherosclerotic lesions that were of a statistically significant lower degree ($p < 0.017$) than that of the control group as demonstrated in Fig. 4. It seems the overall anti-PAF effects exhibited by the marine polar lipids led to a reduction in the formation of foam cells indicating that these lipids possess antiatherogenic properties and thus may explain some of the beneficial effects observed due to fish consumption.

A follow-up study was published that utilized plasma from the same rabbits fed the same fish polar lipids, which investigated the effects of the polar lipids on PAF biosynthesis and PAF catabolism [64]. The specific activity of PAF-AH was decreased in rabbits' platelets of both groups A and B and in rabbits' leukocytes of group A ($p < 0.05$). On the other hand, the specific activity of Lp-PLA_2 was increased in both groups A and B in both leukocytes and platelets ($p < 0.05$). PAF-CPT demonstrated an increased specific activity only in rabbits' leukocytes of group A ($p < 0.05$). Neither of the two groups showed any change in lyso-PAF-AT-specific activity ($p > 0.05$). Free and bound PAF levels increased in group A while decreased in group B ($p < 0.05$). These results indicate the fish polar lipids modulate PAF metabolism upon atherosclerotic conditions in rabbits leading to lower PAF levels and PAF activity in the blood of rabbits with reduced early atherosclerotic

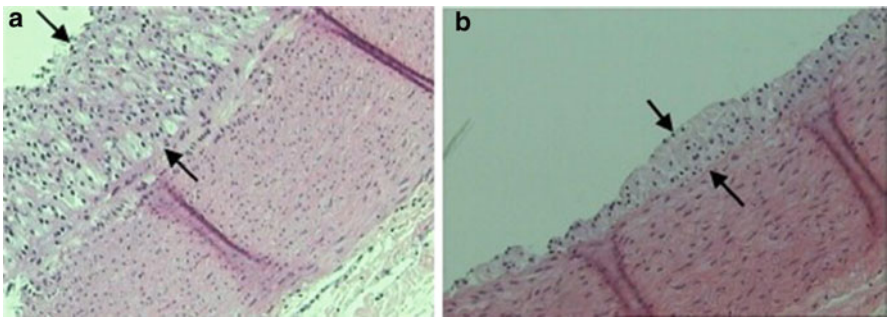


Fig. 4 Representative optic micrographs x100 of aortic wall sections stained with hematoxylin and eosin from the two experimental groups, where atherosclerotic lesions appear as foam cells between the arrows. (**a**) Group A (atherogenic diet); (**b**) group B (atherogenic diet enriched with marine polar lipids)

lesions compared to the control group. Therefore, it can be said that the joint inhibitory effects of these lipids against PAF/PAF-R interactions and the ability to modulate the catabolic and biosynthetic enzymes of PAF are due to the intake of the polar lipids in this investigation. Further studies are required to see if these effects are demonstrated in other animal models and in human studies.

Notably, recent studies have revealed the existence of bioactive polar lipids in several oily fish species, such as sardines. Polar lipids were also present in cod liver oil, which is the main source of marine-derived health [112]. Sardine polar lipid extracts possessed a higher content of bioactive polar lipids that also demonstrated higher antithrombotic activities against platelet aggregation when compared to that of fish oil derived from cod liver oil [112]. Similar studies in Irish organic farmed salmon (*Salmo salar*), another oily fish species, revealed that Atlantic salmon is also a rich source of bioactive polar lipids with potent antithrombotic activities against platelet aggregation [113]. Moreover, it was revealed that the antithrombotic effects of salmon polar lipids were attributed to their anti-PAF effects, since they exhibited potent inhibitory effects against PAF-induced human platelet aggregation and much less potent effect toward thrombin-induced platelet aggregation. It was thus proposed that these bioactive polar lipids seem to act synergistically against platelet aggregation either by inhibiting PAF-induced activities or by very low agonistic effects to its receptor [113]. Structural elucidation of these salmon-derived polar lipids revealed that they also belong to the alkyl-acyl PC and alkyl-acyl PE derivatives that bare ω3 PUFA such as the EPA and DHA at the *sn*-2 position of their glycerol backbone [113]. These results suggest that salmon is a source of bioactive polar lipids rich in ω3 PUFA and that the physiological and pleiotropic beneficial effects of marine polar lipids can be attributed to the coexistence of both important structural elements such as their polar head groups and the constituting PUFA within their structures.

3.2 Dairy Polar Lipids

The negative perception of dairy fats stems from the effort to reduce dietary saturated fatty acid (SFA) intake due to their association with increased cholesterol levels upon consumption and thus an increased risk of CVD development [3, 114]. However, recent research and meta-analyses have demonstrated the benefits of full-fat dairy consumption, based on greater bioavailability of high-value nutrients (such as vitamin D) and anti-inflammatory properties [114]. Dairy products contain a complex lipid profile and are distinguished by the fact that they are the most natural source of short-chain fatty acids (C4–C8, 4–13 wt% total FA), which are generally esterified on the *sn*-3 position of the triglyceride [115]. The nonpolar lipids or neutral lipids (triglycerides or TG; 96–97% of milk lipids), the polar lipids (glycerophospholipids, sphingolipids, glycosphingolipids, glycolipids; 0.2–2% of milk lipids), and cholesterol create an oil in water emulsion to form milk. These lipids form spherical milk fat globules that contain triacylglycerides (0.1–15 μm) that are surrounded in a complex trilaminar membrane (4–12 nm) composed of proteins,

phospholipids, and sphingolipids, suspended in an aqueous liquid phase, which is derived from mammary endothelial cells [116]. This unique structure is the milk fat globule membrane (MFGM), which mainly consists of lipids (40%), proteins (60%), and cholesterol [117]. The membrane of the outer leaflet consists of phospholipids and cholesterol, which stabilizes the triglyceride-rich milk fat globule against coalescence and protects the core from lipolytic degradation and oxidation [32]. The MFGM structure is depicted in Fig. 5.

Other sources of phospholipids in dairy products include MFGM fragments and lipoprotein particles, which are believed to be remnants of the mammary secretory cell membranes. Similar to the MFGM, phospholipids originate from the apical plasma membrane of the mammary gland secretory cell [116–122]. The phospholipids present

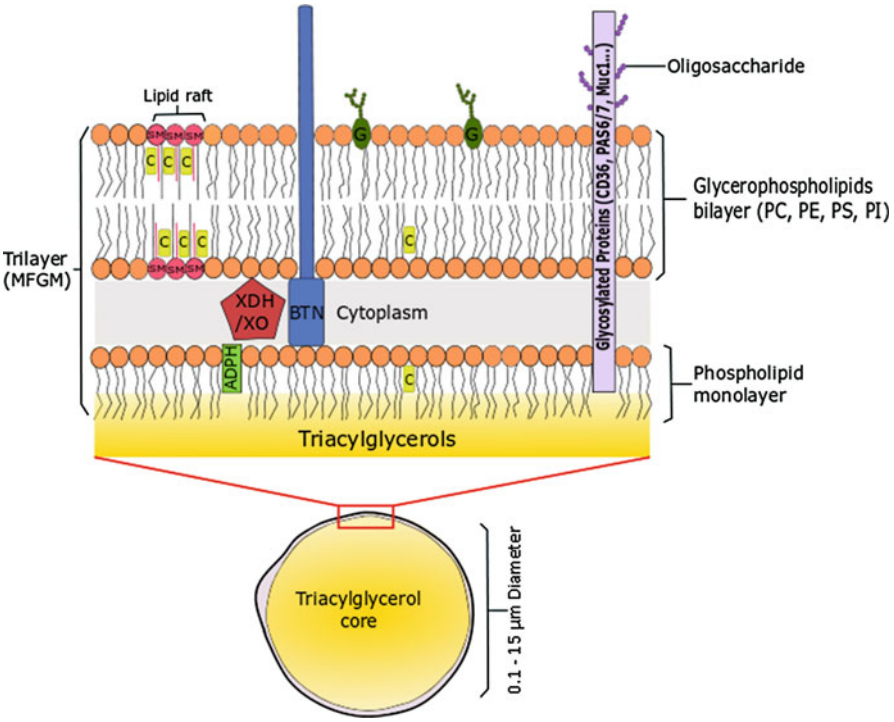


Fig. 5 Illustration of the milk fat globule membrane (MFGM). The sizes in this schematic are not in proportion. A phospholipid monolayer surrounds the triacylglycerol core, followed by a proteinaceous coat connecting the monolayer to the outer phospholipid bilayer. Adipophilin (ADPH) is located in the inner layer polar lipid layer, while xanthine dehydrogenase/oxidase (XDH/XO) is located between both layers. PE, PS, and PI are generally concentrated on the inner surface of the membrane, whereas PC, SM, glycolipids (G), cerebrosides, and gangliosides are mainly located in the external membrane. SM and cholesterol (C) can form rigid domains in the cellular membrane known as lipid rafts. Glycoproteins are distributed over the external membrane surface; these include butyrophilin (BTN), mucin 1 (MUC1), PAS 6/7, and CD36. (Reproduced with permission from Lordan et al. [32])

Table 1 Typical phospholipid composition of bovine, caprine, and ovine milk

Dairy polar lipids	TPL[a]	PC[b]	PE[b]	PI[b]	PS[b]	SM[b]
Bovine milk [118, 130–132]	0.3–1.1	20–40	20–42	0.6–12	2–11	18–35
Ovine milk [133]	0.2–1.0	26–28	26–40	4–7	4–11	22–30
Caprine milk [133]	0.2–1.0	27–32	20–42	4–10	3–14	16–30

Abbreviations: *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PS* phosphatidylserine, *SM* sphingomyelin, *TPL* total polar lipids
[a]Mean values expressed as % of total lipid composition
[b]Expressed as % of total phospholipids

in bovine, caprine, and ovine milks are quantitatively minor lipid constituents of milk; however they possess several beneficial techno-functional properties and are involved in various physiological processes making them nutritionally valuable [32].

Research by our group has demonstrated that bovine, ovine, and caprine dairy products possess polar lipids with potent anti-inflammatory activities against PAF as demonstrated in a series of in vitro experiments on washed rabbit platelets [123–125]. Research has shown that as milk is fermented to yoghurt and then to cheese, the bioactivity of the PAF inhibitors seems to increase [3]. This indicates that the processes of fermentation and lipolysis are crucial to altering the bioactivity of the polar lipid fractions of milk, and this bioactivity increases the further fermentation proceeds [3, 114]. It is thought that the fermentation process alters the fatty acid composition of the polar lipids, thus leading to variation in the biological activity, depending on the microorganisms used. Therefore, yoghurt and cheese seem to exert greater biological activity against PAF than just milk polar lipids. In particular, these effects have been attributed to microorganisms such as *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, which are commonly used in yoghurt production [3, 126]. However, further research is required to identify whether these microorganism may also play a role in the biosynthesis of these bioactive lipids or if they interact with the MFGM. It is also imperative to identify other fermented foods that may contain bioactive polar lipids such as wine, beer, and *kefir* in order to elucidate and establish how fermentation impacts the health effects of various foods.

Research also indicates that polar lipids of caprine and ovine milk and dairy products possess greater bioactivity against PAF than those of bovine milk and dairy products [125, 127]. This may be due to their varied phospholipid content (Table 1). Therefore, further research is required to confirm these observations. Furthermore, polar lipids seem to be bioavailable in in vivo and clinical studies and seem to be able to exert their biological activities [110, 128, 129]; thus whether dairy lipids share the same bioavailability remains to be seen.

3.3 Wine Lipid Microconstituents

Associations between wine consumption and the "French paradox" motivated intensive research into the health benefits of moderate wine consumption. The nutritional

superiority of wine over other alcoholic beverages is thought to be attributed to microconstituents within wine that contain bioactive compounds whose mechanistic pathways are currently under investigation [134]. Alcohol consumption has a complex relationship with human health, and the harmful effects of alcohol are well-known and in contrast to the beneficial effects [135]. Various studies have linked excessive alcohol consumption to various chronic diseases and conditions including cancers and liver cirrhosis [136]. Despite these negative associations, moderate alcohol consumption is associated with a number of health benefits [135]. Research indicates that moderate alcohol consumption (1–2 drinks/day) is associated with positive effects against a number of cardiovascular risk factors [134, 137, 138]. In addition, large epidemiological studies have demonstrated that moderate alcohol consumption significantly reduces cardiovascular risk factors, morbidity, and mortality through a dose-effect relationship that is characterized by a J-shaped curve [138].

Generally, wine contains between 12% and 15% (v/v) ethanol. The ethanol content of wine is associated with a number of beneficial effects including alterations of plasma lipoproteins and modifications of blood platelet function and coagulation [139]. However the benefits of moderate ethanol intake due to alcoholic beverage consumption can only partly explain the protective effects of wine [140]. Mechanistically, lipid and phenolic microconstituents in wine may play a role in attenuating inflammatory processes associated with CVD. Phenolic compounds include phenolic acids (p-coumaric, cinnamic, caffeic, gentisic, ferulic, and vanillic acids), trihydroxy stilbenes (polydatin and resveratrol), and flavonoids (catechin, epicatechin, and quercetin), while their polymerization gives rise to the viniferins and procyanidins. The phenolic content of white wines is considerably lower than red wines due to their manufacturing process. Red wines are usually produced with the grape skins, whereas white wine is generally produced from free-running juices that do not have contact with the skin of the grapes [140, 141]. Initially, the biological activity of wine was attributed to their antioxidant properties; however, currently it is well-established that wine consumption is associated with anti-inflammatory activities [142], and these cardioprotective effects are induced irrespective of their antioxidant properties.

In fact, these anti-inflammatory effects may be attributed to the anti-PAF effects of various lipid and phenolic microconstituents within the wine [140]. Studies show that polar lipids in wine can inhibit the biological actions of PAF [143–146] and modulate enzymes involved in PAF metabolism in vitro [147]. Interestingly, within these studies it was also unexpectedly revealed that white wines, musts, and the specific grapes that produced the white wines possessed polar lipids with potent anti-PAF activities, while structural elucidation of these lipids revealed that they were mostly glycoglycerolipids or glycol-glycerophospholipids and not phenolic compounds [143, 144, 146], supporting the notion that their beneficial effects can be attributed to their anti-PAF effects and not to any putative antioxidant effects.

Clinical studies have also been conducted, which demonstrate that the beneficial effects of wine consumption may be due to bioactive components in wine that affect platelet function. Most notably, in the pursuit for answers to the observed French

paradox, researchers saw a reduction of platelet aggregation due to moderate alcohol consumption [148, 149].

Clinical studies have been conducted that demonstrate that wine consumption may exert its anti-inflammatory effects through the modulation of PAF interaction with the PAF-R and PAF metabolism. A recent study took ten healthy men who participated in four daily trials on separate days. They consumed a standardized meal along with white wine, red wine, an ethanol solution, or water. Blood samples were collected before and after meal consumption and at several time points over a 6-h period, and various hemorheological values were determined. It was found that wine consumption improved platelet sensitivity independently of the alcohol, triglyceride levels were lowered during postprandial elevations, and plasminogen activator inhibitor-1 levels were not impacted negatively by the alcohol consumption. These effects are attributed to the presence of PAF inhibitors in wine [129]. A similar clinical study by the same group also examined the postprandial effects of wine consumption on the PAF metabolic enzymes. The same trial methodology, standardized meal, wines, and controls were used. It was found that consumption of wine, but not alcohol alone, reduced the activity of specific PAF biosynthetic enzymes (PAF-CPT and lyso-PAF AT) during postprandial elevation, thus attenuating postprandial inflammation [150]. Again these effects have been attributed to microconstituents within wine that exhibit potent anti-PAF effects. It seems that the attenuation of the inflammatory may be as a consequence of the attenuation of PAF levels by lipid microconstituents and/or polar lipids that act as PAF inhibitors. Further research is required to further our understanding of this phenomenon and to discern whether these effects are seen in other fermented alcoholic beverages such as beer or spirits.

3.4 Polar Lipids of Olive Oil and By-Products

Virgin olive oil is the main source of dietary lipid in the Mediterranean diet, and it has been linked to numerous health benefits including reduced systolic blood pressure, an enhancing effect of T-cell-mediated function, and an increase in HDL-C [151, 152]. The beneficial effects of olive oil consumption have been attributed to its high concentration of monounsaturated fatty acids (MUFA), namely, oleic acid [153, 154]. However, other seed oils have similar levels of MUFA but do not seem to possess the same biological activity. Therefore, research has turned its attention to other compounds such as polar lipids or phenolic compounds [155]. Polar lipids of virgin olive have demonstrated antagonistic effects against PAF, and following various chromatographic separations, the most active fraction was identified as a glycerol ether glycolipid [156].

A subsequent in vivo study aimed to investigate the effects of virgin olive oil consumption in 40 male hypercholesterolemic white New Zealand rabbits [128]. The rabbits were randomly distributed into four groups. Group A were fed an atherogenic diet containing cholesterol; group B were fed the same atherogenic diet supplemented with 15% (w/w) virgin olive oil; group C were fed atherogenic

diet supplemented with olive oil polar lipids isolated from the same volume of olive oil supplemented in group B; finally, group D were fed the atherogenic diet supplemented with olive oil neutral lipids isolated from the same volume of olive oil supplemented in group B. Baseline measurements were taken for a number of parameters, and the rabbits were fed their respective diets for 45 days before testing and prior to being euthanized. Rabbits that received the polar lipid extract had a significant reduction of early atherosclerotic lesions compared to group A that received only an atherogenic diet, and they also retained the elasticity of the aortic wall. Rabbits that received neutral lipids of olive oil did not exhibit a reduction in early atherosclerotic lesions compared to the control group. PAF levels in the blood were higher for group A and decreased in group D by day 45. In group A, along with increased PAF levels, PAF-AH was also increased, atherosclerotic lesions formed, and vessel wall elasticity was decreased. In groups B and C, blood PAF-AH increased, platelet aggregation was attenuated, less oxidation occurred in the plasma, lesion thickness was reduced, and vessel wall elasticity was retained. However, most of these effects were not observed in animals fed neutral lipids (group D), although blood PAF and plasma oxidation were lower [128]. This study demonstrated for the first time that it was the polar lipid composition of the virgin olive oil that was responsible for the antiatherogenic activities observed [155].

Interestingly, studies have also shown that polar lipids present in the by-products of the olive oil industry possess potent antithrombotic, anti-inflammatory, and anti-PAF activity in vitro and in vivo [157–159]. It has also been shown that these lipids may also beneficially modulate PAF metabolism toward the reduction of PAF levels to homeostatic levels, thus explaining the rationale for their observed in vivo effects on reducing PAF levels [100].

Olive pomace is one of two major by-products of the olive oil industry, the other being olive mill wastewater. Unfortunately, for every 100 kg of olive oil produced, there is 35 kg of olive pomace produced that is generally an underutilized waste product, which has been proposed as a viable alternative to fish oil in the aquaculture industry [160]. In a study by Nasopoulou et al. [107], cultured fish (*Dicentrarchus labrax* and *Sparus aurata*) were fed olive pomace as a substitute for fish oil in fish feed. These fish demonstrated satisfactory growth performance and statistically decreased levels of fatty acids while also possessing potent inhibition of PAF-induced platelet aggregation. A follow-up study demonstrated that the most active polar lipid fractions were specific PC and PE derivatives [83]. Overall, studies carrying out structural elucidation of fish polar lipids from fish fed a diet enriched with olive pomace indicate that the most biologically active lipid fractions contain various diacyl-glycerophospholipid species that seemed to mainly consist of 18:0 or 18:1 fatty acid in the *sn*-1 position and either 22:6 or 20:2 fatty acids in the *sn*-2 position [82, 83]. The proposed structures of these novel bioactive phospholipid species are demonstrated in Fig. 3. Therefore, replacing fish oil in the diet of sea bream and sea bass may improve their cardioprotective effects upon consumption by humans while also tackling the problems associated with by-products of the olive oil industry.

3.5 Various Foods of the Mediterranean Diet

It is well-accepted that lifestyle plays a crucial role in the prevention of CVD [161]. Evidence suggests the most important behavioral risk factors of CVD are an unhealthy diet, physical activity, and harmful use of alcohol and tobacco [162]. Previous research has focused on targeting specific nutrients, particularly the reduction of SFA in an individual's diet; however, recent research indicates that this approach was short-sighted and ineffective and that nutritional interventions in CVD have proved to be an effective strategy with tangible evidence, indicating that the synergistic effects of food combined into a dietary pattern provide the maximum benefit obtainable from nutrition [5, 9, 114, 163]. It seems that most are in agreement that the optimal dietary pattern to reduce CVD includes high consumption of fruits, vegetables, legumes, whole grains, nuts, fish, and poultry, moderate dairy and heart-healthy vegetable oil intake, low red meat intake, and minimal consumption of refined grain products, added sugars, industrial *trans* fats, sugar-sweetened beverages, and processed meat [163, 164]. The Dietary Approaches to Stop Hypertension (DASH) diet and Mediterranean diet reflect these dietary patterns and are palatable, relatively easy to adhere to, and continually recommended for good cardiovascular health [5, 9, 163, 165, 166]. Certainly, while nutritional research should move away from simply focusing on single nutrients as a panacea to prevent CVD, it is important to recognize that it is most likely these nutrients working synergistically together in the diet that leads to the beneficial effects observed against CVD.

There have been several studies published that have identified various food sources containing anti-inflammatory polar lipids. Many of these foods are found in the Mediterranean diet. The traditional Mediterranean diet originates in the olive-growing regions of the Mediterranean and has strong cultural associations with these areas [95]. Various definitions of the Mediterranean diet have been proposed, and the diet has been adopted beyond the Mediterranean region and becomes increasingly medicalized as both a treatment and prevention intervention [167]. Despite these variations, the Mediterranean diet remains based on fresh, seasonal, and local food [166]. The Mediterranean diet is generally characterized by the high consumption of fruit, vegetables, legumes, cereal foods (preferably whole grains), breads, tree nuts, and olive oil, including moderate servings of milk, dairy products, eggs, fish, shellfish, and white meat, and low consumption of processed foods or red meats. Wine is consumed in moderation in non-Islamic countries, and coffee is considered the hot beverage of choice, which nowadays is consumed with sugar [168–170]. In addition, the Mediterranean diet has qualitative cultural and lifestyle elements, such as conviviality, culinary activities, adequate rest, and physical activity [171], which seem to be key for good health and longevity as witnessed in the Blue Zones [5]. However, whether these cultural aspects are vital to the success of the Mediterranean diet or they simply improve the health effects observed through a healthier habitual lifestyle approach requires further in-depth research.

The Mediterranean diet is rich in beneficial fatty acids with a characteristically high content of MUFA and a higher MUFA/SFA ratio than other diets [172, 173]. Due

to the low glycemic index and glycemic loads [174], high dietary fiber [175], and anti-inflammatory effects [176], the Mediterranean diet is synonymous with favorable effects on health status. Evidence suggests that Mediterranean diet is associated with a number of beneficial health effects, including lower incidences of several chronic diseases such as CVD [9, 14], cancer [177, 178], diabetes [179], and neurodegenerative diseases [180, 181]. It has been proposed that these associations are mainly due to the anti-inflammatory nature of the Mediterranean diet [5, 182].

In particular, evidence suggests that these effects may in part be due to the presence of bioactive polar lipids in foods of the Mediterranean diet, which possess inhibitory properties against PAF activities [5, 183]. The polar lipids found in foods of the Mediterranean diet exhibit in vitro and in vivo anti-inflammatory activities through either directly or indirectly inhibiting the PAF/PAF-R pathways and thus PAF activities but also by downregulating its levels through modulating the activities of key metabolic enzymes of PAF by either upregulation of the PAF catabolic enzymes or the downregulation of the basic PAF biosynthetic enzymes [44, 61, 64, 100, 147, 150]. These polar lipid microconstituents in association with various other beneficial components of the Mediterranean diet may be responsible for the observed preventative effects of the Mediterranean diet against the development of CVD. Several of the foods that have demonstrated anti-PAF effects are presented in Table 2 along with other foods and components of the Mediterranean diet that exhibit inhibition of PAF-related inflammatory pathways and manifestations.

Notably, the uptake of dietary polar lipids seems to beneficially affect the functionality of HDL lipoproteins especially in atherosclerotic conditions [5]. HDL-C is generally described as the "good" cholesterol, since it removes excess cholesterol from the blood stream and from atherosclerotic plaques, and it has exhibited anti-inflammatory and antioxidative properties through a plethora of cardioprotective enzymes bonded to HDL, including the aforementioned PAF-AH enzyme activity, which is the main catabolic enzyme of PAF [71]. These HDL-associated activities contribute to the maintenance of endothelial cell homeostasis, which protects the cardiovascular system [191]. Plasma PAF-AH is also found in atherosclerotic lesions, since it comigrates there along with the lipoproteins (i.e., LDL-C), where it is incorporated. Plasma PAF-AH (Lp-PLA_2) mainly plays an anti-inflammatory role in leukocyte/platelet/endothelium activation and seems to suppress atherogenic changes in plasma lipoproteins (such as LDL-C) by promoting the catabolism of PAF and by removing oxidized phospholipids present in Ox-LDL. These oxidized phospholipids mimic the actions of PAF and are generated by oxidative modifications of lipoproteins such as LDL during pro-atherogenic and atherosclerotic events [5, 68, 70, 71]. Thus, during inflammatory cascades that increase PAF levels, this isoform of PAF-AH ($LpPLA_2$) seems to be activated as a homeostatic mechanism to down-regulate these events, by downregulating the levels of PAF and oxidized phospholipids, as a terminator signal [192].

Thus, HDL and its enzymes, including PAF-AH, seem to protect against these manifestations. Dietary intake of bioactive polar lipids, particularly those baring ω3 PUFA, increases HDL-C levels and the incorporation of such anti-inflammatory and antioxidant dietary polar lipids to HDL, thus providing an additional protective

Table 2 Studies on the beneficial impact of microconstituents from foods of the Mediterranean diet, such as polar lipids and vitamins, toward inflammation-related disorders, through their effects on the PAF pathways and metabolism. (Modified with permission from Tsoupras et al. [5])

Studied food and components	Type of study	Results
PL of goat and sheep meat	Ex vivo studies in hPRP	Inhibition of PAF-induced platelet aggregation [193]
PL of red and white wine, musts, grape skins, and yeast	In vitro studies in WRP and in U937 macrophages In vivo postprandial dietary interventions studies in humans	Inhibition of PAF-induced platelet aggregation and modulation of PAF metabolism toward reduced PAF levels [129, 134, 143, 144, 146, 147, 150]
PL of fish (sea bass, sea bream, salmon, etc.)	In vitro studies in WRP, hPRP, and HMC Ex vivo studies in hPRP In vivo studies in hyperlipidemic rabbits	Inhibition of PAF-induced platelet aggregation, modulation of PAF metabolism toward reduced PAF levels, and reduction of the thickness of atherosclerotic lesions in hypercholesterolemic rabbits [61, 64, 82, 83, 104–107, 109–111, 113, 184]
PL of olive oil and olive pomace	In vitro studies in WRP and in HMC In vivo study in hyperlipidemic rabbits	Inhibition of PAF-induced platelet aggregation, modulation of PAF metabolism toward reduced PAF levels, reduction of the thickness of atherosclerotic lesions in hypercholesterolemic rabbits, and regression of the already formed atherosclerotic lesions [100, 128, 156, 157]
PL of seed oils (soybean, corn, sunflower, and sesame oil)	In vitro studies in WRP	Inhibition of PAF-induced platelet aggregation [156]
PL of hen egg	In vitro studies in WRP	Inhibition of PAF-induced platelet aggregation [185]
PL of dairy products (milk, yoghurt, cheese, etc.)	In vitro studies in WRP and ex vivo studies in hPRP	Inhibition of PAF-induced platelet aggregation [114, 123–125]
Lipid extracts from garlic	Ex vivo studies in hPRP	Inhibition of PAF-induced platelet aggregation and de-aggregation of aggregated platelets [186]
Vitamin D and its analogues	In vitro studies in WRP and human leukocytes, ex vivo studies in hPRP, and in vivo studies in hemodialysis patients	Inhibition of PAF-induced platelet aggregation and modulation of PAF metabolism toward reduced PAF levels and reduced levels of several cytokines [38]
Vitamin E	Ex vivo studies in hPRP and whole blood	Inhibition of PAF-induced platelet aggregation [187, 188]

(*continued*)

Table 2 (continued)

Studied food and components	Type of study	Results
Mediterranean-based meals and diets, rich in PL with anti-PAF effects	In vivo studies in humans	Reduction of PAF-induced platelet activity in patients with type 2 diabetes mellitus and metabolic syndrome and healthy subjects
Eugenol in cinnamon and basil	In vivo studies in male Wister rats	Reduction of PAF-induced gastric ulcers [189]
Dietary gangliosides	In vivo studies in male Sprague-Dawley rats	Reduced PAF synthesis and PAF levels [190]
Curcumin	In vitro studies in hPRP	Inhibition of PAF-induced platelet aggregation

Abbreviations: *HMC* human mesangial cells, *hPRP* human platelet-rich plasma, *PAF* platelet-activating factor, *PL* polar lipids, *WRP* washed rabbit platelets

mechanism by increasing plasma PAF-AH activity and by protecting the HDL enzymes (such as PAF-AH) from oxidation-related inactivation. This is in agreement with the beneficial in vitro and in vivo effects of several dietary polar lipids, especially on PAF metabolism and HDL biofunctionality [32].

Overall, the protective outcomes of the adoption of Mediterranean diet toward chronic diseases seem to be associated with the pleiotropic beneficial effects of its bioactive microconstituents that are not only limited to increasing plasma HDL-C levels and functionality and providing better stability against oxidation but mainly on their effects on the levels, activities, and metabolism of key inflammatory mediators such as PAF [5, 32, 44]. However, more in vivo results are required in several chronic disorders and their inflammation-related manifestations in order to further support these findings. In particular, clinical trials implementing dietary patterns such as the Mediterranean diet that are rich in bioactive polar lipids interacting with the PAF/PAF-R pathways and metabolism are required to gain further insight into the role of PAF in chronic diseases.

4 Future Research and Applications of Food Polar Lipids

Systemic inflammation leads to chronic diseases such as CVD; therefore it is imperative to research and understand the mechanisms that govern inflammation. Inflammation is a multifactorial process that leads to occurrence of complex conditions and diseases. Due to the complex etiology of CVD, there is not one single solution or 'panacea' to prevent its occurrence; hence the array of pharmaceutical treatments such as statins and antihypertensives that are available to patients to tackle the various risk factors associated with CVD. Successful dietary and lifestyle interventions are credible alternatives to pharmaceutical treatments that have the potential to tackle multiple risk factors at a time [103], albeit difficult for the

individual that undertakes these changes. Adherence to a healthy dietary pattern such as the Mediterranean diet has demonstrated favorable effects for the primary prevention of CVD. Bioactive components of the foods of the Mediterranean diet play a role in the attenuation of systemic inflammation [5]. PAF plays a critical role in the inflammatory process that seems to be underappreciated. Polar lipids have the ability to disrupt the binding of PAF to its receptor and modulate the PAF metabolic enzymes; therefore these lipids may prove important in the modulation of the inflammatory response. Polar lipids are found in abundance in the Mediterranean diet; thus further studies are required in order to discern their mechanisms and potential in the prevention of CVD and other chronic disease. As CVD is still the leading cause of death worldwide, scientific research on healthy dietary patterns such as the Mediterranean diet and the DASH diet are imperative. Furthermore, the development of novel nutraceutical and pharmaceutical products targeting inflammatory pathways such as the PAF pathway or targeting numerous inflammatory pathways at once will play an integral role in the primary prevention and treatment of CVD in the future.

Furthermore, polar lipids such as phosphatidylcholine (lecithin) are functional food ingredients and are currently used as food additives in a broad range of products including dairy products, instant drinks, baked goods, chocolate, and food supplements. Polar lipids are widely utilized as emulsification agents in the food manufacturing and pharmaceutical industry. The increased accessibility of high-quality polar lipids derived from animal and marine products or by-products will open the field of polar lipid research to new opportunities in food, both for their future use as a superior nutritional source of bioactive molecules with beneficial effects and for use in the food, pharmaceutical, nutraceutical, and cosmetic industries. Further research and development in these areas have the potential to induce significant social, commercial, health, and environmental benefits [32].

Acknowledgments The authors would like to thank the Department of Biological Sciences, University of Limerick, Limerick, Ireland, for their continued support.

References

1. Nicholson SK, Tucker GA, Brameld JM (2008) Effects of dietary polyphenols on gene expression in human vascular endothelial cells. Proc Nutr Soc 67:42–47
2. Wilkins E, Wilson L, Wickramasinghe K et al (2017) European cardiovascular disease statistics 2017. European Heart Network, Brussels
3. Lordan R, Zabetakis I (2017) Invited review: the anti-inflammatory properties of dairy lipids. J Dairy Sci 100:4197–4212
4. Libby P, Ridker PM, Hansson GK (2009) Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol 54:2129–2138
5. Tsoupras A, Lordan R, Zabetakis I (2018) Inflammation, not cholesterol, is a cause of chronic disease. Nutrients 10:604
6. Mozaffarian D, Appel LJ, Van Horn L (2011) Components of a cardioprotective diet new insights. Circulation 123:2870–2891

7. Menotti A, Puddu PE, Lanti M et al (2014) Lifestyle habits and mortality from all and specific causes of death: 40-year follow-up in the Italian Rural Areas of the Seven Countries Study. J Nutr Health Aging 18:314–321
8. Stampfer MJ, Hu FB, Manson JE et al (2000) Primary prevention of coronary heart disease in women through diet and lifestyle. N Engl J Med 343:16–22
9. Sanches Machado d'Almeida K, Ronchi Spillere S, Zuchinali P et al (2018) Mediterranean diet and other dietary patterns in primary prevention of heart failure and changes in cardiac function markers: a systematic review. Nutrients 10, 58
10. Martínez-González MA, Salas-Salvadó J, Estruch R et al (2015) Benefits of the Mediterranean diet: insights from the PREDIMED study. Prog Cardiovasc Dis 58:50–60
11. Estruch M, Sanchez-Quesada J, Beloki L et al (2013) The induction of cytokine release in monocytes by electronegative low-density lipoprotein (LDL) is related to its higher ceramide content than native LDL. Int J Mol Sci 14:2601
12. Panagiotakos DB, Notara V, Kouvari M et al (2016) The Mediterranean and other dietary patterns in secondary cardiovascular disease prevention: a review. Curr Vasc Pharmacol 14:442–451
13. de Lorgeril M (2013) Mediterranean diet and cardiovascular disease: historical perspective and latest evidence. Curr Atheroscler Rep 15:370
14. Shen J, Wilmot KA, Ghasemzadeh N et al (2015) Mediterranean dietary patterns and cardiovascular health. Annu Rev Nutr 35:425–449
15. Nasopoulou C, Zabetakis I (2015) Marine oils and inflammation. In: Zabetakis I (ed) Marine oils: from sea to pharmaceuticals. Nova Science Publishers, New York, p 179
16. Ravnskov U, Diamond DM, Hama R et al (2016) Lack of an association or an inverse association between low-density-lipoprotein cholesterol and mortality in the elderly: a systematic review. BMJ 6:e010401
17. Libby P, Ridker PM, Maseri A (2002) Inflammation and atherosclerosis. Circulation 105:1135–1143
18. Herder C, de las Heras Gala T, Carstensen-Kirberg M et al (2017) Circulating levels of interleukin 1-receptor antagonist and risk of cardiovascular disease: meta-analysis of six population-based cohorts. Arterioscler Thromb Vasc Biol 37:1222
19. Welsh P, Grassia G, Botha S et al (2017) Targeting inflammation to reduce cardiovascular disease risk: a realistic clinical prospect? Br J Pharmacol 174:3898
20. Woodward M, Rumley A, Welsh P et al (2007) A comparison of the associations between seven hemostatic or inflammatory variables and coronary heart disease. J Thromb Haemost 5:1795–1800
21. Welsh P, Murray HM, Ford I et al (2011) Circulating interleukin-10 and risk of cardiovascular events. Arterioscler Thromb Vasc Biol 31:2338
22. The Emerging Risk Factors Collaboration (2012) C-reactive protein, fibrinogen, and cardiovascular disease prediction. N Engl J Med 367:1310–1320
23. Moss JW, Ramji DP (2016) Cytokines: roles in atherosclerosis disease progression and potential therapeutic targets. Future Med Chem 8:1317–1330
24. Capra V, Bäck M, Barbieri SS et al (2013) Eicosanoids and their drugs in cardiovascular diseases: focus on atherosclerosis and stroke. Med Res Rev 33:364–438
25. Serhan CN (2007) Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol 25:101–137
26. Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 233:674–688
27. Spite M, Serhan CN (2010) Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. Circ Res 107:1170–1184
28. Serhan CN (2014) Novel pro-resolving lipid mediators in inflammation are leads for resolution physiology. Nature 510:92–101
29. Buckley Christopher D, Gilroy Derek W, Serhan Charles N (2014) Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity 40:315–327

30. Norris PC, Dennis EA (2012) Omega-3 fatty acids cause dramatic changes in TLR4 and purinergic eicosanoid signaling. Proc Natl Acad Sci USA 109:8517–8522
31. Aung T, Halsey J, Kromhout D et al (2018) Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: meta-analysis of 10 trials involving 77 917 individuals. JAMA Cardiol 3:225–234
32. Lordan R, Tsoupras A, Zabetakis I (2017) Phospholipids of animal and marine origin: structure, function, and anti-inflammatory properties. Molecules 22:1964
33. Schwingshackl L, Hoffmann G (2014) Mediterranean dietary pattern, inflammation and endothelial function: a systematic review and meta-analysis of intervention trials. Nutr Metab Cardiovasc Dis 24:929–939
34. Demopoulos C, Pinckard R, Hanahan DJ (1979) Platelet-activating factor. Evidence for 1-*O*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). J Biol Chem 254:9355–9358
35. Ishii S, Shimizu T (2000) Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. Prog Lipid Res 39:41–82
36. Honda Z, Ishii S, Shimizu T (2002) Platelet-activating factor receptor. J Biochem 131: 773–779
37. Palur Ramakrishnan AVK, Varghese TP, Vanapalli S et al (2017) Platelet activating factor: a potential biomarker in acute coronary syndrome? Cardiovasc Ther 35:64–70
38. Verouti SN, Tsoupras AB, Alevizopoulou F et al (2013) Paricalcitol effects on activities and metabolism of platelet activating factor and on inflammatory cytokines in hemodialysis patients. Int J Artif Organs 36:87–96
39. Clay KL, Johnson C, Worthen GS (1991) Biosynthesis of platelet activating factor and 1-*O*-acyl analogues by endothelial cells. Biochim Biophys Acta 1094:43–50
40. Tordai A, Franklin RA, Johnson C et al (1994) Autocrine stimulation of B lymphocytes by a platelet-activating factor receptor agonist, 1-palmitoyl-2-acetyl-sn-glycero-3-phosphocholine. J Immunol 152:566–573
41. Castro Faria Neto HC, Stafforini DM, Prescott SM et al (2005) Regulating inflammation through the anti-inflammatory enzyme platelet-activating factor-acetylhydrolase. Mem Inst Oswaldo Cruz 100:83–91
42. Yost CC, Weyrich AS, Zimmerman GA (2010) The platelet activating factor (PAF) signaling cascade in systemic inflammatory responses. Biochimie 92:692–697
43. Demopoulos CA, Karantonis HC, Antonopoulou S (2003) Platelet activating factor – a molecular link between atherosclerosis theories. Eur J Lipid Sci Technol 105:705–716
44. Tsoupras AB, Iatrou C, Frangia C et al (2009) The implication of platelet activating factor in cancer growth and metastasis: potent beneficial role of PAF-inhibitors and antioxidants. Infect Disord Drug Targets (Formerly Current Drug Targets-Infectious Disorders) 9: 390–399
45. Melnikova V, Bar-Eli M (2007) Inflammation and melanoma growth and metastasis: the role of platelet-activating factor (PAF) and its receptor. Cancer Metastasis Rev 26:359
46. Reznichenko A, Korstanje R (2015) The role of platelet-activating factor in mesangial pathophysiology. Am J Pathol 185:888–896
47. Braquet P, Hosford D, Braquet M et al (1989) Role of cytokines and platelet-activating factor in microvascular immune injury. Int Arch Allergy Immunol 88:88–100
48. Ocana JA, Romer E, Sahu R et al (2018) Platelet-activating factor–induced reduction in contact hypersensitivity responses is mediated by mast cells via cyclooxygenase-2–dependent mechanisms. J Immunol 200:4004
49. Koltai M, Hosford D, Guinot P et al (1991) Platelet activating factor (PAF). A review of its effects, antagonists and possible future clinical implications (Part I). Drugs 42:9–29
50. Koltai M, Hosford D, Guinot P et al (1991) PAF. A review of its effects, antagonists and possible future clinical implications (Part II). Drugs 42:174–204
51. Negro Alvarez JM, Miralles Lopez JC, Ortiz Martinez JL et al (1997) Platelet-activating factor antagonists. Allergol Immunopathol 25:249–258

52. Singh P, Singh IN, Mondal SC et al (2013) Platelet-activating factor (PAF)-antagonists of natural origin. Fitoterapia 84:180–201
53. Feige E, Mendel I, George J et al (2010) Modified phospholipids as anti-inflammatory compounds. Curr Opin Lipidol 21:525–529
54. Papakonstantinou VD, Lagopati N, Tsilibary EC et al (2017) A review on platelet activating factor inhibitors: could a new class of potent metal-based anti-inflammatory drugs induce anticancer properties? Bioinorg Chem Appl 2017:6947034
55. Marathe GK, Prescott SM, Zimmerman GA et al (2001) Oxidized LDL contains inflammatory PAF-like phospholipids. Trends Cardiovasc Med 11:139–142
56. Snyder F (1985) Chemical and biochemical aspects of platelet activating factor: a novel class of acetylated ether-linked choline-phospholipids. Med Res Rev 5:107–140
57. Snyder F (1995) Platelet-activating factor and its analogs: metabolic pathways and related intracellular processes. Biochim Biophys Acta 1254:231–249
58. Snyder F, Fitzgerald V, Blank ML (1996) Biosynthesis of platelet-activating factor and enzyme inhibitors. In: Nigam S, Kunkel G, Prescott SM (eds) Platelet-activating factor and related lipid mediators 2: roles in health and disease. Springer US, Boston, pp 5–10
59. Shindou H, Hishikawa D, Harayama T et al (2009) Recent progress on acyl CoA: lysophospholipid acyltransferase research. J Lipid Res 50:S46–S51
60. Imaizumi TA, Stafforini DM, Yamada Y et al (1995) Platelet-activating factor: a mediator for clinicians. J Intern Med 238:5–20
61. Tsoupras AB, Fragopoulou E, Nomikos T et al (2007) Characterization of the de novo biosynthetic enzyme of platelet activating factor, DDT-insensitive cholinephosphotransferase, of human mesangial cells. Mediat Inflamm 2007:27683
62. Tsoupras AB, Chini M, Mangafas N et al (2011) Platelet-activating factor and its basic metabolic enzymes in blood of naive HIV-infected patients. Angiology 63:343–352. https://doi.org/10.1177/0003319711420608
63. Tsoupras AB, Chini M, Mangafas N et al (2012) Platelet-activating factor and its basic metabolic enzymes in blood of naive HIV-infected patients. Angiology 63:343–352
64. Nasopoulou C, Tsoupras AB, Karantonis HC et al (2011) Fish polar lipids retard atherosclerosis in rabbits by down-regulating PAF biosynthesis and up-regulating PAF catabolism. Lipids Health Dis 10:1–18
65. Detopoulou P, Nomikos T, Fragopoulou E et al (2009) Platelet activating factor (PAF) and activity of its biosynthetic and catabolic enzymes in blood and leukocytes of male patients with newly diagnosed heart failure. Clin Biochem 42:44–49
66. Papakonstantinou VD, Chini M, Mangafas N et al (2014) In vivo effect of two first-line ART regimens on inflammatory mediators in male HIV patients. Lipids Health Dis 13:90–90
67. Marathe GK, Zimmerman GA, Prescott SM et al (2002) Activation of vascular cells by PAF-like lipids in oxidized LDL. Vasc Pharmacol 38:193–200
68. Stafforini DM (2009) Biology of platelet-activating factor acetylhydrolase (PAF-AH, lipoprotein associated phospholipase A2). Cardiovasc Drugs Ther 23:73–83
69. Karasawa K, Inoue K (2015) Overview of PAF-degrading enzymes. Enzymes 38:1–22
70. Stafforini DM, Zimmerman GA (2014) Unraveling the PAF-AH/Lp-PLA(2) controversy. J Lipid Res 55:1811–1814
71. Tellis CC, Tselepis A (2014) Pathophysiological role and clinical significance of lipoprotein-associated phospholipase A2 (Lp-PLA2) bound to LDL and HDL. Curr Pharm Des 20:6256–6269
72. Appleyard CB, Hillier K (1995) Biosynthesis of platelet-activating factor in normal and inflamed human colon mucosa: evidence for the involvement of the pathway of platelet-activating factor synthesis *de novo* in inflammatory bowel disease. Clin Sci 88:713–717
73. Detopoulou P, Nomikos T, Fragopoulou E et al (2013) Platelet activating factor in heart failure: potential role in disease progression and novel target for therapy. Curr Heart Fail Rep 10:122–129

74. Detopoulou P, Fragopoulou E, Nomikos T et al (2012) Baseline and 6-week follow-up levels of PAF and activity of its metabolic enzymes in patients with heart failure and healthy volunteers – a pilot study. Angiology 64:522–528
75. Vance JE, Vance DE (2008) Biochemistry of lipids, lipoproteins and membranes. Elsevier, Oxford, UK
76. Andreyev AY, Fahy E, Guan Z et al (2010) Subcellular organelle lipidomics in TLR-4-activated macrophages. J Lipid Res 51:2785–2797
77. Quehenberger O, Armando AM, Brown AH et al (2010) Lipidomics reveals a remarkable diversity of lipids in human plasma. J Lipid Res 51:3299–3305
78. Dowhan W, Bogdanov M, Mileykovskaya E (2008) Functional roles of lipids in membranes. In: Biochemistry of lipids, lipoproteins and membranes, 5th edn. Elsevier, Amsterdam, pp 1–37
79. Paradies G, Petrosillo G, Paradies V et al (2010) Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. J Pineal Res 48:297–310
80. Yin H, Zhu M (2012) Free radical oxidation of cardiolipin: chemical mechanisms, detection and implication in apoptosis, mitochondrial dysfunction and human diseases. Free Radic Res 46:959–974
81. Musatov A, Robinson NC (2012) Susceptibility of mitochondrial electron-transport complexes to oxidative damage. Focus on cytochrome c oxidase. Free Radic Res 46:1313–1326
82. Sioriki E, Smith TK, Demopoulos CA et al (2016) Structure and cardioprotective activities of polar lipids of olive pomace, olive pomace-enriched fish feed and olive pomace fed gilthead sea bream (*Sparus aurata*). Food Res Int 83:143–151
83. Nasopoulou C, Smith T, Detopoulou M et al (2014) Structural elucidation of olive pomace fed sea bass (*Dicentrarchus labrax*) polar lipids with cardioprotective activities. Food Chem 145:1097–1105
84. Minihane AM, Vinoy S, Russell WR et al (2015) Low-grade inflammation, diet composition and health: current research evidence and its translation. Br J Nutr 114:999–1012
85. Galland L (2010) Diet and inflammation. Nutr Clin Pract 25:634–640
86. Shivappa N, Bonaccio M, Hebert JR et al (2018) Association of pro-inflammatory diet with low-grade inflammation: results from the Moli-sani study. Nutrition 54:182
87. O'Keefe JH, Gheewala NM, O'Keefe JO (2008) Dietary strategies for improving post-prandial glucose, lipids, inflammation, and cardiovascular health. J Am Coll Cardiol 51:249–255
88. de Koning EJP, Rabelink TJ (2002) Endothelial function in the post-prandial state. Atheroscler Suppl 3:11–16
89. Hyson D, Rutledge JC, Berglund L (2003) Postprandial lipemia and cardiovascular disease. Curr Atheroscler Rep 5:437–444
90. Jansen F, Yang X, Franklin BS et al (2013) High glucose condition increases NADPH oxidase activity in endothelial microparticles that promote vascular inflammation. Cardiovasc Res 98:94–106
91. Shrestha C, Ito T, Kawahara K et al (2013) Saturated fatty acid palmitate induces extracellular release of histone H3: a possible mechanistic basis for high-fat diet-induced inflammation and thrombosis. Biochem Biophys Res Commun 437:573–578
92. Bessueille L, Magne D (2015) Inflammation: a culprit for vascular calcification in atherosclerosis and diabetes. Cell Mol Life Sci 72:2475–2489
93. Nathan C (2002) Points of control in inflammation. Nature 420:846–852
94. Le Gouic AV, Harnedy PA, FitzGerald RJ (2018) Bioactive peptides from fish protein by-products. In: Mérillon J-M, Ramawat KG (eds) Bioactive molecules in food. Springer International Publishing, Cham, pp 1–35
95. Renzella J, Townsend N, Jewell J et al (2018) What national and subnational interventions and policies based on Mediterranean and Nordic diets are recommended or implemented in the WHO European Region, and is there evidence of effectiveness in reducing noncommunicable diseases? World Health Organization, WHO Regional Office for Europe, Copenhagen

96. de Lorgeril M, Salen P, Martin JL et al (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation 99:779–785
97. Knoops KT, de Groot LC, Kromhout D et al (2004) Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. JAMA 292:1433–1439
98. Lerner A, Matthias T (2015) Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. Autoimmun Rev 14:479–489
99. Rauber F, da Costa Louzada ML, Steele E et al (2018) Ultra-processed food consumption and chronic non-communicable diseases-related dietary nutrient profile in the UK (2008–2014). Nutrients 10:587
100. Tsoupras AB, Fragopoulou E, Iatrou C et al (2011) In vitro protective effects of olive pomace polar lipids towards platelet activating factor metabolism in human renal cells. Curr Top Nutraceutical Res 9:105
101. Petsini F, Fragopoulou E, Antonopoulou S (2018) Fish consumption and cardiovascular disease related biomarkers: a review of clinical trials. Crit Rev Food Sci Nutr. https://doi.org/10.1080/10408398.2018.1437388
102. Din JN, Newby DE, Flapan AD (2004) Omega 3 fatty acids and cardiovascular disease – fishing for a natural treatment. BMJ 328:30–35
103. Megson IL, Whitfield PD, Zabetakis I (2016) Lipids and cardiovascular disease: where does dietary intervention sit alongside statin therapy? Food Funct 7:2603–2614
104. Rementzis J, Antonopoulou S, Argyropoulos D et al (1996) Biologically active lipids from *S. scombrus*. In: Nigam S., Kunkel G., Prescott S.M. (eds) Platelet-activating factor and related lipid mediators 2. Advances in Experimental Medicine and Biology 416, Springer, pp 65–72
105. Panayiotou A, Samartzis D, Nomikos T et al (2000) Lipid fractions with aggregatory and antiaggregatory activity toward platelets in fresh and fried cod (*Gadus morhua*): correlation with platelet-activating factor and atherogenesis. J Agric Food Chem 48:6372–6379
106. Nasopoulou C, Nomikos T, Demopoulos C et al (2007) Comparison of antiatherogenic properties of lipids obtained from wild and cultured sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). Food Chem 100:560–567
107. Nasopoulou C, Stamatakis G, Demopoulos CA et al (2011) Effects of olive pomace and olive pomace oil on growth performance, fatty acid composition and cardio protective properties of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). Food Chem 129:1108–1113
108. Nasopoulou C, Psani E, Sioriki E et al (2013) Evaluation of sensory and in vitro cardio protective properties of sardine (*Sardina pilchardus*): the effect of grilling and brining. Food Nutr Sci 4:940
109. Sioriki E, Nasopoulou C, Demopoulos CA et al (2015) Comparison of sensory and cardioprotective properties of olive-pomace enriched and conventional gilthead sea bream (*Sparus aurata*): the effect of grilling. J Aquat Food Prod Technol 24:782–795
110. Nasopoulou C, Karantonis HC, Perrea DN et al (2010) In vivo anti-atherogenic properties of cultured gilthead sea bream (*Sparus aurata*) polar lipid extracts in hypercholesterolaemic rabbits. Food Chem 120:831–836
111. Nasopoulou C, Karantonis HC, Andriotis M et al (2008) Antibacterial and anti-PAF activity of lipid extracts from sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). Food Chem 111:433–438
112. Morphis G, Kyriazopoulou A, Nasopoulou C et al (2016) Assessment of the in vitro anti-thrombotic properties of sardine (*Sardina pilchardus*) fillet lipids and cod liver oil. Fishes 1:1–15
113. Tsoupras A, Lordan R, Demuru M et al (2018) Structural elucidation of Irish organic farmed salmon (*Salmo salar*) polar lipids with antithrombotic properties. Mar Drugs 16, 176

114. Lordan R, Tsoupras A, Mitra B et al (2018) Dairy fats and cardiovascular disease: do we really need to be concerned? Foods 7:29
115. Lecomte M, Bourlieu C, Michalski M-C (2017) Nutritional properties of milk lipids: specific function of the milk fat globule. In: Collier RJ, Preedy VR (eds) Dairy in human health and disease across the lifespan. Academic, Cambridge, MA, pp 435–452
116. Le TT, Phan TTQ, Camp JV et al (2015) Milk and dairy polar lipids: occurrence, purification, nutritional and technological properties. In: Ahmad MU, Xu X (eds) Polar lipids: biology, chemistry, and technology. AOCS Press, Urbana, pp 91–143
117. Dewettinck K, Rombaut R, Thienpont N et al (2008) Nutritional and technological aspects of milk fat globule membrane material. Int Dairy J 18:436–457
118. Rombaut R, Dewettinck K (2006) Properties, analysis and purification of milk polar lipids. Int Dairy J 16:1362–1373
119. Lopez C (2011) Milk fat globules enveloped by their biological membrane: unique colloidal assemblies with a specific composition and structure. Curr Opin Colloid Interface Sci 16:391–404
120. Lopez C, Briard-Bion V, Ménard O (2014) Polar lipids, sphingomyelin and long-chain unsaturated fatty acids from the milk fat globule membrane are increased in milks produced by cows fed fresh pasture based diet during spring. Food Res Int 58:59–68
121. Rodríguez-Alcalá LM, Fontecha J (2010) Major lipid classes separation of buttermilk, and cows, goats and ewes milk by high performance liquid chromatography with an evaporative light scattering detector focused on the phospholipid fraction. J Chromatogr A 1217:3063–3066
122. Contarini G, Povolo M (2013) Phospholipids in milk fat: composition, biological and technological significance, and analytical strategies. Int J Mol Sci 14:2808
123. Tsorotioti SE, Nasopoulou C, Detopoulou M et al (2014) In vitro anti-atherogenic properties of traditional Greek cheese lipid fractions. Dairy Sci Technol 94:269–281
124. Poutzalis S, Anastasiadou A, Nasopoulou C et al (2016) Evaluation of the in vitro anti-atherogenic activities of goat milk and goat dairy products. Dairy Sci Technol 96:317–327
125. Megalemou K, Sioriki E, Lordan R et al (2017) Evaluation of sensory and in vitro anti-thrombotic properties of traditional Greek yogurts derived from different types of milk. Heliyon 3:Article e00227
126. Antonopoulou S, Semidalas CE, Koussissis S et al (1996) Platelet-activating factor (PAF) antagonists in foods: a study of lipids with PAF or anti-PAF-like activity in cow's milk and yogurt. J Agric Food Chem 44:3047–3051
127. Lordan R, Zabetakis I (2017) Ovine and caprine lipids promoting cardiovascular health in milk and its derivatives. Adv Dairy Res 5:176
128. Karantonis HC, Antonopoulou S, Perrea DN et al (2006) In vivo antiatherogenic properties of olive oil and its constituent lipid classes in hyperlipidemic rabbits. Nutr Metab Cardiovasc Dis 16:174–185
129. Xanthopoulou MN, Kalathara K, Melachroinou S et al (2017) Wine consumption reduced postprandial platelet sensitivity against platelet activating factor in healthy men. Eur J Nutr 56 (4):1485. https://doi.org/10.1007/s00394-016-1194-0
130. Küllenberg D, Taylor LA, Schneider M et al (2012) Health effects of dietary phospholipids. Lipids Health Dis 11:1
131. Cohn J, Kamili A, Wat E et al (2010) Dietary phospholipids and intestinal cholesterol absorption. Nutrients 2:116
132. Weihrauch JL, Son Y-S (1983) Phospholipid content of foods. J Am Oil Chem Soc 60:1971–1978
133. Zancada L, Pérez-Díez F, Sánchez-Juanes F et al (2013) Phospholipid classes and fatty acid composition of ewe's and goat's milk. Grasas Aceites 64:304–310
134. Fragopoulou E, Choleva M, Antonopoulou S et al (2018) Wine and its metabolic effects. A comprehensive review of clinical trials. Metabolism 83:102

135. Temple NJ (2016) What are the health implications of alcohol consumption? In: Wilson T, Temple NJ (eds) Beverage impacts on health and nutrition, 2nd edn. Springer International Publishing, Cham, pp 69–81
136. Schwarzinger M, Thiébaut SP, Baillot S et al (2017) Alcohol use disorders and associated chronic disease – a national retrospective cohort study from France. BMC Public Health 18:43
137. Arranz S, Chiva-Blanch G, Valderas-Martínez P et al (2012) Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. Nutrients 4:759
138. de Gaetano G, Costanzo S, Di Castelnuovo A et al (2016) Effects of moderate beer consumption on health and disease: a consensus document. Nutr Metab Cardiovasc Dis 26:443–467
139. Rimm EB, Williams P, Fosher K et al (1999) Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 319:1523–1528
140. Fragopoulou E, Demopoulos CA, Antonopoulou S (2009) Lipid minor constituents in wines. A biochemical approach in the French paradox. Int J Wine Res 1:131–143
141. Soleas GJ, Diamandis EP, Goldberg DM (1997) Wine as a biological fluid: history, production, and role in disease prevention. J Clin Lab Anal 11:287–313
142. Dell'Agli M, Buscialà A, Bosisio E (2004) Vascular effects of wine polyphenols. Cardiovasc Res 63:593–602
143. Fragopoulou E, Antonopoulou S, Tsoupras A et al (2004) Antiatherogenic properties of red/white wine, musts, grape-skins, and yeast. In: 45th international conference on the bioscience of lipids. Elsevier, University of Ioannina, Greece, p 66
144. Fragopoulou E, Nomikos T, Tsantila N et al (2001) Biological activity of total lipids from red and white wine/must. J Agric Food Chem 49:5186–5193
145. Fragopoulou E, Antonopoulou S, Demopoulos CA (2002) Biologically active lipids with antiatherogenic properties from white wine and must. J Agric Food Chem 50:2684–2694
146. Fragopoulou E, Nomikos T, Antonopoulou S et al (2000) Separation of biologically active lipids from red wine. J Agric Food Chem 48:1234–1238
147. Xanthopoulou MN, Asimakopoulos D, Antonopoulou S et al (2014) Effect of Robola and Cabernet Sauvignon extracts on platelet activating factor enzymes activity on U937 cells. Food Chem 165:50–59
148. Renaud SC, Beswick AD, Fehily AM et al (1992) Alcohol and platelet aggregation: the Caerphilly Prospective Heart Disease Study. Am J Clin Nutr 55:1012–1017
149. Renaud S, de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339:1523–1526
150. Argyrou C, Vlachogianni I, Stamatakis G et al (2017) Postprandial effects of wine consumption on Platelet Activating Factor metabolic enzymes. Prostaglandins Other Lipid Mediat 130:23
151. Sikand G, Kris-Etherton P, Boulos NM (2015) Impact of functional foods on prevention of cardiovascular disease and diabetes. Curr Cardiol Rep 17:39
152. Rozati M, Barnett J, Wu D et al (2015) Cardio-metabolic and immunological impacts of extra virgin olive oil consumption in overweight and obese older adults: a randomized controlled trial. Nutr Metab 12:28
153. López-Miranda J, Pérez-Jiménez F, Ros E et al (2010) Olive oil and health: summary of the II international conference on olive oil and health consensus report. Nutr Metab Cardiovasc Dis 20:284–294
154. Covas M-I, de la Torre R, Fitó M (2015) Virgin olive oil: a key food for cardiovascular risk protection. Br J Nutr 113:S19–S28
155. Karantonis HC (2017) Antiatherogenic properties of olive oil glycolipids. In: Kiritsakis A, Shahidi F (eds) Olives and olive oil as functional foods. John Wiley & Sons Ltd, Chichester UK
156. Karantonis HC, Antonopoulou S, Demopoulos CA (2002) Antithrombotic lipid minor constituents from vegetable oils. Comparison between olive oils and others. J Agric Food Chem 50:1150–1160

157. Tsantila N, Karantonis HC, Perrea DN et al (2007) Antithrombotic and antiatherosclerotic properties of olive oil and olive pomace polar extracts in rabbits. Mediat Inflamm 2007:36204
158. Tsantila N, Karantonis HC, Perrea DN et al (2010) Atherosclerosis regression study in rabbits upon olive pomace polar lipid extract administration. Nutr Metab Cardiovasc Dis 20:740–747
159. Karantonis HC, Tsantila N, Stamatakis G et al (2008) Bioactive polar lipids in olive oil, pomace and waste byproducts. J Food Biochem 32:443–459
160. Nasopoulou C, Zabetakis I (2013) Agricultural and aquacultural potential of olive pomace a review. J Agric Sci 5:116
161. Masana L, Ros E, Sudano I et al (2017) Is there a role for lifestyle changes in cardiovascular prevention? What, when and how? Atheroscler Suppl 26:2–15
162. Lim SS, Vos T, Flaxman AD et al (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the global burden of disease study 2010. Lancet 380:2224–2260
163. Ravera A, Carubelli V, Sciatti E et al (2016) Nutrition and cardiovascular disease: finding the perfect recipe for cardiovascular health. Nutrients 8:363
164. Anand SS, Hawkes C, de Souza RJ et al (2015) Food consumption and its impact on cardiovascular disease: importance of solutions focused on the globalized food system: a report from the workshop convened by the World Heart Federation. J Am Coll Cardiol 66:1590–1614
165. Vogt TM, Appel LJ, Obarzanek EVA et al (1999) Dietary approaches to stop hypertension. J Acad Nutr Diet 99:S12–S18
166. Willett WC, Sacks F, Trichopoulou A et al (1995) Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr 61:1402s–1406s
167. Trichopoulou A, Martínez-González MA, Tong TY et al (2014) Definitions and potential health benefits of the Mediterranean diet: views from experts around the world. BMC Med 12:112
168. World Cancer Research Fund, American Institute for Cancer Research (2007) Food, nutrition, physical activity, and the prevention of cancer: a global perspective. American Institute for Cancer Research, Washington, DC
169. Davis C, Bryan J, Hodgson J et al (2015) Definition of the Mediterranean diet: a literature review. Nutrients 7:9139–9153
170. Castro-Quezada I, Román-Viñas B, Serra-Majem L (2014) The Mediterranean diet and nutritional adequacy: a review. Nutrients 6:231
171. Bach-Faig A, Berry EM, Lairon D et al (2011) Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr 14:2274–2284
172. Bos MB, de Vries JHM, Feskens EJM et al (2010) Effect of a high monounsaturated fatty acids diet and a Mediterranean diet on serum lipids and insulin sensitivity in adults with mild abdominal obesity. Nutr Metab Cardiovasc Dis 20:591–598
173. Serra-Majem L, Bes-Rastrollo M, Román-Viñas B et al (2009) Dietary patterns and nutritional adequacy in a Mediterranean country. Br J Nutr 101:S21–S28
174. Rodríguez-Rejón AI, Castro-Quezada I, Ruano-Rodríguez C et al (2014) Effect of a Mediterranean diet intervention on dietary glycemic load and dietary glycemic index: the PREDIMED study. J Nutr Metab 2014:985373
175. Estruch R, Martínez-González MA, Corella D et al (2009) Effects of dietary fibre intake on risk factors for cardiovascular disease in subjects at high risk. J Epidemiol Community Health 63:582–588
176. Estruch R, Martinez-Gonzalez MA, Corella D et al (2006) Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med 145:1–11
177. Turati F, Bravi F, Polesel J et al (2017) Adherence to the Mediterranean diet and nasopharyngeal cancer risk in Italy. Cancer Causes Control 28:89–95
178. Giacosa A, Barale R, Bavaresco L et al (2013) Cancer prevention in Europe: the Mediterranean diet as a protective choice. Eur J Cancer Prev 22:90–95

179. Rossi M, Turati F, Lagiou P et al (2013) Mediterranean diet and glycaemic load in relation to incidence of type 2 diabetes: results from the Greek cohort of the population-based European Prospective Investigation into Cancer and Nutrition (EPIC). Diabetologia 56:2405–2413
180. Sofi F, Abbate R, Gensini GF et al (2010) Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr 92:1189–1196
181. Sofi F, Macchi C, Abbate R et al (2013) Mediterranean diet and health. Biofactors 39:335–342
182. Chrysohoou C, Panagiotakos DB, Pitsavos C et al (2004) Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: the Attica study. J Am Coll Cardiol 44:152–158
183. Detopoulou P, Demopoulos C, Karantonis H et al (2015) Mediterranean diet and its protective mechanisms against cardiovascular disease: an insight into platelet activating factor (PAF) and diet interplay. Ann Nutr Disord Ther 2:1–10
184. Nasopoulou C, Gogaki V, Stamatakis G et al (2013) Evaluation of the in vitro anti-atherogenic properties of lipid fractions of olive pomace, olive pomace enriched fish feed and gilthead sea bream (*Sparus aurata*) fed with olive pomace enriched fish feed. Mar Drugs 11:3676
185. Nasopoulou C, Gogaki V, Panagopoulou E et al (2013) Hen egg yolk lipid fractions with antiatherogenic properties. Anim Sci J 84:264–271
186. Apitz-Castro R, Cabrera S, Cruz MR et al (1983) Effects of garlic extract and of three pure components isolated from it on human platelet aggregation, arachidonate metabolism, release reaction and platelet ultrastructure. Thromb Res 32:155–169
187. Violi F, Pratico D, Ghiselli A et al (1990) Inhibition of cyclooxygenase-independent platelet aggregation by low vitamin E concentration. Atherosclerosis 82:247–252
188. Kakishita E, Suehiro A, Oura Y et al (1990) Inhibitory effect of vitamin E (alpha-tocopherol) on spontaneous platelet aggregation in whole blood. Thromb Res 60:489–499
189. Capasso R, Pinto L, Vuotto ML et al (2000) Preventive effect of eugenol on PAF and ethanol-induced gastric mucosal damage. Fitoterapia 71:S131–S137
190. Park EJ, Suh M, Thomson B et al (2005) Dietary ganglioside decreases cholesterol content, caveolin expression and inflammatory mediators in rat intestinal microdomains. Glycobiology 15:935–942
191. Rizzo M, Otvos J, Nikolic D et al (2014) Subfractions and subpopulations of HDL: an update. Curr Med Chem 21:2881–2891
192. Marathe GK, Pandit C, Lakshmikanth CL et al (2014) To hydrolyze or not to hydrolyze: the dilemma of platelet-activating factor acetylhydrolase. J Lipid Res 55:1847–1854
193. Poutzalis S, Lordan R, Nasopoulou C et al. (2018) Phospholipids of goat and sheep origin: Structural and functional studies. Small Ruminant Research 167:39–47

Neuroprotective and Antiaging Essential Oils and Lipids in Plants

20

Mamali Das and Kasi Pandima Devi

Contents

Abstract

Age-related neurological disorders, such as Alzheimer's and Parkinson's disease, have a huge medical and economical impact in both the industrialized and nonindustrialized countries. Neurodegenerative diseases alone affect 74 million people worldwide and among them, 6.8 million die every year. Essential oils (EOs) and plant lipids (PLs) are used since long time in traditional medicine for their ability to manage a wide range of diseases. There are numerous reports on the neuroprotective and antiaging potentials and mechanism of PLs and EOs.

M. Das · K. Pandima Devi (✉)
Department of Biotechnology, Alagappa University [Science Campus], Karaikudi, Tamil Nadu, India
e-mail: mamagujrat.das@gmail.com; devikasi@yahoo.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_89

Several clinically important EOs and their components from *Mentha piperita, Eucalyptus globulus, Nigella sativa, Jasminum sambac, Rosmarinus officinalis*, and plant-derived lipids like stearidonic acid (SDA) from Echium oil, stigmasterol, β-sitosterol, from *Datura innoxa*, palmitic acid, linoleic acid from *Celastrus paniculatus*, and many more plants are reported for their neuroprotective and antiaging effects. This chapter aims to emphasize on the current finding on EOs and PLs tested against aging-associated neurodegenerative disorders like Alzheimer disease (AD) and possible molecular mechanism of their neuroprotective effects.

Keywords
Essential oils · Plant lipids · Alzheimer's disease · Cholinesterase inhibitors · Antioxidants · Amyloid-β · NFTs · Dementia · BACE1

1 Introduction

Aging is a time-dependent natural process that affects almost all living organisms. The oldest definition by Harman defines aging as the progressive accumulation of changes with time that are associated with the ever-increasing susceptibility to disease and death [1]. Though aging is one of the most popular topics among mankind from the historical time period, inauguration of aging research could be addressed back to only 30 years with the discovery of first long-lived strains in *Caenorhabditis elegans* (*C. elegans*) [2]. Currently, age is regarded as the main risk factor for prevalent diseases, including cardiovascular diseases, neurodegenerative disorders, and cancer [3]. Aging leads to accumulation of genetic damage which promotes aging-associated diseases [4, 5]. The epigenetic changes like alteration in DNA methylation, posttranslational histone modification, and chromatin remodeling also augment aging process [6]. Additionally, chronic expression of unfolded or misfolded proteins contributes to the development of some specific and fatal age-related neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and cataracts (Fig. 1) [7]. Aging also reduces efficacy of the respiratory chain by dysfunctioning mitochondrial membrane potential thus increasing electron leakage and reducing ATP generation mediated by activation of inflammasomes [8], therefore mitochondrial dysfunction and aging process has been suspected to be linked.

Essential oils (EOs) represent a mixture of highly complex, naturally occurring plant secondary metabolites abundantly present in flowers, leaves, seeds, rhizomes, and barks and are usually isolated via hydrodistillation and cold pressing methods [9]. They are used as spiritual, mental, and physical healing agents from very ancient time period [10] and nowadays are used as traditional medicines, aromatherapy, massage therapies, as well as in cosmetics, perfumes, and food industries [11, 12]. In Egypt, Greece, and Rome, EOs extracted from aromatic plants were employed for the prevention and treatment of various diseases [13]. EOs act as natural safeguards in plants with antimicrobial, herbivores repellent, and insect attractants and thus are

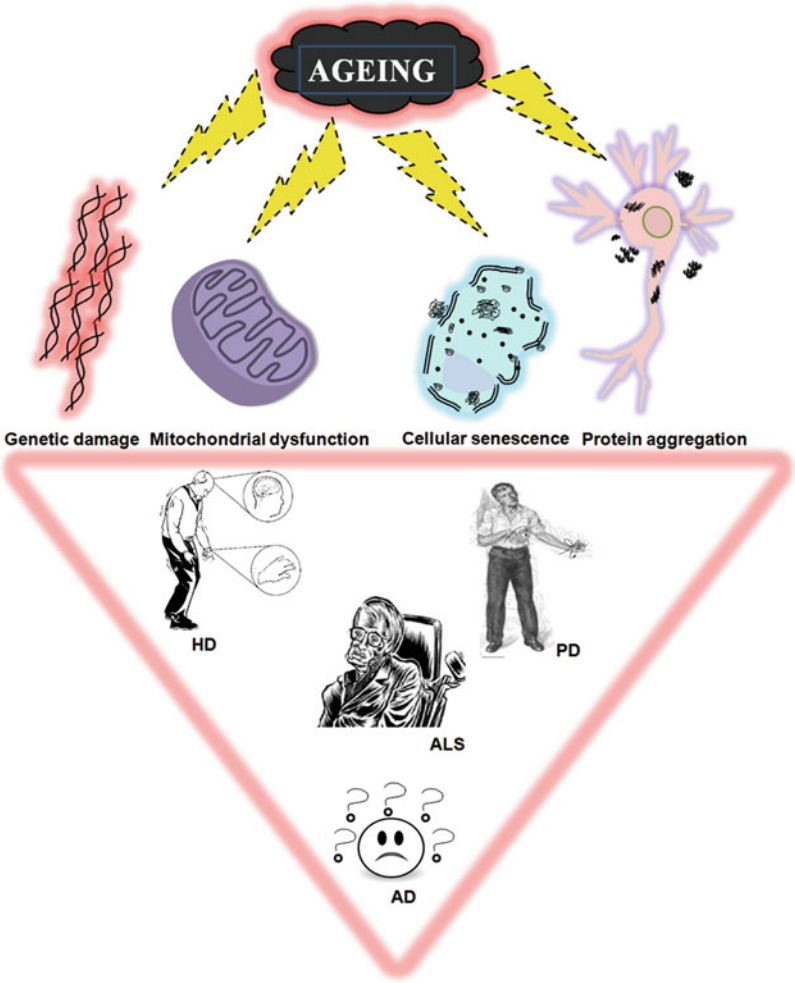


Fig. 1 Aging as a cause of neurodegenerative diseases like AD: Alzheimer's disease, HD: Huntington's disease, PD: Parkinson's disease, and ALS: amyotrophic lateral sclerosis

hugely used as antibacterial, insecticidal, and antifungal agents. Recently, the use of EOs has dragged much scientific attention for the management of several neurological diseases due to improved knowledge on their structure and other biological activities.

Lipids which are basically fatty acids (straight carbon chain, with hydrogen atoms at one end and a carboxyl group (–COOH) at the other end) are important component of fat-soluble part of plants, animals, and microorganisms. Some naturally synthesized fatty acids like omega-9 (ω-9), which need not to be obtained through diets, are termed as nonessential fatty acids [14], while some fatty acids like omega-3 (ω-3) and omega-6 (ω-6) fatty acids (Fig. 2) which form a major part of neuronal tissue

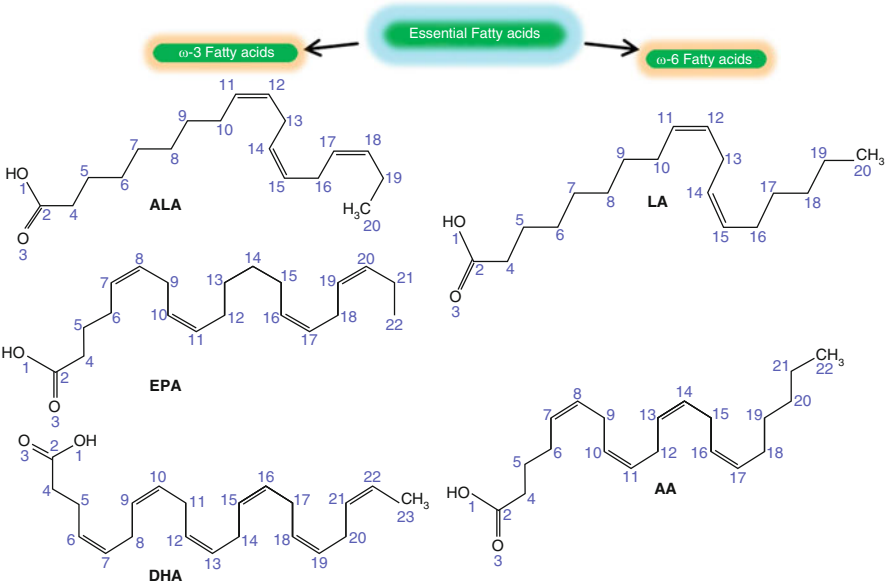


Fig. 2 Major essential fatty acids. ALA: alpha linoleic acid, EPA: eicosapentanoic acid, DHA: docosahexanoic acid, LA: linoleic acid, and AA: arachidonic acid

cannot be synthesized by the body itself and need to be obtained through plant and marine fish oil are considered as essential. In recent years, these ω-3 fatty acids have been found to be critical for the development of human nervous system particularly during neonatal phase [15]. At the same time, its deficiency in adults lead to severe abnormalities in learning, neural, and visual systems and also may cause obesity, cardiovascular disease, inflammation, and cancer. Therefore, investigation of health benefits of dietary supplementation of ω-3 fatty acids has become a promising area for drug developing bodies.

2 Chemistry of Essential Oils

Essential oils are concentrated hydrophobic liquids with several volatile aromatic compounds and have many synonyms like volatile oils, ethereal oils, or aetherolea. Usually, they consist of low molecular weight compounds with terpenes and aliphatic compounds in variable concentrations [16]. Some EOs have usually higher concentrations (20–95%) of two or three components, whereas, other components are present in minor amounts. For instance, linalool constitutes 68% of the *Coriandrum sativum* EO, whereas, α/β thuyone and camphor constitute 57% and 24% of *Artemisia Herbaalba* EO, respectively. Similarly, D-limonene constitutes >80% of citrus peel oils, α-phellandrene and limonene make 36% and 31% of *Anethum graveolens* leaf EO, respectively. The chemical composition of EOs vary

both numerically and stereochemically depending on several factors like extraction techniques, climate, plant origin, soil composition, vegetative cycle stage, and age [17]; therefore, they are needed to be extracted from the same plant part and harvested under the same growing conditions and in most efficient season. Essential oils combat wide range of health issues that are listed in Table 1.

3 Chemistry of Plant Lipids

Lipids including fatty acids, fats, oils, steroids (sterols), waxes, cutin, suberin, glycerophospholipids (phospholipids), glyceroglycolipids (glycosylglycerides), terpenes, and tochopherols are ubiquitous compounds in plants. Several groups of lipids have been shown to provide health benefits either through modification of tissue fatty acid composition or induction of cell signaling pathways. While some health benefits are derived from consumption of short- to medium-chain fatty acids, evidence suggests that the polyunsaturated fatty acids (PUFAs) are the most important bioactive lipids. Among the essential PUFAs, the most important are ω-3 and ω-36 fatty acids. PUFAs are found mostly in plant seed oils and are important substrates for the biosynthesis of cellular hormones (eicosanoids) and other signaling compounds that modulate human health. They are essential for storing metabolic energy, provide protection against pathogens and dehydration, they carry electrons, and absorb light. Gas-liquid chromatography (GLC) and normal-phase high-performance liquid chromatography (HPLC) analysis of fatty acids and fat-soluble

Table 1 Common essential oils and their health benefits

Name of essential oil	Effective for
Lemon	Toning, circulation, and respiration
Lavender	Acne and relaxation
Orange	Respiration and skin moisturizer
Pine	Anxiety, dump skin, and muscle ache
Rose	Obstruct menses and dry skin
Rosemary	Dandruff, digestion, and pain relief
Sandalwood	Insomnia and respiratory infection
Sage	Joint pain, fever, and appetite loss
Tea tree	Fungal infection in skin
Patchouli	Sexuality problem and damaged skin
Peppermint	Poor digestion and sore feet
Cinnamon	Poor digestion and nausea
Ginger	Stomach upset and muscle ache
Clove	Respiratory and skin infections
Fennel	Menstrual dysfunction and constipation
Jasmine	Depression
Grapefruit	Depression
Cedarwood	Eczema and acne

bioactives from Datura and Hyoscyamus seeds showed major fatty acid as linoleic acid followed by oleic, palmitic, and stearic acids. Also, the crude seed extract was rich in phytosterols, like stigmasterol, β-sitosterol, lanosterol, D5-avenasterol, and sitostanol. Datura seed extracts showed stronger radical scavenging activity than Hyoscyamus species [18]. Similarly, studies on the lipid profile of Indian *Celastrus paniculatus* seed oil revealed oleic, palmitic, and linoleic as the major fatty acids followed by glycolipids and phospholipids in its oil. Phytosterol like β-sitosterol were also found in high amount with campesterol and stigmasterol in low amount. *Celastrus paniculatus* oil exhibited stronger radical scavenging activity [19]. Plants especially leafy vegetables and nuts are rich in ω-3 PUFAs, and these are currently the main source to obtain α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [20]. ALA is commonly present in plant sources walnuts, flax seeds, butternuts, red and black currant seeds, pumpkin seeds, wheat germ, soy and canola oil, and leafy green plants like Purslane in high concentrations. *Perilla frutescens* seed oil is also a rich source of ω-3 linolenic acid [21]. Among the plant sources, flax seeds have the highest concentration of ALA.

4 Are Aging and Neurodegenerative Diseases Related?

As we age, along with some visible changes like hair and skin, our brain and central nervous system also undergo the aging process. This is one of the facts that people are more likely to suffer from many neurological problems after the age of 65. Aging makes the patients more vulnerable to irreversible diseases like Alzheimer's disease, Parkinson's disease, and stroke and thus has been considered as a major risk factor of the above said diseases. With advances in molecular biology, many age-related signaling pathways like rapamycin (TOR) [22], and insulin/IGF-1 signaling, have been found to be connected with neurodegeneration [23]. Most of the age-related neurodegenerative diseases like AD are characterized by accumulation of disease-specific misfolded proteins in the central nervous system which leads to neurodegeneration, synaptic dysfunction, and neuroinflammation in central nervous system, causing several pathological effects [24] (Table 2).

5 Alzheimer's Disease

Alzheimer's disease (AD) is the fifth leading age-related neurological disorders in the world and is characterized by cognitive dysfunction, memory loss, psychological changes, and disability to perform day-to-day activities due to oxidative stress-induced scarcity in cholinergic neurotransmission, accumulation of amyloid plaques (amyloid-β, Aβ), and neurofibrillary tangles (NFTs) in the brain [25, 26]. The risk of AD considerably increases with aging, affecting 7–10% of 65, and 40% of people over 80 years of age. It is a multifactorial dementia; therefore, exploration of pathway-based inhibitors is the most helpful way in the anti-AD drug development. In this context, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)

Table 2 Some of the common age-related neurological disorders and their pathological effects

S. No.	Age-associated neurodegenerative diseases	Pathological hallmarks	Disease consequences
1	Alzheimer's disease	Aggregated β-amyloid and tau in cerebral cortex and hippocampus, oxidative stress, activated microglia	Mental decline, difficulty in thinking and understanding, delusion, disorientation, forgetfulness, inability to create new memories, depression, and hallucination
2	Parkinson's disease	Aggregated α-synuclein in substantia nigra areas of the midbrain and basal forebrain	Bradykinesia, rigidity and rest tremor, sleep disturbance, executive dysfunction, depression, psychosis, and confusion
3	Amyotrophic lateral sclerosis or Lou Gehrig's disease	Aggregated superoxide dismutase-1 (SOD1) in spinal cord and motor cortex	Cramping, muscle weakness, problems with coordination, fatigue or feeling faint, shortness of breath vocal cord spasm, apathy, difficulty raising the foot, difficulty swallowing, drooling, lack of restraint, severe constipation, and tremor
4	Huntington's disease	Aggregated huntingtin (Htt) protein	Jerky body movements, mood swinging, reduced mental abilities, coordination

inhibitor compounds such as donepezil, galanthamine, rivastigmine, tacrine, etc. represent majority of drugs approved for clinical use in the symptomatic AD relief, while EOs target several pathways and thus provide better healing.

6 Neuroprotective Effects of Essential Oils

6.1 Effect on Cholinesterase Activity

Cholinesterases are one of the potential AD targets and a variety of EOs show promising anticholinesterase activity. EOs from the leaves and flowers of *Polygonum hydropiper* are recently reported to show free radicals scavenging and AChE, BChE inhibitory activity [27]. Extensive GC-MS analysis of the leaf and plant extract showed caryophylene oxide (41.42%) as the major EO in leaf and decahydronaphthalene (38.29%) as major EO of the flower. Also, EO from *Rumex hastatus* has reported anticholinesterase and antiradical potentials with IC50 values of 32.54 and 97.38 mg/ml for AChE and BChE, respectively, and IC50 values of 3.71 and 6.29 mg/ml for antioxidant assays [28]. *Narcissus poeticus* L. flower oil also show *in vitro* AChE, BChE inhibitory activity [29]. EOs like thymol and

p-cymene (19% and 16.1% concentration, respectively) from *Origanum ehrenbergii* showed potential antioxidant and AChE, BChE inhibitory activity [30]. Similarly, the anti-AChE activity of spathulenol from *Marlierea racemosa* Vell. were evaluated by de Souza et al. [31]. Cistaceae family (*Cistus creticus, Cistus monspeliensis, Cistus salvifolius, Cistus villosus*, *and Cistus libanotis*) were investigated for antioxidant and cholinesterase inhibitory potentials [32]. EOs from *C. monspeliensis and C. libanotis* were found to be most potent antioxidants. *C. salvifolius* exhibited AChE inhibitory, while, *C. libanotis, C. creticus*, *and C. salvifolius* showed BChE inhibitory activity.

6.2 Effect on Learning and Memory (Cognition)

Learning is the ability to acquire new and modify existing information, while, memory refers to the storing and retrieving ability of this information, and cognition is the combination of these two vital processes. In AD, deterioration of cholinergic neurons lead to cognitive deficits [33], and therefore, a cholinergic boost may potentially revert the cognitive dysfunction [34]. Based on this idea, several nicotinic and muscarinic agonists were tested, but the process failed due to limited efficacy, toxicity, and bioavailability problems [35]. EOs are reported for their beneficial effects in Alzheimer and dementia by several research groups. Shimizu et al. reported the effect of EOs from *Eucalyptus globulus* Labill. and *Lavandula angustifolia* Mill. on persistent attention [36, 37]. *Rosmarinus officinalis* L. of family Lamiaceae which is frequently used in diet formulations was also found to be a great source of EOs with strong antiradical, antibacterial, antifungal, and anticancer properties [38]. Rosemary EOs are also reported as neurostimulants, moderate AChE inhibitors, locomotor activity enhancers, vigor motivators, and cerebral cortex stimulators [39].

6.3 Effect on Aβ Aggregation

Formation of senile Aβ plaques and NFTs are important hallmarks of AD [40]. Aβ originates from enzymatic hydrolysis of the amyloid precursor protein (APP). The APP is cleaved by α, β, and γ secretase enzymes in a sequential manner, leading to the formation of $A\beta_{17–42}$ by α- and γ-secretases and neurotoxic $A\beta_{1–42}$ by β and γ secretases [41]. An imbalance in the generation and removal of Aβ results in its neuronal accumulation and becomes the starting point for neurodegeneration and neuroinflammation in AD. As a result, inhibition of β-amyloid cleaving enzyme 1 (BACE1) or β-secretase becomes an important target in management of AD. 6-gingerol, a predominant constituent of ginger EO, showed the neuroprotective potential against $A\beta_{25–35}$-mediated oxidative and nitrosative cell death in SH-SY5Y cells via inhibition of DNA fragmentation, interruption in mitochondrial membrane potential (MMP), activation of caspase-3 and increased Bax/Bcl-2 ratio, suppression of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [42].

6-gingerol also upregulate the expression of mRNA and proteins responsible for the synthesis of c-glutamylcysteine ligase, implicated in the biosynthesis of glutathione. Similarly, the EO from SuHeXiang Wan (SHXW), a Chinese traditional medicinal prescription consisting of 15 crude herbs whose essential oil has been shown to have anticonvulsant and antioxidative activity used as traditional medicine to treat seizures, infantile convulsions, AD, and other neurodegenerative disorders [43]. Pre-inhalation of SHXW EO revitalized the memory of AD animal model injected with $A\beta_{1-42}$, suppressed $A\beta_{1-42}$ mediated c-jun N-terminal kinase (JNK), protein kinases (p38), and tau phosphorylation in the hippocampus of animals. Also, SHXW EO decreases Aβ-mediated apoptosis and ROS production by upregulation of HO1 and Nrf2 expression in SH-SY5Y cells. Consecutively, thymol and carvacrol on cognitive function, it was found that both the EOs improved cognitive functions in animal models [44]. Most importantly, both samples were safe at their highest concentrations. Also, EO from *Coriandrum sativum* var. microcarpum (coriander) had antidepressant, anxiolytic, and antioxidant potentials upon inhalation in $A\beta_{1-42}$ rat models of AD [45]. Similarly, EOs of *Zataria multiflora* Boiss. (Lamiaceae) showed profound cognitive and neuroprotective effects in AD animal models by improving their cognitive abilities in a concentration-dependent manner [46].

6.4 Effect on Oxidative Stress

Generation of free radicals during aerobic respiration is an essential part of living beings. These free radicals readily attack DNA, proteins, fatty acids, and other essential molecules and therefore are implicated in a variety of disorders including AD, cancer, aging, and inflammation, and several plant extracts has been found to have potential radical scavenging activities [47–52]. Free radicals are neutralized to nonradical forms by various enzymes including CAT, SOD, and hydroperoxidase. But, when free radical generation is abnormally high, then administration of free radical scavengers from outside becomes necessary. Furthermore, in aging brain and AD patients, mitochondrial dysfunction lead to excessive production of free radicals and subsequently result in pathological abnormalities. Aβ is a potent initiator of ROS and RNS production which rapidly cause oxidative damage of neural, microglial, and cerebrovascular cells and tissues [53]. Investigation of the neuroprotective effects of lavender (*Lavandula angustifolia* ssp. Angustifolia Mill. and *Lavandula hybrida* Rev) revealed that its EO showed antioxidant and antiapoptotic potentials [54]. Lavender EO mediates its protective effects via strong antioxidant activities. In addition, there are numerous examples of EOs from chamomile, clove, thyme, eucalyptus, juniper, basil, cumin, cinnamon, and coriander to possess considerable antioxidative potentials [55–57]. Similarly, dietary intake of oregano oil has been reported to delay lipid oxidation in animal models [58]. EOs from *Achillea millefolium* were reported to scavenge hydroxyl free radicals via inhibition of lipid peroxidation [59]. Studies on EOs from *Salvia multicaulis* and *Salvia cryptantha* showed antioxidant activities higher than the standard drugs [60]. Many aroma components of EO like linalool, α-terpinene, 1,8- cineole, β-terpinolene, β-terpinene,

menthon, thymol, eugenol, and isomenthone have reported antioxidant potentials [61]. Similarly, the EO of *Melissa officinalis* L. rich in menthone, geranial, isomenthone, and citronellal has reported antioxidative properties [62]. A comprehensive account of possible neuroprotective mechanism of EOs is given in Fig. 3.

7 Neuroprotective Effects of Plant Lipids

7.1 Contribution of Long Chain ω-3 PUFAs to Brain Function and Its Molecular Mechanism

Brain is a repository of DHA and AA that are highly susceptible to free radical-induced damage [63]. Oxidative damage of the brain is characterized by increased lipid peroxidation, which deteriorates neuronal functions [64]. Recent studies shows participation of DHA in brain pathological activities and involvement in regulation of many metabolic and inflammatory pathways, and postmortem samples from AD brains show gradual decline of DHA in patient's brain [65]. Also, studies reveal that higher dietary intake of DHA can reduce the risk of cognitive impairment or AD [66–68]. It has also been found that ω-3 PUFAs cannot benefit patients with already diagnosed AD progression but can enhance learning and memory function only in age-related cognitive decline in healthy elderly populations [69]. Recently, through a study carried out in rats, it has been found that DHA deficiency activates caspases in AD models and intensify the age-related decline of glutamatergic transmission in learning and memory functions [70]. On the other hand, DHA supplementation diminish oxidative stress or lipid peroxidation and protect against memory loss in AD rat models [71] by reducing the accumulation of neuronal Aβ and tau protein [72]. This information may help researchers to design application of ω-3 PUFAs for better relief from age-related cognitive consequences.

DHA that is transferred from the maternal circulation to fetal brain plays a crucial role in synaptogenesis. DHA is incorporated into fetal brain through fatty acid transport protein-4 (FATP 4) [73]. However, the amount of maternal DHA in the synapses and neural membranes depends on the dietary intake [74–78]. Studies have shown that DHA acts as an endogenous ligand for retinoic acid receptors (RAR) and retinoid x receptors (RXR) [79] which decrease with age and correlated with age-related memory deficits. Dyall and colleagues suggest that DHA supplementation reverse the decrease of RAR and RXR which alleviate the memory deficits and increase neurogenesis [80]. Patients diagnosed with AD show lower DHA levels in plasma and brain [81, 82] which not only could be due to lower dietary intake of ω-3 fatty acids but also due to increased oxidation of PUFAs [83, 84]. Preclinical evidence suggests that a DHA-enriched diet reduces amyloid formation [85, 86]. Akbar and coworkers provided additional evidence that DHA is highly enriched in neuronal membranes and provides neuroprotection via PI3, Akt pathway which is a critical signaling pathway for neuronal survival [87]. Dietary supplementation of DHA has been shown to increase the levels of hippocampal BDNF (brain-derived

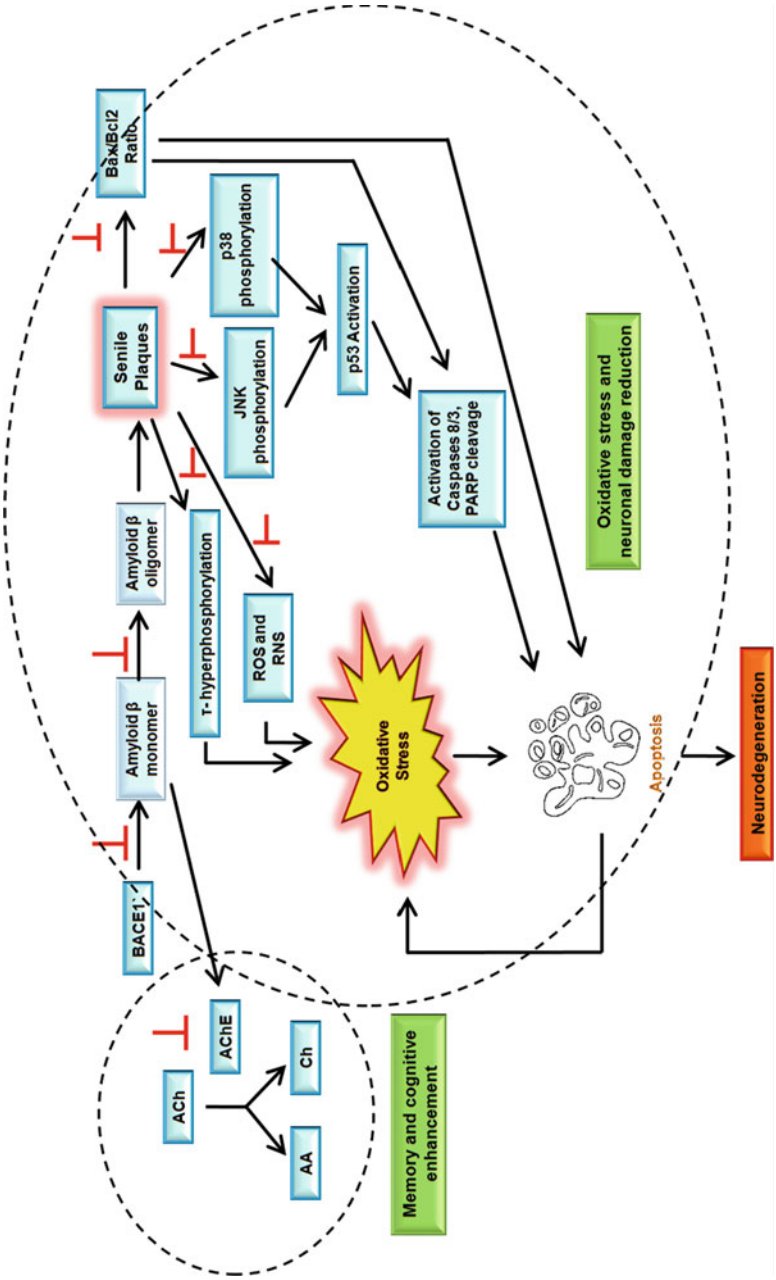


Fig. 3 Overview of possible molecular mechanism of essential oils executing multifactorial neuroprotective effects in Alzheimer's disease condition. EOs mitigate AChE, BACE1 activity; Aβ oligomer formation; τ, JNK, and p38 phosphorylation; Bax/Bcl2 ratio; and ROS and RNS production, thus enhancing memory and cognitive power and simultaneously reducing oxidative stress and neuronal apoptosis

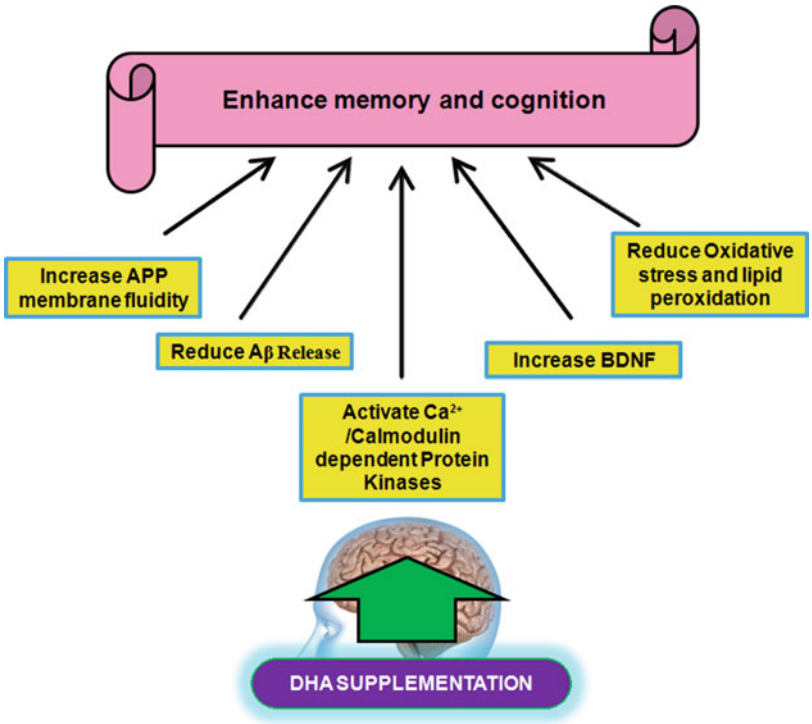


Fig. 4 Outline of possible molecular mechanism of DHA executing multifarious neuroprotective effects in Alzheimer's disease condition. DHA reduce overall Aβ-induced neurodegeneration and apoptosis by increasing APP membrane fluidity, thus reducing Aβ release, activating Ca^{2+}/calmodulin-dependent kinases, and increasing brain-derived neurotrophic factor and reducing oxidative stress and lipid peroxidation

neurotrophic factor which is essential for maintaining synaptic plasticity and cell survival [88], and activation of Akt is known to be linked to increase in BDNF. Also, DHA-rich diet activates Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) which is critical for learning and memory and plays a crucial role in induction and maintenance of long-term potentiation in hippocampus [89, 90]. Literature survey also propose that DHA modulates multiple cellular functions like enhanced membrane fluidity of amyloid precursor protein (APP) and reduced amyloid-β release by shifting the mechanism towards nonamyloidogenic pathway [91]. DHA is also suggested to assist N-methyl-D aspartate (NMDA) responses [92] and block K^+ channels [93], thus directly affecting memory and learning [94]. Most recently, DHA supplementation has also been shown to amend gene expression at the transcriptional level, by activating members of peroxisome proliferator-activated receptor (PPAR) family [95] and stabilizing mRNA of several enzymes associated with glucose and lipid metabolism [96]. Figure 4 gives a broad outline of DHA imparting neuroprotective effect through various mechanisms.

7.2 DHA Depletion and Cognitive Impairment

Studies in AD animal models suggest that DHA deficiency in neural tissue leads to behavioral changes similar to that in patients with AD. Furthermore, experimental evidence suggests that DHA decreases with age, particularly in regions of the hippocampus which are crucial for higher brain functions such as memory formation and cognition [97, 98]. Decreased DHA levels are reported to detrimentally affect the major excitatory neurotransmitter, glutamate, which contributes to the integrity of brain function in learning memory performance [99]. Recently, it has been demonstrated that DHA protects rat cortical neurons from soluble Aβ oligomer-induced neurodegeneration and apoptosis [100, 101].

8 Conclusions

In recent times, plant-based therapies have got considerable attention owing to their comparative safety, potency, and efficacy on multiple targets. Among the natural products, EOs from aromatic and medicinal plants is of great interest because of their antioxidant, cholinesterase inhibitory, anti-amyloid properties, and high availability at the target due to their lipophilic character. Thus, EOs can be very effective alternative for the management of AD in comparison to synthetic drugs which are associated with severe side effects. Also, numerous EOs have been reported to possess strong antioxidant potentials and can be effectively used in free radical-induced disorders including neurological diseases and aging. This chapter also highlighted the potentiality of long chain ω-3 fatty acids to reduce low-grade inflammation in the early stages of age-related neurodegenerative diseases like AD.

Acknowledgment The authors gladly acknowledge the bioinformatics infrastructure facility, Alagappa University, funded by the Department of Biotechnology, Ministry of Science and Technology, Government of India (No. BT/BI/25/015/2012). Mamali Das acknowledges DST–PURSE for offering Research Fellowship.

References

1. Harman D (1981) The aging process. Proc Natl Acad Sci U S A 78(11):7124–7128
2. Klass MR (1983) A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. Mech Ageing Dev 22(3):279–286
3. Piper MD, Partridge L (2016) Protocols to study aging in Drosophila. Methods Mol Biol 1478:291–302
4. Moskalev AA, Smit-McBride Z et al (2012) Gadd45 proteins: relevance to aging, longevity and age-related pathologies. Ageing Res Rev 11(1):51–66
5. Talens RP, Christensen K et al (2012) Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs. Aging Cell 11(4):694–703
6. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE (2009) Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 78:959–991

7. Green DR, Galluzzi L, Kroemer G (2011) Mitochondria and the autophagy – inflammation – cell death axis in organismal aging. Science 333(6046):1109–1112
8. Edris AE (2007) Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res 21(4):308–323
9. Guenther E (1950) The essention oils. D. Van Nostrand Company, London
10. Smith-Palmer A, Stewart J, Fyfe L (2001) The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiol 18(4):463–470
11. Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol 94(3):223–253
12. Keville K, Green M (2012) Aromatherapy: a complete guide to the healing art. Crossing Press, Berkeley
13. Alabdulkarim B, Bakeet ZA, Arzoo S (2012) Role of some functional lipids in preventing diseases and promoting health. J King Saud Uni – Sci 24(4):319–329
14. Innis SM (2008) Dietary omega 3 fatty acids and the developing brain. Brain Res 1237:35–43
15. Bishop NA, Lu T, Yankner BA (2010) Neural mechanisms of ageing and cognitive decline. Nature 464(7288):529
16. González-Burgos E, Carretero ME, Gómez-Serranillos MP (2011) Sideritis spp.: uses, chemical composition and pharmacological activities – a review. J Ethnopharmacol 135(2):209–225
17. Angioni A, Barra A, Coroneo V, Dessi S, Cabras P (2006) Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers. J Agric Food Chem 54(12):4364–4370
18. Ramadan MF, Zayed R, El-Shamy H (2007) Screening of bioactive lipids and radical scavenging potential of some solanaceae plants. Food Chem 103(3):885–890
19. Hassanien MF, Kinni SG, Moersel JT (2010) Bioactive lipids, fatty acids and radical scavenging activity of Indian *Celastrus paniculatus* oil. J Appl Bot Food Qual 83(2):157–162
20. Eckert GP, Franke C, Nöldner M, Rau O, Wurglics M, Schubert-Zsilavecz M, Müller WE (2010) Plant derived omega-3-fatty acids protect mitochondrial function in the brain. Pharmacol Res 61(3):234–241
21. Longvah T, Deosthale YG (1991) Chemical and nutritional studies on Hanshi (Perilla frutescens), a traditional oilseed from Northeast India. J Am Oil Chem Soc 68(10):781–784
22. Talboom JS, Velazquez R, Oddo S (2015) The mammalian target of rapamycin at the crossroad between cognitive aging and Alzheimer's disease. NPJ Aging Mech Dis 1:15008. https://doi.org/10.1038/1:npjamd2015.8
23. van Ham TJ, Breitling R, Swertz MA, Nollen EA (2009) Neurodegenerative diseases: lessons from genome-wide screens in small model organisms. EMBO Mol Med 1(8–9):360–370
24. Passe TJ, Rajagopalan P, Tupler LA, Byrum CE, Macfall JR, Krishnan KR (1997) Age and sex effects on brain morphology. Prog Neuropsychopharmacol Biol Psychiatry 21(8):1231–1237
25. Nussbaum RL, Ellis CE (2003) Alzheimer's disease and Parkinson's disease. N Engl J Med 348(14):1356–2364
26. Reitz C (2012) Alzheimer's disease and the amyloid cascade hypothesis: a critical review. Int J Alzheimers Dis 2012:369808. https://doi.org/10.1155/2012/369808
27. Ayaz M, Junaid M, Ullah F, Sadiq A, Khan MA, Ahmad W, Shah MR, Imran M, Ahmad S (2015) Comparative chemical profiling, cholinesterase inhibitions and anti-radicals properties of essential oils from *Polygonum hydropiper* L: a preliminary anti-Alzheimer's study. Lipids Health Dis 14(1):141
28. Ahmad S, Ullah F, Sadiq A, Ayaz M, Imran M, Ali I, Zeb A, Ullah F, Shah MR (2016) Chemical composition, antioxidant and anticholinesterase potentials of essential oil of *Rumex hastatus* D. Don collected from the North West of Pakistan. BMC Complement Altern Med 16 (1):29
29. Okello EJ, Dimaki C, Howes MJ, Houghton PJ, Perry EK (2008) In vitro inhibition of human acetyl-and butyryl-cholinesterase by *Narcissus poeticus* L.(Amaryllidaceae) flower absolute. Int J Essent Oil Ther 2(3):105–110

30. Loizzo MR, Menichini F, Conforti F, Tundis R, Bonesi M, Saab AM, Statti GA, de Cindio B, Houghton PJ, Menichini F, Frega NG (2009) Chemical analysis, antioxidant, antiinflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss and *Origanum syriacum* L. essential oils. Food Chem 117(1):174–180
31. Souza A, Silva MC, Cardoso-Lopes EM, Cordeiro I, Sobral ME, Young MC, Moreno PR (2009) Differential acetyl cholinesterase inhibition by volatile oils from two specimens of *Marlierea racemosa* (Myrtaceae) collected from different areas of the Atlantic Rain Forest. Nat Prod Commun 8:1143–1146
32. Loizzo MR, Jemia MB, Senatore F, Bruno M, Menichini F, Tundis R (2013) Chemistry and functional properties in prevention of neurodegenerative disorders of five Cistus species essential oils. Food Chem Toxicol 59:586–594
33. Schliebs R, Arendt T (2011) The cholinergic system in aging and neuronal degeneration. Behav Brain Res 221(2):555–563
34. Anand R, Gill KD, Mahdi AA (2014) Therapeutics of Alzheimer's disease: past, present and future. Neuropharmacology 76:27–50
35. Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M (2010) Alzheimer's disease: clinical trials and drug development. Lancet Neurol 9(7):702–716
36. Shimizu K, Gyokusen M, Kitamura S, Kawabe T, Kozaki T, Ishibashi K, Izumi R, Mizunoya W, Ohnuki K, Kondo R (2008) Essential oil of lavender inhibited the decreased attention during a long-term task in humans. Biosci Biotechnol Biochem 72(7):1944–1947
37. Shimizu Y, Imayoshi Y, Kato M, Maeda K, Iwabuchi H, Shimomura K (2009) Volatiles from leaves of field-grown plants and shoot cultures of Gynura bicolor DC. Flavour Fragr J 24(5):251–258
38. Faixova Z, Faix S (2008) Biological effects of rosemary (Rosmarinus officinalis L) essential oil (a review). Folia Vet 52(3–4):135–139
39. Hongratanaworakit T (2009) Simultaneous aromatherapy massage with rosemary oil on humans. Sci Pharm 77(2):375–388
40. Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat Rev Mol Cell Biol 8(2):101
41. De Strooper B, Vassar R, Golde T (2010) The secretases: enzymes with therapeutic potential in Alzheimer disease. Nat Rev Neurol 6(2):99–107
42. Lee C, Park GH, Kim CY, Jang JH (2011) [6]-gingerol attenuates β-amyloid-induced oxidative cell death via fortifying cellular antioxidant defense system. Food Chem Toxicol 49(6):1261–1269
43. Hong YK, Park SH, Lee S, Hwang S, Lee MJ, Kim D, Lee JH, Han SY, Kim ST, Kim YK, Jeon S (2011) Neuroprotective effect of SuHeXiang Wan in Drosophila models of Alzheimer's disease. J Ethnopharmacol 134(3):1028–1032
44. Azizi Z, Ebrahimi S, Saadatfar E, Kamalinejad M, Majlessi N (2012) Cognitive-enhancing activity of thymol and carvacrol in two rat models of dementia. Behav Pharmacol 23(3):241–249
45. Cioanca O, Hritcu L, Mihasan M, Trifan A, Hancianu M (2014) Inhalation of coriander volatile oil increased anxiolytic–antidepressant-like behaviors and decreased oxidative status in beta-amyloid (1–42) rat model of Alzheimer's disease. Physiol Behav 131:68–74
46. Majlessi N, Choopani S, Kamalinejad M, Azizi Z (2012) Amelioration of amyloid β-induced cognitive deficits by Zataria multiflora Boiss. essential oil in a rat model of Alzheimer's disease. CNS Neurosci Ther 18(4):295–301
47. Ayaz M, Sadiq A, Junaid M, Ullah F, Subhan F, Ahmed J (2017) Neuroprotective and anti-aging potentials of essential oils from aromatic and medicinal plants. Front Aging Neurosci 9:168
48. Ahmad S, Ullah F, Ayaz M, Sadiq A, Imran M (2015) Antioxidant and anticholinesterase investigations of *Rumex hastatus* D. Don: potential effectiveness in oxidative stress and neurological disorders. Biol Res 48(1):20

49. Kamal Z, Ullah F, Ayaz M, Sadiq A, Ahmad S, Zeb A, Hussain A, Imran M (2015) Anticholinesterse and antioxidant investigations of crude extracts, subsequent fractions, saponins and flavonoids of *Atriplex laciniata* L.: potential effectiveness in Alzheimer's and other neurological disorders. Biol Res 48(1):21
50. Sadiq A, Mahmood F, Ullah F, Ayaz M, Ahmad S, Haq FU, Khan G, Jan MS (2015) Synthesis, anticholinesterase and antioxidant potentials of ketoesters derivatives of succinimides: a possible role in the management of Alzheimer's. Chem Cent J 9(1):1–9
51. Shah SM, Ayaz M, Khan AU, Ullah F, Farhan, Shah AU, Iqbal H, Hussain S (2015) 1, 1-diphenyl, 2-picrylhydrazyl free radical scavenging, bactericidal, fungicidal and leishmanicidal properties of *Teucrium stocksianum*. Toxicol Ind Health 31(11):1037–1043
52. Ullah F, Ayaz M, Sadiq A, Hussain A, Ahmad S, Imran M, Zeb A (2016) Phenolic, flavonoid contents, anticholinesterase and antioxidant evaluation of *Iris germanica* var; florentina. Nat Prod Res 30(12):1440–1444
53. Engel J, Pedley TA, Aicardi J (2008) Epilepsy: a comprehensive textbook. Lippincott Williams & Wilkins, Philadelphia
54. Hancianu M, Cioanca O, Mihasan M, Hritcu L (2013) Neuroprotective effects of inhaled lavender oil on scopolamine-induced dementia via anti-oxidative activities in rats. Phytomedicine 20(5):446–452
55. Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A, Saija A (2005) Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem 89(4):549–554
56. El-Ghorab A, Shaaban HA, El-Massry KF, Shibamoto T (2008) Chemical composition of volatile extract and biological activities of volatile and less-volatile extracts of juniper berry (*Juniperus drupacea* L.) fruit. J Agric Food Chem 56(13):5021–5025
57. Wei A, Shibamoto T (2010) Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. J Agric Food Chem 58(12):7218–7225
58. Botsoglou NA, Florou-Paneri P, Christaki E, Giannenas I, Spais AB (2004) Performance of rabbits and oxidative stability of muscle tissues as affected by dietary supplementation with oregano essential oil. Arch Anim Nutr 58(3):209–218
59. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A, Akpulat HA (2003) Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. millefolium Afan. (Asteraceae). J Ethnopharmacol 87(2):215–220
60. Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissiou M, Sokmen A (2004) Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chem 84(4):519–525
61. El-massry KF, El-Ghorab AH (2006) Effect of essential oils and non-volatile extracts of some aromatic plants on Cu^{++}-induced oxidative modification of human low-density lipoprotein (LDL). J Essent Oil Bear Plants 9(3):292–299
62. Mimica-Dukic N, Bozin B, Sokovic M, Simin N (2004) Antimicrobial and antioxidant activities of *Melissa officinalis* L.(Lamiaceae) essential oil. J Agric Food Chem 52(9):2485–2459
63. Söderberg M, Edlund C, Kristensson K, Dallner G (1991) Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids 26(6):421
64. Gemma C, Vila J, Bachstetter A, Bickford PC (2007) Oxidative stress and the aging brain: from theory to prevention. In: Riddle DR, editor. Brain Aging: Models, Methods, and Mechanisms. Boca Raton (FL): CRC Press/Taylor & Francis
65. Adibhatla RM, Hatcher JF (2008) Altered lipid metabolism in brain injury and disorders. Subcell Biochem 49:241–268
66. Chiurchiù V, Orlacchio A, Maccarrone M (2016) Is modulation of oxidative stress an answer? The state of the art of redox therapeutic actions in neurodegenerative diseases. Oxidative Med Cell Longev 2016:7909380

67. Ramirez-Ramirez V, Macias-Islas MA, Ortiz GG, Pacheco-Moises F, Torres-Sanchez ED, Sorto-Gomez TE, Cruz-Ramos JA, Orozco-Aviña G, Celis De La Rosa AJ (2013) Efficacy of fish oil on serum of TNFα, IL-1β, and IL-6 oxidative stress markers in multiple sclerosis treated with interferon beta-1b. Oxid Med Cell Longev 2013:709493
68. Lauritzen LA, Hansen HS, Jørgensen MH, Michaelsen KF (2001) The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 40(1):1–94
69. Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, Tucker KL, Kyle DJ, Wilson PW, Wolf PA (2006) Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 63(11):1545–1550
70. Yurko-Mauro K, Alexander DD, Van Elswyk ME (2015) Docosahexaenoic acid and adult memory: a systematic review and meta-analysis. PLoS One 10(3):e0120391
71. Kotani S, Sakaguchi E, Warashina S, Matsukawa N, Ishikura Y, Kiso Y, Sakakibara M, Yoshimoto T, Guo J, Yamashima T (2006) Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. Neurosci Res 56(2):159–164
72. Chiu CC, Su KP, Cheng TC, Liu HC, Chang CJ, Dewey ME, Stewart R, Huang SY (2008) The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: a preliminary randomized double-blind placebo-controlled study. Prog Neuropsychopharmacol Biol Psychiatry 32(6):1538–1544
73. Koletzko B, Larqué E, Demmelmair H (2007) Placental transfer of long-chain polyunsaturated fatty acids (LC-PUFA). J Perinat Med 35(S1):S5–11
74. Bazan NG, Molina MF, Gordon WC (2011) Docosahexaenoic acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. Annu Rev Nutr 31:321–351
75. Martin RE, Bazan NG (1992) Changing fatty acid content of growth cone lipids prior to synaptogenesis. J Neurochem 59(1):318–325
76. Larqué E, Krauss-Etschmann S, Campoy C, Hartl D, Linde J, Klingler M, Demmelmair H, Caño A, Gil A, Bondy B, Koletzko B (2006) Docosahexaenoic acid supply in pregnancy affects placental expression of fatty acid transport proteins. Am J Clin Nutr 84(4):853–861
77. De Vriese SR, Matthys C, De Henauw S, De Backer G, Dhont M, Christophe AB (2002) Maternal and umbilical fatty acid status in relation to maternal diet. Prostaglandins Leukot Essent Fatty Acids 67(6):389–396
78. Dunstan JA, Mori TA, Barden A, Beilin LJ (2004) Effects of n-3 polyunsaturated fatty acid supplementation in pregnancy on maternal and fetal erythrocyte fatty acid composition. Eur J Clin Nutr 58(3):429
79. de Urquiza AM, Liu S, Sjöberg M, Zetterström RH, Griffiths W, Sjövall J, Perlmann T (2000) Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. Science 290(5499):2140–2144
80. Dyall SC (2015) Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. Front Aging Neurosci 7:52
81. Plourde M, Fortier M, Vandal M, Tremblay-Mercier J, Freemantle E, Begin M, Pifferi F, Cunnane SC (2007) Unresolved issues in the link between docosahexaenoic acid and Alzheimer's disease. Prostaglandins Leukot Essent Fatty Acids 77(5):301–308
82. Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH (2000) Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids 35(12):1305–1312
83. Greiner RS, Moriguchi T, Hutton A, Slotnick BM, Salem N (1999) Rats with low levels of brain docosahexaenoic acid show impaired performance in olfactory-based and spatial learning tasks. Lipids 34(1):S239–S243
84. Bazan NG (2009) Neuroprotectin D1-mediated anti-inflammatory and survival signaling in stroke, retinal degenerations, and Alzheimer's disease. J Lipid Res 50:S400–S405

85. Cole GM, Frautschy SA (2010) DHA may prevent age-related dementia. J Nutr 140(4):869–874
86. Hashimoto M, Tanabe Y, Fujii Y, Hagiwara R, Yamasaki H, Shido O (2002) Mechanism of improvement of spatial cognition with dietary docosahexaenoic acid. Nihon yakurigaku zasshi. Folia Pharmacol Jpn 120(1):54P–56P
87. Akbar M, Calderon F, Wen Z, Kim HY (2005) Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. Proc Natl Acad Sci U S A 102(31):10858–10863
88. Wu A, Ying Z, Gomez-Pinilla F (2008) Docosahexaenoic acid dietary supplementation enhances the effects of exercise on synaptic plasticity and cognition. Neuroscience 155(3):751–759
89. Calon F, Lim GP, Morihara T, Yang F, Ubeda O, Salem N, Frautschy SA, Cole GM (2005) Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. Eur J Neurosci 22(3):617–626
90. Elgersma Y, Sweatt JD, Giese KP (2004) Mouse genetic approaches to investigating calcium/calmodulin-dependent protein kinase II function in plasticity and cognition. J Neurosci 24(39):8410–8415
91. Grimm MO, Kuchenbecker J, Grösgen S, Burg VK, Hundsdörfer B, Rothhaar TL, Friess P, De Wilde MC, Broersen LM, Penke B, Péter M (2011) Docosahexaenoic acid reduces amyloid β production via multiple pleiotropic mechanisms. J Biol Chem 286(16):14028–14039
92. Nishikawa M, Kimura S, Akaike N (1994) Facilitatory effect of docosahexaenoic acid on N-methyl-D-aspartate response in pyramidal neurones of rat cerebral cortex. J Physiol 475(1):83–93
93. Poling JS, Karanian JW, Salem N, Vicini S (1995) Time-and voltage-dependent block of delayed rectifier potassium channels by docosahexaenoic acid. Mol Pharmacol 47(2):381–390
94. Horrocks LA, Farooqui AA (2004) Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. Prostaglandins Leukot Essent Fatty Acids 70(4):361–372
95. Jump DB (2002) Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr Opin Lipidol 13(2):155–164
96. Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, Salem N, Frautschy SA, Cole GM (2005) A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. J Neurosci 25(12):3032–3040
97. Fernandes JS, Mori MA, Ekuni R, Oliveira RM, Milani H (2008) Long-term treatment with fish oil prevents memory impairments but not hippocampal damage in rats subjected to transient, global cerebral ischemia. Nutr Res 28(11):798–808
98. Pomponi M, Pomponi M (2008) DHA deficiency and Alzheimer's disease. Clin Nutr 27(1):170
99. Su HM (2010) Mechanisms of n-3 fatty acid-mediated development and maintenance of learning memory performance. J Nutr Biochem 21(5):364–373
100. Pasinetti GM, Wang J, Ho L, Zhao W, Dubner L (2015) Roles of resveratrol and other grape-derived polyphenols in Alzheimer's disease prevention and treatment. Biochim Biophys Acta 1852(6):1202–1208
101. Patel KR, Scott E, Brown VA, Gescher AJ, Steward WP, Brown K (2011) Clinical trials of resveratrol. Ann N Y Acad Sci 1215(1):161–169

Protective Effect of Omega 3 Fatty Acids EPA and DHA in the Neurodegenerative Disease

21

Edwin E. Martínez Leo, Rafael A. Rojas Herrera, and Maira R. Segura Campos

Contents

Abstract

Neurodegenerative diseases (ND) are characterized by the death of neurons in different regions of the nervous system, followed by functional deterioration. The most frequent pathologies are the group of dementias such as Parkinson's disease (PD) and Alzheimer's disease (AD), which represent an important impact on society and quality of life of people, mainly in the elderly group. Omega 3 is a polyunsaturated fatty acid, whose anti-inflammatory effect has been related to benefits on the neuroinflammatory processes characteristic of ND. Although the clinical evidence is unclear, epidemiological studies report improvement in cognitive performance and provide evidence on its neuroprotective effect in specific regions of the nervous system. The objective of this review is to determine the neuroprotective effects of omega 3 fatty acids on neurodegenerative disease.

E. E. Martínez Leo · R. A. Rojas Herrera · M. R. Segura Campos (✉)
Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico
e-mail: rafael.rojas@correo.uady.mx; maira.segura@correo.uady.mx

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_90

Keywords
Neuroinflammation · Neuroregenery · Functional nutrition · Neuroprotection

1 Neurodegenerative Disease: Epidemic of the Twenty-First Century

The ND integrate a heterogeneous group of diseases with involvement of the central nervous system (CNS) characterized by a progressive neuronal loss in specific brain areas or anatomic systems. This progressive loss of nerve cells is what causes the neurological and neuropsychological signs and symptoms characteristic of this group of diseases [52]. ND affect several activities of the organism, such as balance, movement, speech, respiration, and functions of the heart. Some ND are Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Friedreich's ataxia (AF), Huntington's disease (HD), Parkinson's disease (PD), and spinal muscular atrophy [40].

Currently, intervention in issues related to ND faces a complex context. There are multiple sociological factors such as increase of the life expectancy, increase of the population and aging, greater difficulty of scientific-technical advances, and predictable chronification of the diseases. Likewise, the presence of socioeconomic factors (increased job insecurity, impoverishment of the middle classes, increased healthcare costs, and weakening public social and health investment) has been circumstances that, in their combination, have increased the prevalence of ND [16].

According to the report of the World Health Organization (WHO), the ND affect around the world about one billion people. The report *Neurological Disorders: Public Health Challenges* shows that around a billion people are affected worldwide; 50 million suffer from epilepsy and 24 million suffer from Alzheimer's and other dementias. Neurological disorders affect people from all countries, without distinction of sex, educational level, and economic status. Given the above, the WHO advocates that neurological care be integrated into primary healthcare, in order to prevent ND, through strategies that may also directly affect the aspects of metabolic alteration [59, 60].

As the global aging rate increases, the impact of ND will be felt in both developed and developing countries. According to Rita Levi-Montalcini, Nobel Prize in Medicine, "the burden of neurological disorders is reaching significant proportions in countries where the percentage of people over 65 years of age increases" [59]. Until 2015 and because of its prevalence, the three main ND were Alzheimer's and other dementias, reporting 35 billion global cases, Parkinson's with 23 billon worldwide cases, and multiple sclerosis with 2 billion [61].

2 The Nervous System as an Immuno-Privileged Site

The nervous system (NS) is one of the smallest and at the same time most complex of the organism. It consists of an intricate, specialized, and highly organized network of cells called neurons and glial cells. The NS carries out a complex set of activities that

allow a human being to feel different smells, produce speech, and remember past events. It also provides signals that control body movements and regulate the functioning of tissues and organs [47].

The NS is subdivided into CNS, consisting of encephalon and spinal cord, and the peripheral nervous system (PNS), which includes all nerve tissues located in the periphery. The brain is the main constituent organ of the CNS, whose cognitive function involving networks of cells including neurons, one of the most important cell populations in the NS, is involved. Neurons are the main functional units of the CNS and have the ability to communicate with each other quickly and efficiently through electrical or chemical signals that are translated as action potentials [2]. The CNS is protected from damage and injury, by the blood-brain barrier (BBB), which is a physical barrier located between blood vessels and brain tissue, with a very high selective permeability that limits the access of molecules to protect the brain from any type of pathogen or toxic substance. The foregoing allows this system to be immunologically specialized [10].

The CNS is a specialized and essential system in the survival and defense of injuries to the organism, for which reason it has been classified as immunoprivileged. The anatomical sites that present this condition are able to tolerate the introduction of antigens without initiating an inflammatory immune response [22]. Although there is still debate between the homeostatic maintenance of the CNS and the immune system, it is well known that immune cells (lymphocytes and macrophages) play an important role in neurodegeneration [10]. In the particular case of the CNS, the lymphocytes have the capacity to enter through the cerebrospinal fluid in the choroid plexus or the cerebral parenchyma. Said extravasation of immune cells is mediated by the adhesion molecules ICAM (intercellular adhesion molecules) and VCAM (vascular cell adhesion molecules), which are present in the endothelium of the blood-brain barrier. This allows the CNS to develop a more regulated immune response, compared to other sites in the body [47].

Conversely, not all nerve cells produce action potentials; such is the case of glia. Glial cells differ from neurons, since they do not have synaptic contacts and have the ability to divide throughout life. The main functions of glial cells are to (1) maintain an ionic medium in nerve cells, (2) modulate the speed of propagation of nerve signals, (3) modulate the synaptic action by controlling the uptake of neurotransmitters, (4) provide a foundation for neural development, and (5) contribute (or prevent, in some cases) the survival of a neuronal lesion [35].

Glial cells are classified mainly into two groups: (1) macroglia, which include astrocytes and oligodendrocytes, of ectodermal origin, and (2) microglia, of mesodermal origin, which invade the CNS during embryonic development at the time of vascularization [15]. Microglia constitute part of the macrophages and constitute between 5% and 10% of the neuronal cell population [36].

Under normal conditions, the microglia is at rest, but if there is an injury or infection, mechanisms that promote neurotoxic activities are activated, producing reactive oxygen species (ROS) and reactive nitrogen species (RNS) and secreting prostaglandins, chemokines, and cytokines. They favor an inflammation status. If the activation of the microglia remains until it becomes chronic, it leads to an

imbalance in the inflammatory response, generating a cycle of inflammation and tissue damage [35]. Inflammation is defined as the response of vascularized living tissue to the lesion, and in this sense, it is a complex cascade of physiological responses to harmful stimuli from the environment [12].

Another factor that triggers inflammation is oxidative stress (OXS). Several studies show that a state of chronic-systemic oxidation plays a crucial role in the pathogenesis of ND [9, 44]. OXS is defined as the electrochemical imbalance between prooxidant substances (ROS and ROS) and antioxidant substances, in favor of the former. The body has antioxidant defense mechanisms, both enzymatic (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)), and nonenzymatic (glutathione), for protection against prooxidants. However, before the chronic development of the disease, such as obesity and diabetes, there is a depletion of the antioxidant systems, which favors the perpetuation of OXS [33]. Particularly, the brain is susceptible to OXS lesions because it is an organ with high energy use and metabolic demand, so minimal imbalances of the redox state favor tissue damage and activation of neuroinflammatory mechanisms [8].

The inflammation present in ND, both acute and chronic, occurs in response to injury or alteration in the CNS. The chronic and unbalanced inflammatory response is a source of damage to the integrity and function of the CNS cells, since the neural tissues have a restricted cell regeneration and are extremely vulnerable to immune and inflammatory processes [46]. Inflammation determines the progression of neuronal death in ND. In this sense, the excessive and destructive immune response becomes a chronic and persistent process that leads to progressive and irreversible neurodegeneration inducing a vicious circle that causes cognitive deterioration, giving rise to the progression of chronic ND such as AD and PD [12].

3 Neurodegenerative Disease and Neuroinflammation

The term neuroinflammation describes an extensive process of physiopathological mechanisms and phenomena mediated by alterations in the glial cell morphology. Neuroinflammation is a reactive state of the immunological component of the CNS. This concept of neuroinflammation encompasses the response or set of responses that occur in the CNS to any damage occurring in the tissue [6].

As previously described, microglia play an important role in the development of the neuroinflammatory response. This cell has toll-like receptors, involved in the recognition of damage to the CNS by chemical agents, such as ROS and RNS, and production of proinflammatory cytokines. Given the exposure to inflammation molecules, the cells of the microglia are activated, and this results in a series of processes that involve the morphological transformation of an inactive branched state to an active phagocytic ameboid and the proliferation and migration toward sites of injury by chemotaxis [32].

The activation of the microglia causes a release of products that may have neurotoxic effects within which they are the proinflammatory cytokines interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), interferon gamma

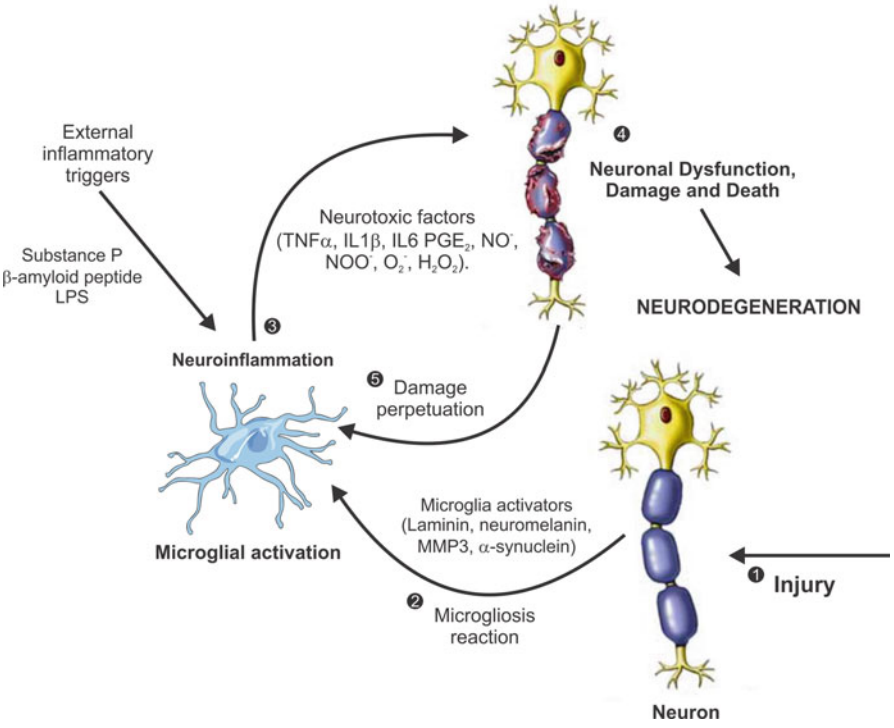


Fig. 1 Microglial activation model conducive to neurodegeneration

(IFN), oxidizing compounds such as ROS and RNs, as well as excitatory amino acids such as glutamate, all act together to modulate the inflammatory processes that affect the permeability of the BBB [12, 30] (Fig. 1).

IL-1β is one of the most important cytokines in the progression of the neuroinflammatory process, playing an important role in the development of acute neuronal injuries. Likewise, it has been demonstrated that the administration of the IL-1 receptor antagonist (IL-1ra) prior to brain injury significantly reduces the death of bark neurons in rats. The use of transgenic animals with specific modifications of the genes that express IL-1β has shown that their deficiency exhibits a reduction of necrosis despite the fact that the animals are subjected to different neuronal aggressions [15].

Also, IL-1β induces the expression of inducible nitric oxide synthase (iNOS), responsible for triggering the release of nitric oxide (NO-) in glial cells and causing nitrosative stress. The RNS are oxidative molecules with special attachment to lipid peroxidation, which favors mainly the rupture of cell membranes in CNS. Lipid peroxidation is the most frequent oxidative damage at the cellular level, produced particularly by the hydroxyl radicals (OH-) and peroxynitrite (ONOO-), on the polyunsaturated fatty acids of cell membranes. Initially, a fatty acid is oxidized by leaving a hydrogen atom of a methylene group to the OH- that acts as an oxidizing

agent, producing peroxyl radical. This, in turn, repeats the chain process until irreversible tissue damage occurs [18].

A second type of glia cell involved in neuroinflammatory processes are the astrocytes that reside in the CNS. Its activation leads to a condition called astrocytosis, where the number and size of the glial fibrillary acidic protein (GFAP), a cytoskeletal protein that contributes to hypertrophy and hyperplasia of astrocytes, increases. This leads to the generation of a glial scar in the area of tissue necrosis, excluding non-neuronal cells, and serves as a complement in spaces of neuronal loss (Zhang et al. 2010a).

For its part, among the main functions of astrocytes is its participation in the CNS immune response. Active astrocytes produce a variety of molecules, which are involved in the initiation, progression, and regulation of the inflammatory response. In addition to the production of pro- and anti-inflammatory cytokines, there is a greater expression of the enzyme genes cyclooxygenase 2 (COX-2) and iNOS as well as higher production of NO-, an important molecule in the development of oxidative stress and neuronal necrosis (Zhang et al. 2010). Table 1 lists the main substances released by glial cells that participate in neuroinflammatory processes.

The constant and prolonged activation of glial cells and astrocytes, as described previously, leads to a chronic state of inflammation, which perpetuates the release of proinflammatory molecules. The above contributes to the development of neurodegenerative processes, for example, AD, Parkinson's, and Huntington's, which are characterized by slow and progressive neuronal loss in certain areas of the brain such as the hippocampus, the striatum, the black substance of compact part (SNpc), and the cerebral cortex [23].

It is important to point out that neuroinflammation is only the mechanism of immunological protection against CNS damage, so its activation is of benefit for the protection of the integrity of the NS. However, chronic inflammation is characterized by the prolonged activation of the microglia and the perpetuation of mediators of inflammation, which increases the cellular oxidative and nitrosative state. In view of the above, a response chain is generated between oxidative stress-inflammation-

Table 1 Molecules released by microglia and astrocytes during the neuroinflammatory process

Microglia	Astrocytes
Complement proteins Neurotrophic factors Proinflammatory cytokines (IL-1, IL-6, IL-17, TNF-α) ROS (OH-, O_2^-, H_2O_2) RNS (NO^-, $ONOO^-$) iNOS	Complement proteins Neurotrophic factors (NGF, NFDB, CNF, GDFI-1) Proinflammatory cytokines (IL-1, IL-6, IL-17, TNF-α) ROS (OH^-, O_2^-, H_2O_2) RNS (NO^-, $ONOO^-$) iNOS COX-2

NGF neuronal growth factor
NFDB neurotrophic factor derived from the brain
CNF niliary neurotrophic factor
GDFI-1 growth factor derived from insulin-1

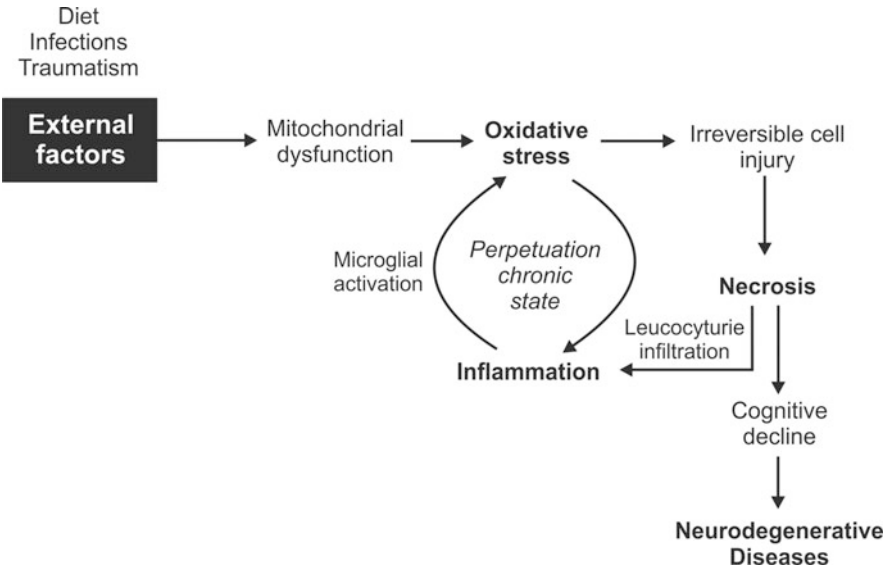


Fig. 2 Schematic representation of the neurodegeneration process through inflammation

oxidative stress, which favors necrosis, increasing cognitive decline and the development of NS, as described in Fig. 2 [7].

3.1 Alzheimer's Disease

The greatest evidence of the role of chronic neuroinflammation of the CNS as a trigger for neurodegenerative disease is in studies of AD. Considered as the most common neurodegenerative disease and the main cause of dementia in older adults, approximately 10% of all people over 65 and up to 50% of those over 85 are diagnosed with this disease [50].

AD is an irreversible and progressive disorder characterized by the loss of neurons mainly in the cerebral cortex and hippocampus, which leads to memory loss, cognitive deterioration, and changes in personality. Although its etiology is unclear, it is suggested that the accumulation of β-amyloid proteins in the neuritic plaques and tau protein residues are responsible for the attraction and activation of glial cells, triggering reactive changes in the microglia, and release of pro-inflammatory mediators and ROS that contribute to the necrosis of neuronal cells, leading to chronic and progressive neurodegeneration [49].

It should be noted that genetic susceptibility, trauma, diet rich in fats and simple sugars, alterations in cholesterol homeostasis, deficiency of cyanocobalamin, and iron overload are among the risk factors described. However, although none of the aforementioned appears to be a direct etiological factor, they do act as signaling

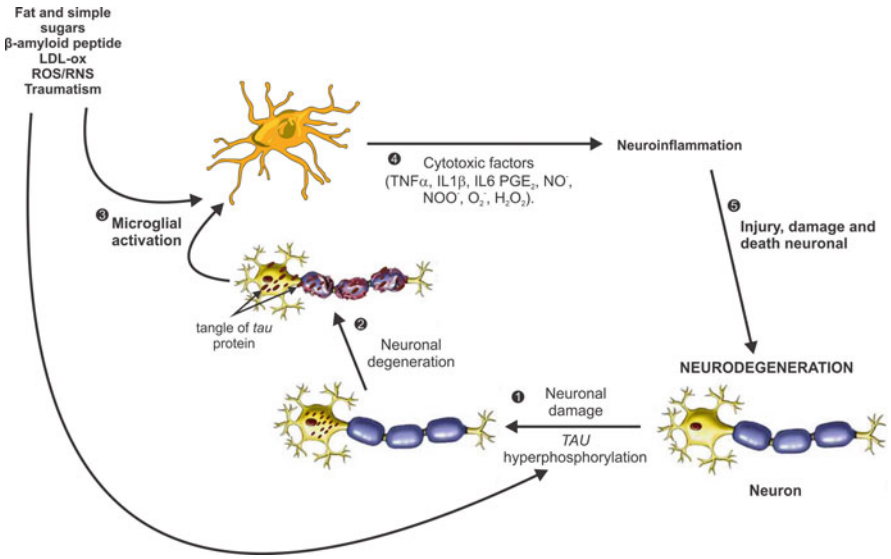


Fig. 3 Microglial activation model responsible for neuroinflammation in AD

devices at the CNS level, triggering alterations in the redox state, activation of glial cells, and protein abnormalities. Therefore, the constant and prolonged exposure of any of these factors could be an important trigger that favors a chronic neuroinflammation response [31].

One of the main approaches about the development of AD is the tau protein, which plays a fundamental role in the maintenance of microtubules of neurons and as a component of axonal transport. Currently, it is known that tau hyperphosphorylation leads to the self-aggregation of this protein, triggering the disarticulation of microtubules, disorders in neuronal activity, and loss in its ability to transmit messages. The above is translated into a cellular lesion that leads to the activation of microglia, release of proinflammatory cytokines, and the development of neuroinflammation (Fig. 3). The persistent alteration of the signaling mechanism produces necrosis of nerve cells and cognitive deterioration, eventually causing the process of neurodegeneration [49, 57].

Currently, evidence of the importance of inflammation in neuronal damage comes from immunohistochemical and molecular biology studies done in the brain tissues of patients with AD, which revealed the distinctive features of inflammation, including the activation of microglia and astrocytes, the expression of cytokines, and the invasion of immune cells. Studies performed in patients with AD reveal high concentrations of proinflammatory cytokines in the blood, mainly IL-6, TNF-α, IL-1β, IL-12, and IL-18. For its part, it has also been shown that the chronic treatment of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen, significantly reduces the risk of developing AD [50].

3.2 Parkinson's Disease (PD)

PD is the second most common neurodegenerative disorder associated with age and of unknown origin, affecting 4.1 to 4.6 million people over 50 years of age worldwide, and it is predicted that by 2030 this figure will double due to the increase in hope of life [60].

PD is characterized by the presence of disorders in involuntary movements, associated with the selective loss of dopaminergic neurons in the SNpc and by the presence of intraneuronal cytoplasmic protein inclusions (Lewy bodies) in the surviving neurons, as well as in other central regions and peripheral of the CNS. The accumulation of Lewy bodies in neurons is associated with the activation of microglia, the increase of proinflammatory cytokines, and the decrease of some neurotrophic products, which together lead to degenerative changes in the SNpc and locus coeruleus, specifically in dopaminergic neurons. In this process pro-inflammatory, it is believed that the major histocompatibility class II complex (MHC-II), half of the neurodegeneration process, since an increase in its expression due to the proinflammatory activation of the microglia has been related to a greater neuronal death in these regions [25]. Although several causes of PD are described, recent studies show that neuroinflammation and microglial activation play an important role in its pathogenesis, derived from a microenvironment of neurological damage that breaks down the homeostatic mechanisms that promote cell survival [51].

The degeneration of dopaminergic neurons produces a decrease in dopamine levels that causes a loss of synaptic regulation in basal ganglia. The basal ganglia are collections of nerve cell bodies located subcortically near the base of the brain. They include the striatum (caudate and putamen), subthalamic nucleus (NST), pale globe external (Gpe) and internal (Gpi), ventral nucleus of the thalamus, and the SNpc and SN pars reticulata (SNpr). These nuclei are responsible for motor control, since they are interconnected with the cortex and the brain stem and the thalamus [41].

Dopaminergic neurons are very sensitive to any pathological change that occurs in the brain. In response to the damage, the microglia increases the secretion of neurotrophic factors and proinflammatory cytokines and the activation of nitric oxide synthase, NADPH oxidase, and cyclooxygenase, which cause damage to the neuron and the reactivity of astrocytes; therefore a progression of neurodegeneration occurs in a self-powered way [51].

Activated microglia produces large amounts of superoxide radicals and is the main source of oxidative stress responsible for the death of dopaminergic cells in PD (Wang et al. 2015) (Fig. 4). In postmortem brains, high levels of proinflammatory cytokines have been observed and in animal models active microglia from early stages of neuronal degeneration [26, 39].

The in vivo correlation between microglial activation and the corresponding loss of dopaminergic neurons in early PD was studied by Ouchi in 2005. This correlation supports the hypothesis of neuroinflammatory response mediated by microglia that contributes significantly to the degenerative process and suggests the importance of

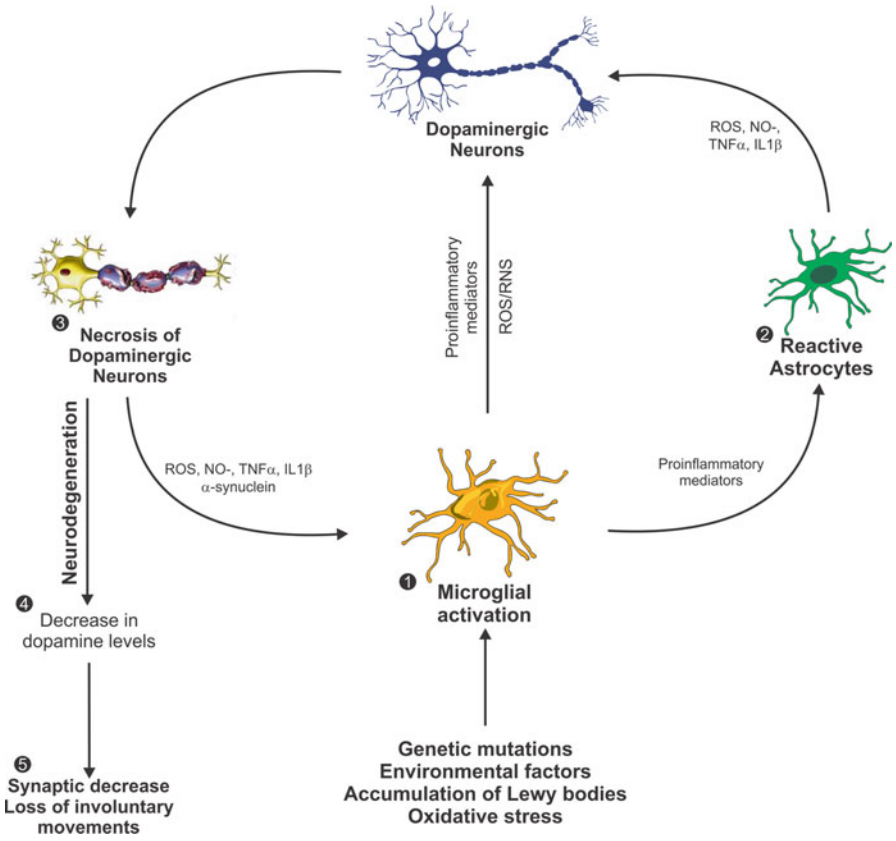


Fig. 4 Representation of the inflammatory mechanisms involved in the development of PD

early therapeutic intervention with neuroprotective drugs [42]. Currently, research is aimed at finding effective and preventive treatments for ND, paying special attention to the search for methods to normalize this phenomenon of excessive reactivity in the cells of the microglia, without inhibiting their elementary functions or causing a reduction in total microglia [58].

4 Advances in the Nutritional Treatment of ND

Scientific and technological advances in nutrition and health make it necessary to have effective interventions in the nutritional management of ND. Functional nutrition is an area of nutriology whose axis is the use of bioactive compounds in food, at specific doses, for the restoration of homeostasis lost in the disease. In the particular case of ND, its mechanisms of biochemical and metabolic alteration are associated

with neuroinflammation process, which is why the use of bioactive compounds with anti-inflammatory effect is a potential neuroprotective agent in ND [34].

The most widely studied anti-inflammatory and used in clinical practice is omega 3. Its anti-inflammatory activity lies in being a substrate of cyclooxygenase (COX) and lipoxygenase (LOX), for the synthesis of prostaglandins, thromboxanes, and leukotrienes of the anti-inflammatory series [34].

4.1 Anti-inflammatory Characteristics of Omega 3

The fatty acids of the omega 3 family are long-chain polyunsaturated fatty acids (PUFA) that present their first unsaturation in the third carbon. Among the main representatives of the family of omega 3 fatty acids is α-linolenic acid, present in foods of plant origin and which is metabolized into eicosapentaenoic acid (EPA) and subsequently into docosahexaenoic acid (DHA) in animal organisms [54].

EPA and DHA are important structural components of the phospholipids of cell membranes. Diets rich in EPA and DHA increase the proportion of these fatty acids in cell membranes, particularly in lymphocytes, which in addition to reducing the content of arachidonic acid (AA) in the membranes of these cells, by a competitive effect, decreases the generation of proinflammatory products derived from ω-6 PUFA. EPA is also a substrate for COX (1 and 2) and lipoxygenase-5, which competes with AA for the generation of eicosanoids, but in the case of EPA, these have anti-inflammatory properties. Due to the above, dietary supplementation with EPA can reduce the formation of prostaglandin E2 (PGE2), thromboxane A2 (TXA2), and leukotriene B4 (LTB4) and maintain the levels of prostaglandin I2, potent vasodilator, thromboxane A3, aggregation inhibitor platelet, and leukotriene B5 anti-inflammatory non-chemotactic that inhibits cell adhesion (Table 2) [55].

While the products of AA metabolism (PGE2, TXA2, and LTB4) have pro-inflammatory properties, the products of EPA conversion have antagonistic properties that decrease inflammation, vasoconstriction, and platelet aggregation and may even antagonize the effects, typically proinflammatory effects of eicosanoids derived from AA [1].

Table 2 Metabolic effects of eicosanoids derived from arachidonic acid (AA) and eicosapentaenoic acid (EPA)

Cyclooxygenase pathway				Lipoxygenase pathway	
Endothelial cells		Platelets		Leukocytes	
AA	EPA	AA	EPA	AA	EPA
Prostacyclin I2 Vasoconstrictor Platelet aggregation	Platelet antiaggregation Vasodilator	Thromboxane A2 Platelet aggregation Vasoconstrictor	Thromboxane A3 Vasodilator	Leukotriene B4 Proinflammatory Chemotactic Cell adhesion	Leukotriene B5 Anti-inflammatory Non-chemotactic Inhibits cell adhesion

The production of proinflammatory cytokines is regulated by the availability of eicosanoids derived from AA, which can be modulated by the intake of ω-3 PUFA, which act at the gene level, since the expression of the genes for cytokines and cell adhesion molecules it is reduced in response to the ω-3 PUFA exposure. In addition, ω-3 PUFA directly affect the intracellular signaling pathways associated with the activation of transcription factors, such as nuclear factor κβ (NF-κβ) and peroxisome proliferator-activated receptors (PPARs) that regulate the expression of a series of genes whose products are proinflammatory [1].

Supplementation with EPA and DHA is also able to reduce the production of proinflammatory cytokines, such as IL-1, IL-6, and TNF-α, which are released when macrophages and monocytes are activated. Although these cytokines are potent activators of immune function, the excess activity of these substances contributes to pathological inflammation, a situation observed in metabolic diseases of inflammatory origin [17].

4.2 Potential Neuroprotective Effect of Omega 3 (ω-3) in ND

Several studies have shown the neuroprotective role of ω-3 fatty acids in various circumstances such as the ND to exhibit benefits in the modulation of neuronal activity, the regulation of immune cell response, and also the production of lipid mediators pro-resolution and more potent anti-inflammatories, such as resolvins [20, 28, 29]. Also, neuroprotective effect is observed in lesions induced by ischemia and by excitotoxicity produced by neurotransmitters (glutamate, mainly) [21].

The neuroprotective effects of ω-3 are due to multiple factors and may be related to a series of molecular effects at the neuronal level, especially in the CNS. Studies in rodents indicate that long-chain ω-3 fatty acids play a role in cognitive and behavioral functions. Although ω-3, due to its chemical structure (with numerous double bonds), are more vulnerable to oxidative stress (characteristic of metabolic and neurodegenerative diseases), in cells in general and especially neurons, they can reduce the damage caused by oxidative stress through neuroprotectins (docosanoids derived from DHA) [5]. In addition, ω-3 regulate the expression of neuroprotective genes, such as the expression of the antiapoptotic Bcl2 gene [4].

According to Chen et al. [13], ω-3 supplementation inhibits microglial activation and the subsequent inflammatory response by regulating the translocation and nuclear secretion of HMGB1 and its activation in the signaling pathway TLR4/NF-κβ, pathways responsible for neuroinflammation after injury neuronal. The above is an important evidence that leads to the neuroprotective effects exhibited by ω-3 fatty acids.

Chronic ω-3 fatty acid deficiency increases anxiety in rodents, particularly in stressful conditions [19]. Also, chronic DHA deficiency is accompanied by anxiety and learning and memory impairments that have been associated with changes in the processes of neurotransmission [11].

Specifically, the diet deficient in alpha-linolenic acid reduces the dopaminergic neurotransmission of the nucleus accumbens of murine models. Through in vivo

microdialysis experiments, elevated basal levels of dopamine and its metabolites (DOPAC and HVA) were observed in rats subjected to a diet deficient in fatty acids, in comparison with controls, indicating alterations in dopaminergic neurotransmission in the nucleus accumbens [67].

Other investigations focused on the neuroprotective effects of ω-3 on AD reveal the presence of low levels of plasma DHA. In an animal model (mouse with Alzheimer's disease), when administering a diet enriched with ω-3 (EPA + DHA), a reduction in the accumulation of β-amyloid peptide (peptide with neurotoxic actions) was observed in more than 70% of the cases [27].

The administration of 15 mg of EPA and DHA per g of intake per day in female rats, treated on the second day of pregnancy up to 14 days after calving, significantly reduced brain damage and improved long-term neurological outcomes up to 5 weeks after a neonatal hypoxic ischemic injury. The ω-3 polyunsaturated fatty acids exerted an anti-inflammatory effect in microglia both in the in vivo model of ischemic-hypoxic and in vitro microglial cultures subjected to inflammatory stimuli by inhibiting the activation of NF-κβ and the subsequent release of inflammatory mediators (Zhang et al. 2010).

In more recent studies, mice induced to neuropathic pain by partial ligation of the sciatic nerve received oral treatment of a fish oil concentrate of 2.3 g/Kg of weight, and reduced levels of TNF-α in the spinal cord, from the myeloperoxidase activity (MPO) sciatica and the expression of activation transcription factor 3 (ATF-3), an important marker of neuronal injury, were observed. Likewise, the diet high in ω-3 improved the sciatic functional index (SCI), as well as the electrophysiological record, which corroborates the increase in the expression of GAP43 and the total number of myelin fibers observed in the sciatic nerve. These results point to the regenerative and possibly protective properties of a combined oral administration of EPA and DHA after a peripheral nerve injury, as well as its anti-neuroinflammatory activity, evidencing the ω-3 fatty acids that promise therapeutic results for the treatment of neurodegeneration [48].

4.2.1 Alzheimer's

The consumption of ω-3 fatty acids is associated with a reduction of neuroinflammation markers in AD [24, 53]. Limited evidence in human models shows that ω-3 improve the metabolic neuroinflammatory alterations present in AD. An intervention study reveals a decrease in the loss of gray matter volume after the combined treatment of DHA/EPA [62]. Yassine et al. [63] showed significant associations between low serum concentrations of DHA with increased cerebral amyloid load, smaller brain volume (SBV), and alteration in nonverbal memory, in volunteers without cognitive or mild impairment.

Correlative epidemiological studies suggest that a high intake of foods rich in ω-3 is associated with greater cognitive performance and possibly less neuronal deterioration and risk of AD [38]. Results recently presented at the Alzheimer's Association International Conference in Toronto indicate that blood DHA levels are significantly associated with superior cognitive ability in two large population-based studies, totaling more than 5000 individuals [56].

It should be noted that although clinical trials of ω-3 supplementation following the diagnosis of AD do not show significant effects, studies have shown its potential preventive effect, associated with its anti-inflammatory effects [43, 45, 64]. Recently published data from the MAPT (Multidomain Alzheimer Preventive Trial) report no significant benefit in elderly participants who received 800 mg/day of DHA supplements for more than 3 years [3].

4.2.2 Parkinson's

Evidence from clinical studies about ω-3 fatty acid supplementation in PD is still very limited. In animal models, it has been observed that DHA induces a recovery of the dopaminergic system after an extensive Parkinson's injury. After the induction of dopaminergic denervation with 6-hydroxydopamine (6-OHDA), a high intake of DHA led to a) increased levels of dopamine in the striatum, b) greater dopaminergic terminals in striated body, and c) increase of the soma area of dopaminergic neurons. Although the cell count remained unchanged, such improvement of key components in the dopaminergic system suggests that DHA activated the compensatory mechanisms that contribute to the functionality and recovery of CNS [14]. Therefore, these data suggest that DHA induced neuroregeneration and could be used after diagnosis of PD.

Recently Mori et al. [37] demonstrated that supplementation of 3 g/Kg of weight (18% EPA and 12% DHA) in Wistar rats mitigates the loss of SNpc neurons and nerve terminals in the striatum, after an induction of damage with 6-OHDA. This protective effect was associated with reductions in iNOS-immunoreactive cell density and reactivity of microglia and astrocytes, thereby reducing dopaminergic damage in PD.

5 Conclusion

The scientific evidence shows the potential neuroprotective effect of omega 3 fatty acids on ND, associated with the reduction of neuroinflammation. Although more clinical studies are necessary to obtain ω-3 dosages in the prevention and treatment of ND, the trials in animal and epidemiological models are an important reference for its recommendation as part of a nutritional treatment.

References

1. Agrawal A, Alharthi A, Vailati-Riboni M, Zhou Z, Loor J (2017) Expression of fatty acid sensing G-protein coupled receptors in peripartal Holstein cows. J Anim Sci Biotechnol 8(20):1–10
2. Allen NJ, Barres BA (2009) Glia – more than just brain glue. Nature 457:675–677
3. Andrieu S, Guyonnet S, Coley N, Cantet C, Bonnefoy M, Bordes S et al (2017) Effect of long-term omega 3 polyunsaturated fatty acid supplementation with or without multidomain intervention on cognitive function in elderly adults with memory complaints (MAPT): a randomised, placebo-controlled trial. Lancet Neurol 16(5):377–389

4. Bazan NG (2006) Cell survival matters: docosahexaenoic acid signaling, neuroprotection and photoreceptors. Trends Neurosci 29:263–271
5. Bazan NG (2009) Neuroprotectin D1-mediated anti-inflammatory and survival signaling in stroke, retinal degenerations, and Alzheimer's disease. J Lipid Res 50:S400–S405
6. Becher B, Spath S, Goverman J (2016) Cytokine networks in neuroinflammation. Nat Rev Immunol 17:49. https://doi.org/10.1038/nri.2016.123
7. Block ML, Hong JS (2005) Microglia and inflammation mediated neurodegeneration: multiple triggers with a common mechanism. Prog Neurobiol 76(77):98
8. Cai L, Wu X, Lv Y, Xu Y, Mi G, Li J (2015) The neuroprotective and antioxidant activities of protein hydrolysates from grass carp (*Ctenopharyngodon idella*) skin. J Food Sci Technol 52(6):3750–3755
9. Canet-Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S et al (2004) The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid driven mitochondrial localization. Proc Natl Acad Sci USA 101:9103–9108
10. Carson M, Doose J, Melchior B, Schmid C, Ploix C (2006) CNS immune privilege: hiding in plain sight. Immunol Rev 213:48–65
11. Chalon S (2006) Omega-3 fatty acids and monoamine neurotransmission. Prostaglandins Leukot Essent Fatty Acids 75(4–5):259–269
12. Chen W, Zhang X, Huang W (2016) Role of neuroinflammation in neurodegenerative diseases. Mol Med Rep 13:3391–3396
13. Chen X, Wu S, Chen C, Xie B, Fang Z et al (2017) Omega-3 polyunsaturated fatty acid supplementation attenuates microglial-induced inflammation by inhibiting the HMGB1/TLR4/NF-κβ pathway following experimental traumatic brain injury. J Neuroinflammation 14:143, 1–12
14. Coulombe K, Saint-Pierre M, Cisbani G, St-Amour I, Gibrat C, Giguere-Rancourt A et al (2016) Partial neurorescue effects of DHA following a 6-OHDA lesion of the mouse dopaminergic system. J Nutr Biochem 30:133–142
15. Cunningham C, Martínez V, Noctor S (2013) Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 33(10):4216–4233
16. Finkel L, Arroyo M, Crespo C, Garcés M (2014) Estudio sobre las enfermedades neurodegenerativas en España y su impacto económico y social. Resumen Ejecutivo Alianza Española de Enfermedades Neurodegenerativas, 2–4
17. Flachs P, Rossmeisl M, Kopecky J (2014) The effect of n-3 fatty acids on glucose homeostasis and insulin sensitivity. Physiol Res 63(1):S93–S118
18. Galicia M, Gutiérrez G (2014) Papel del estrés oxidativo en el desarrollo de la enfermedad hepática alcohólica. Rev Gastroenterol Mex 79:135–144
19. Harauma A, Moriguchi T (2011) Dietary n-3 fatty acid deficiency in mice enhances anxiety induced by chronic mild stress. Lipids 46(5):409–416
20. Heng LJ, Qi R, Yang RH, Xu GZ (2015) Docosahexaenoic acid inhibits mechanical allodynia and thermal hyperalgesia in diabetic rats by decreasing the excitability of DRG neurons. Exp Neurol 271:291–300
21. Hogyes E, Nyakas C, Kiliaan A, Farkas T, Penke B, Luiten PG (2003) Neuroprotective effect of developmental docosahexaenoic acid supplement against excitotoxic brain damage in infant rats. Neuroscience 119:999–1012
22. Hong S, Van Kaer L (1999) Immune privilege: keeping an eye on natural killer T cells. J Exp Med 190(9):1197–1200
23. Hong H, Kim BS, Im H (2016) Pathophysiological role of neuroinflammation in neurodegenerative diseases and psychiatric disorders. Int Neurourol J 20(1):S2–S7
24. Hopperton KE, Trepanier M-O, Giuliano V, Bazinet RP (2016) Brain omega-3 polyunsaturated fatty acids modulate microglia cell number and morphology in response to intracerebroventricular amyloid-b 1-40 in mice. J Neuroinflammation 13(1):257
25. Johnson ME, Bobrovskaya L (2014) An update on the rotenone models of Parkinson's disease: their ability to reproduce the features of clinical disease and model gene environment interactions. Neurotoxicology 46:101–116

26. Langston J, Forno L, Tetrud J, Reeves A, Kaplan J, Karluk D (1999) Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. Ann Neurol 46:598–605
27. Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, Salem N Jr (2005) A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. J Neurosci 25:3032–3040
28 Lobo BW, Lima CK, Teixeira MS, Silva NL, Takiya CM, Ramos MF et al (2016) Fish oil attenuates persistent inflammatory pain in rats through modulation of TNF-alpha and resolvins. Life Sci 152:30–37
29 Lu Y, Zhao LX, Cao DL, Gao YJ (2013) Spinal injection of docosahexaenoic acid attenuates carrageenan-induced inflammatory pain through inhibition of microglia-mediated neuro-inflammation in the spinal cord. Neuroscience 241:22–31
30 Lyman M, Lloyd D, Ji X, Vizcaychipi M, Ma D (2014) Neuroinflammation: the role and consequences. Neurosci Res 79:1–12
31 Maccioni RB (2008) Nuevas Avenidas hacia el diagnóstico y tratamiento de los desórdenes cognitivos: enfermedad de Alzheimer. Medwave 8(11). https://doi.org/10.5867/medwave.2008.11.3660
32 Marinelli C, Di Liddo R, Facci L, Bertalot T, Conconi M, Zusso M, Skaper S, Giusti P (2015) Ligand engagement of toll-like receptors regulates their expression in cortical microglia and astrocytes. J Neuroinflammation 12(244):1–20
33 Martínez E, Acevedo J, Segura M (2016) Biopeptides with antioxidant and anti-inflammatory potential in the prevention and treatment of diabesity disease. Biomed Pharmacother 83:816–826
34 Martínez E, Villavicencio T, Segura M (2017) Functional foods and chemoprevention in cancer. In: Grumezescu A, Holban A (eds) Therapeutic foods, 1st edn. Academic, London. ISBN 9780128115176
35 Mizuno T (2015) Neuron–microglia interactions in neuroinflammation. Clin Exp Neuroimmunol 6:225–231
36 Morales I, Farías G, Maccioni R (2010) La neuroinflamación como factor detonante del desarrollo de la enfermedad de Alzheimer. Rev Chil Neuropsiquiatr 48(1):49–57
37 Mori M, Delattre A, Carabelli B, Pudell C, Bortolanza M et al (2017) Neuroprotective effect of omega-3 polyunsaturated fatty acids in the 6-OHDA model of Parkinson's disease is mediated by a reduction of inducible nitric oxide synthase. Nutr Neurosci 21:1–11
38 Morris MC (2016) Nutrition and risk of dementia: overview and methodological issues. Ann N Y Acad Sci 1367(1):31–37
39 Muñoz AM, Rey P, Parga J, Guerra MJ, Labandeira-Garcia JL (2005) Glial over expression of heme oxygenase-1: a histochemical marker for early stages of striatal damage. J Chem Neuroanat 29:113–126
40 National Institute of Neurological Disorders and Stroke (2017) Neurodegenerative diseases
41 Obeso JA, Lanciego JL (2011) Past, present, and future of the pathophysiological model of the Basal Ganglia. Front Neuroanat 5:39–47
42 Ouchi Y, Yoshikawa E, Sekine Y, Futatsubashi M, Kanno T, Ogusu T, Torizuka T (2005) Microglial activation and dopamine terminal loss in early Parkinson's disease. Ann Neurol 57:168–175
43 Quinn JF, Raman R, Thomas RG, Yurko-Mauro K, Nelson EB, Van Dyck C et al (2010) Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. J Am Med Assoc 304(17):1903–1911
44 Reed TT (2011) Lipid peroxidation and neurodegenerative disease. Free Radic Biol Med 51:1302–1319
45 Salem N, Vandal M, Calon F (2015) The benefit of docosahexaenoic acid for the adult brain in aging and dementia. Prostaglandins Leukot Essent Fat Acids 92:15–22
46 Shabab T, Khanabdali R, Zorofchian S, Abdul H, Mohan G (2016) Neuroinflammation pathways: a general review. Int J Neurosci 127(7):624–633

47 Shrestha R, Millington O, Brewer J, Bushell T (2013) Is central nervous system an immune-privileged site? Kathmandu Univ Med J 41(1):102–107
48 Silva R, Oliveira J, Santos B, Dias F, Martinez A, Lima C, Miranda A (2017) Long-chain Omega-3 fatty acids supplementation accelerates nerve regeneration and prevents neuropathic pain behavior in mice. Front Pharmacol 8:1–12
49 Smith J, Das A, Ray S, Banik N (2012) Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain Res Bull 87:10–20
50 Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N (2010) A meta-analysis of cytokines in Alzheimer's disease. Biol Psychiatry 68:930–941
51 Tansey M, Goldberg M (2010) Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis 37(3):510–518
52 Torrell G (2015) Enfermedades neurodegenerativas. Actualización Med Familia 11(7):374–383
53 Trepanier M-O, Hopperton KE, Orr SK, Bazinet RP (2016) N-3 poly-unsaturated fatty acids in animal models with neuroinflammation: an update. Eur J Pharmacol 785:187e206
54 Twining C, Lawrence P, Winkler D, Flecker A, Brenna J (2017) Conversion efficiency of alpha linolenic acid to omega-3 highly unsaturated fatty acids in aerial insectivore chicks. J Exp Biol 221:jeb165373. https://doi.org/10.1242/jeb.165373
55 Valenzuela R, Tapia G, González M, Valenzuela A (2011) Omega-3 fatty acids (EPA and DHA) and its application in diverse clinical situations. Rev Chilena Nutr 38(3):356–367
56 Van Duijn CM, van der Lee SJ, Ikram MA, Hofman A, Hankemeier T, Amin N et al (2016) Metabolites associated with cognitive function in the Rotterdam study and Erasmus Rucphen family study. Alzheimers Dement 12(7):P165
57 Venneti S, Wiley CA, Kofler J (2009) Imaging microglial activation during neuroinflammation and Alzheimer's disease. J Neuroimmune Pharmacol 4(2):227–243
58 Wes P, Sayed F, Bard F, Gan L (2016) Targeting microglia for the treatment of Alzheimer's disease. Glia 64(6):1–23
59 WHO (2007) Los trastornos neurológicos afectan a millones de personas en todo el mundo. Informe de la OMS
60 WHO (2012) Reporte de prevalencias en enfermedades no transmisibles y salud mental
61 WHO (2016) Reporte de enfermedades crónico no transmisibles
62 Witte AV, Kerti L, Hermannstadter HM, Fiebach JB, Schreiber SJ, Schuchardt JP et al (2014) Long-chain omega-3 fatty acids improve brain function and structure in older adults. Cerebral Cortex (New York, N.Y.: 1991) 24(11):3059–3068
63 Yassine HN, Feng Q, Azizkhanian I, Rawat V, Castor K, Fonteh AN et al (2016) Association of serum docosahexaenoic acid with cerebral amyloidosis. JAMA Neurol 73(10):1208–1216
64 Yurko-Mauro K, Alexander DD, Van Elswyk ME (2015) Docosahexaenoic acid and adult memory: a systematic review and meta-analysis. PLoS One 10(3):e0120391
65 Zhang D, Hu X, Qian L, O'Callaghan J, Hong J (2010a) Astrogliosis in CNS pathologies: is there a role for microglia? Mol Neurobiol 41:232–241
66 Zhang W, Hu X, Yang W, Gao Y, Chen J (2010b) Omega-3 polyunsaturated fatty acid supplementation confers long-term neuroprotection against neonatal hypoxic–ischemic brain injury through anti-inflammatory actions. Stroke 41:2341–2347
67 Zimmer L, Delion-Vancassel S, Durand G et al (2000) Modification of dopamine neurotransmission in the nucleus *accumbens* of rats deficient in n-3 polyunsaturated fatty acids. J Lipid Res 41(1):32–40

Application of Lipid Nanocarriers for the Food Industry

22

Zahra Rafiee and Seid Mahdi Jafari

Contents

Abstract

Bioactive compounds effectively contribute to human health, and hence, they have recently attracted great attention in order to fortify food products and develop novel functional foods. However, employment of bioactive compounds in food matrices has some limitations. Most of the bioactive substances are readily decomposed in food as well as within the gastrointestinal tract that cause remarkable losses in their efficiency. Furthermore, they display low water solubility, poor bioavailability, and insufficient dispersibility. Other problems are

Z. Rafiee · S. M. Jafari (✉)
Department of Food Materials and Process Design Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
e-mail: zahra_rafiee@yahoo.com; smjafari@gau.ac.ir

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_93

related to their interaction with food ingredients and unfavorable effects on sensory attributes of food products. Lipid-formulation nanoencapsulation technologies including nanoliposomes, nanoemulsions, lipid nanoparticles (SLNs, solid lipid nanoparticles, and NLCs, nanostructured lipid carriers), and nano-phytosomes potentially help to solve these issues. These nanodelivery systems provide more stability, solubility in different media, functionality, bioavailability, targeting properties, and the ability of controlled release in food and pharmaceutical practices. This chapter reviews lipid-based nanocarriers in terms of production methods, types, characteristics, and composition for incorporation of different bioactive compounds. Also, food applications of various bioactive compounds incorporated in the commonly used lipid-based nanocarriers are highlighted. In this sense, the relevant recent studies have been discussed.

Keywords
Bioactive compounds · Nanoencapsualtion · Lipid-based nanocarriers · Functional foods · Food applications

1 Introduction

Health concerns have recently increased the tendency for development of functional foods and novel food products fortified with bioactive compounds and nutraceuticals especially phytochemicals [14, 205]. However, there are some limitations for direct utilization of bioactive compounds in food matrices. Most of the bioactive substances suffer from low stability and decomposition when exposed to unfavorable conditions during food processing and storage (e.g., light, oxygen, and moisture) as well as within the gastrointestinal tract (GIT) which reduces their efficiency and bioactivity [1, 139]. Moreover, they exhibit low water solubility, poor bioavailability, and insufficient dispersibility in food systems, interact with food ingredients, and negatively influence sensory properties of food systems [39, 46, 51, 66].

Food-grade delivery systems for nanoencapsulation of bioactive compounds represent an efficient way to solve these drawbacks. Among different nanodelivery techniques, lipid-based nanocarriers (Fig. 1) including nanoliposomes, nanoemulsions, lipid nanoparticles (SLNs, solid lipid nanoparticles, and NLCs, nanostructured lipid carriers) and nano-phytosomes have received a great attention owing to their beneficial characteristics [6, 64]. They have great potentials to accommodate and release various bioactive compounds (hydrophilic, lipophilic, and amphiphilic compounds) in a sustained and controlled manner, improve solubility and encapsulation efficiency of hydrophobic bioactive compounds, decrease their volatility, and enhance their target specificity. Lipid-based nanocarriers also promote effectiveness and bioavailability by enhancing stability of entrapped compounds in food mediums and during digestion [43, 79, 192]. Furthermore, these systems are biocompatible and can be fabricated by inexpensive and safe components for large-scale industrial practices via simple and available production technologies without the use of organic solvents [79, 98]. This chapter will review lipid-based nanocarriers in terms of

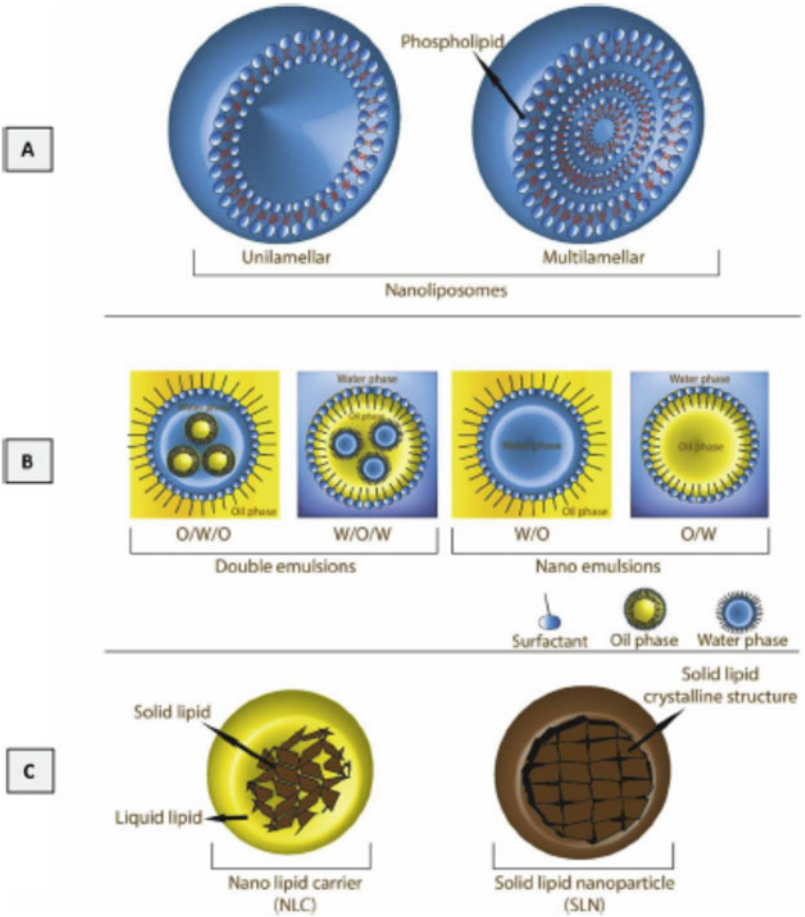


Fig. 1 Schematic illustration of three important nanostructured lipid-based encapsulation formulations: nanoliposomes (**a**), nanoemulsions (**b**) plus NLCs and SLNs (**c**). (Reprinted with permission from Ref. [6])

production methods, types, characteristics, and composition. Also, food applications of different bioactive compounds incorporated in the commonly used lipid-based nanocarriers are highlighted. Future trends will also be discussed in the last section.

2 Nanoencapsulation by Lipid-Based Nanocarriers

Encapsulation is described as an effective and practical technology for incorporating different unstable compounds within carrier materials which can occur in micro- and nanoscale. According to nanotechnology definition, an average size under 1000 nm is referred to as nanoparticle; although for therapeutic and pharmaceutical delivery

systems, it should be lower than 100 nm [54, 194]. Many studies have indicated superiority of nanocarriers to micro ones. Compared to microencapsulation systems, nanosized carriers offer more stability, solubility in different media, functionality, bioavailability, absorption, homogeneity, targeting properties, and the ability of controlled release in food and pharmaceutical practices which are associated with their greater surface area and larger reactivity [48, 52]. Moreover, food formulations containing nanoparticles with sizes smaller than 100 nm have acceptable sensory attributes due to their high optical transparency [114, 115, 160].

There are several methods for preparing nanoencapsulated bioactive compounds which can be categorized into five different classes including lipid-based techniques, specialized-equipment techniques, nature-inspired techniques, biopolymer-based techniques, and disparate techniques [79]. Nanoencapsulation technology should be selected on the basis of various factors such as release pattern, cost, the nature and physicochemical features of the wall and core materials, purpose of encapsulation, etc. [6, 167].

Diverse types of wall materials can be applied to incorporate bioactives including proteins, lipids, and carbohydrates. Carbohydrate- and protein-based nanocapsules cannot be applied in industrial applications because of occurring complex chemical or heat treatments which make it difficult to control processing conditions. In contrast, lipid-based nanocarriers not only have scaling-up potentials and low toxic effects but also offer greater encapsulation potency [54, 199]. The presence of emulsifiers is mostly essential to incorporate hydrophobic bioactive substances (e.g., flavonoids, fatty acids, aromas, phytosterols, lipophilic vitamins, and carotenoids) due to their non-solubility nature. Furthermore, utilization of digestible lipids provides sufficient blended micelles for solubilization and carrying lipophilic compounds which consequently facilitate their intestinal absorption [78, 164]. Accordingly, lipid-formulation nanoencapsulation technologies have received remarkable attention as novel and promising vehicles for bioactive ingredients in food and pharmaceutical industries. Nanoliposomes, nanoemulsions, SLNs, NLCs, and nano-phytosomes are basic kinds of lipid-based nanocarriers which are described in the following sections [50, 64, 79].

2.1 Nanoemulsions

The emulsion is defined as a system composed of two immiscible liquids (mostly water and oil) where one is dispersed as the droplet form (the dispersed or internal phase) in other one (the continuous or external phase). Emulsions can be classified into three main types in terms of particle size of droplets: microemulsion (10–100 nm), macroemulsion (0.5–100 μm), and nanoemulsion (known as miniemulsion, 100–500 nm) [80, 83].

The nanoemulsions are formulated in well-optimized mixtures consisting of at least three components being oil, aqueous phases, and surfactant(s). The oil phase of oil-in-water (O/W) nanoemulsions and water phase of water-in-oil (W/O) nanoemulsions are generally hosting the lipophilic and hydrophilic bioactive ingredients,

respectively [131, 132]. The bioactive compounds are usually solubilized in the related phase prior to the formation of nanoemulsions. The lipophilic phase can be comprised of triacylglycerols (long-chain, medium-chain, and short-chain triglycerides), essential oils, mineral oils, fat substitutes, waxes, or combination of them. In addition to the oil and water phases, the preparation of nanoemulsions requires the use of surfactant(s) which is adsorbed on newly formed droplet surfaces and facilitates droplet disruption and protects droplets toward aggregation [128].

As illustrated in Table 1, nanoemulsions can be generally produced by two different techniques: high-energy (e.g., ultrasonication, electrified coaxial liquid jets, microfluidization, high-pressure homogenization, and high shear mixing) or low-energy emulsification approaches [81, 82]. High-energy techniques need high levels of mechanical energy to provide intense disruptive forces for generating fine droplets. In contrast, low-energy procedures are based on utilizing the internal chemical energy of the oil-water-surfactant mixtures, and emulsification is merely carried out via simple stirring [79]. It is possible to fabricate nanoemulsions by modifying the system conditions. For instance, large amounts of emulsifiers, a high volume fraction of dispersed phase, and changes in the hydrophilic-lipophilic balance of matrix through modifying parameters (e.g., composition or temperature) lead to generation of nanoemulsions by microemulsification, catastrophic inversion, and phase inversion methods (inversion temperature and phase inversion composition), respectively [9, 183]. Low-energy methods are not suitable for scaling up and industrial purposes [11, 12]. High-energy techniques are highly favorable for producing ultra-fine droplets, but they may degrade susceptible compounds [84]. Furthermore, nanoemulsions can be manufactured by cubosomes and colloidosomes as well as microfluidic channels [6].

Nanoemulsions possess distinct features such as a small droplet size, optical transparency, high surface area, and high kinetic stability which make them appropriate candidates for the delivery of bioactive compounds [137, 138]. They display good potentials to improve solubility, absorption, and bioavailability of incorporated bioactive compounds [182, 186]. However, some adverse phenomena may take place in nanoemulsion systems like Ostwald ripening, flocculation, creaming, coalescence, and gelling, which negatively influence their storage stability. On the other hand, they require high levels of emulsifiers and particular devices for nanoencapsulation [6, 127, 172].

2.2 Nanoliposomes

Liposomes are self-assembled spherical vesicles consisting of at least one concentric lipid bilayer that encloses an aqueous phase [145]. Dispersing phospholipids in aqueous solutions results in orientation of hydrophobic groups toward the inside of the sphere with the hydrophilic heads toward outside. Therefore, a spherical bilayer membrane is formed which surrounds the aqueous core [47, 54]. Liposomes are categorized into different classes of large unilamellar vesicles (LUVs), small unilamellar vesicles (SUVs), giant unilamellar vesicles (GUVs), multivesicular

Table 1 Overview of nanoemulsion preparation techniques (Reprinted with permission from Ref. [6])

Technology	Process steps
Hot homogenization technique	1. Melting the lipid and dissolving/dispersing the bioactive compound in the lipid 2. Dispersing the bioactive-loaded lipid in hot aqueous surfactant mixture 3. Premixing using a stirrer to form a coarse pre emulsion 4. High-pressure homogenization at temperatures above lipid melting point 5. Hot O/W nanoemulsion 6. Solidification of the nanoemulsion by cooling down to room temperature
Cold homogenization technique	1. Melting the lipid and dissolving/dispersing the bioactive compound in the lipid 2. Solidification of the bioactive-loaded lipid in liquid nitrogen or dry ice 3. Grinding in a powder mill (50–100 μm) 4. Dispersing the powder in an aqueous surfactant dispersion medium (premix) 5. High-pressure homogenization at room temperature or below
High-pressure homogenization	1. The lipid is pushed with high pressure (100–2000 bars) through a very high shear stress 2. Disruption of particles down to the submicrometer or nanometer range
Solvent emulsification-evaporation method	1. Lipids and bioactive compounds are dissolved in a water-immiscible organic solvent with low boiling point 2. The solution is then emulsified in the aqueous emulsifier solution 3. Evaporate in rotary evaporation at 50–60 °C
Solvent emulsification-diffusion technique	1. Both the solvent and water are mutually saturated in order to ensure the initial thermodynamic equilibrium of both liquids 2. Lipid and bioactive compound are dissolved in water-saturated solvent, and this organic phase is stirred using mechanical stirrer 3. After formulation of O/W emulsion, water in typical ratios from 1:5 to 1:10 is added to the system in order to allow solvent diffusion into the continuous phase, thus leading to the aggregation of the lipid in the nanoparticles
Microemulsion technique	1. Lipids are melted, and bioactive compound is incorporated into molten lipid 2. Mixture of water, co-surfactant, and surfactant is heated to the same temperature as lipids and added under mild stirring to the lipid melt 3. A transparent, thermodynamically stable system is formed when the compounds are mixed in correct ratios for microemulsion formation 4. This microemulsion is then dispersed in a cold aqueous medium under mild mechanical mixing of hot microemulsion with water in a ratio in the range 1:25–1:50
Melting dispersion method	1. Bioactive compound and solid lipid are melted in an organic solvent regarded as oil phase. Simultaneously water phase is also

(*continued*)

Table 1 (continued)

Technology	Process steps
	heated to the same temperature as oil phase 2. The oil phase is added to a small volume of water phase, and the resulting emulsion is stirred at high speed for few hours 3. The emulsion is cooled down to room temperature to yield nanoparticles
Ultrasonication technique	1. The core material is melted 2. Addition of phospholipids along with an aqueous medium 3. Dispersing the melted material at increased temperatures by ultrasonication
Solvent injection	1. Lipids are dissolved in a water-miscible solvent (e.g., acetone, isopropanol, and methanol) or water-miscible solvent mixture 2. Quickly injected into an aqueous solution of surfactants through an injection needle
Double emulsion technique	1. Bioactive compound (mainly hydrophilic ones) is dissolved in aqueous solution emulsified in melted lipid 2. The primary emulsion is stabilized by adding stabilizer that is dispersed in aqueous phase containing hydrophilic emulsifier 3. Emulsion is stirred and filtered

vesicles (MVVs), and multilamellar vesicles (MLVs) regarding their size and lamellarity [68].

A wide variety of methods have been employed for producing nanoliposomes. These techniques are generally divided into mechanical (extrusion, ultrasonication by probe or bath sonicator, microfluidization, and high-pressure homogenization) and nonmechanical methods (reversed-phase evaporation, freeze-drying-rehydration, freeze-thawing, depletion of mixed detergent-lipid micelles, and injection methods) [203]. Mozafari [141] proposed a method for liposome preparation based on heating treatment. Today, novel methods such as dense gas techniques, supercritical fluid technology, dual asymmetric centrifugation, membrane contactor technology, cross-flow filtration technology, and freeze-drying of double emulsions method have been developed for preparing nanoliposomal formulations [76, 121, 207]. Table 2 presents advantages and disadvantages of various preparation methods of nanoliposomes.

Nanoliposomes are mostly used in food, bioprocessing, and pharmaceutical industries for incorporation of bioactive compounds to enhance their solubility, bioavailability, and functionality, provide controlled and sustained release, and protect them against adverse conditions. Also, nanoliposomes can be manufactured from natural and safe components (e.g., soy, egg, or milk) and are biocompatible and targetable. Additionally, liposomal ingredients such as phospholipids and sphingolipids have potential health benefits for humans [51, 171]. Owing to amphiphilic nature, liposomes are capable of encapsulating bioactive compounds with different degrees of solubility. Hydrophobic compounds are distributed within the lipid bilayers, while hydrophilic substances are enclosed inside aqueous space. Compounds with intermediate polarity also can be located between aqueous

Table 2 Overview of conventional and novel methods for nanoliposome production (Reprinted with permission from Ref. [6])

Technology		Advantages	Disadvantages
Conventional methods	Thin-film dispersion (Bangham method)	Rapidly formed; low energy input to form. Highest stability and transition cooperatively	Usually yields large and multilamellar vesicles with low encapsulation efficiencies (EEs)
	Ethanol/ether injection	Organic solvent residue, ready to nozzle blockage in ether system, time-consuming, sterilization issue	Highly heterogeneous dispersion; lower encapsulation efficiency vs. LUVs. Nearly impossible to determine mass
	Ultrasonication: probe	Can be performed directly on hydrated MLVs. Preferred method to form SUVs Excellent for reduction of large MLVs to more homogenous dispersion of SUVs	Probe sonication can degrade sample/localized overheating Requires constant cooling. Liposomes are metastable (low storage stability) Low volume required for effective treatment
	Ultrasonication: bath	Less destructive to liposomes, more homogenous product, and greater reproducibility vs. probe sonicator. Increased sample volume capability. Increased control over sample temperature	Requires extensive sonication to obtain minimum size limit of SUV; not always possible. Nonhomogenous product; requires removal of large vesicles by chromatographic/ centrifugal methods
	Reverse-phase evaporation	Higher entrapment efficiency reported for variety of molecules with MLVs (proteins, nucleic acids, etc.)	Heterogeneous distribution of MLVs and unilamellar vesicles; additional homogenization required. Solvent exposure may inhibit protein activity. Incomplete removal of organic solvent
	Freeze-dried rehydration vesicles	High entrapment efficiency. Improved homogeneity in mixing of lipid species/liposome. Useful for forming antigen-entrapping vesicles	Dehydration best controlled by freeze-drying; long time to process
	Microfluidic channel	Control of particle size, production of vesicles with diameter up to 29 nm	Not suitable for bulk production, organic solvent use, with agitation over treatment, damage of the liposome structure, and the leakage of encapsulated components

(*continued*)

Table 2 (continued)

Technology		Advantages	Disadvantages
	Detergent depletion	No solvent used; protein activity retained. No mechanical energy input. Homogenous distribution of liposomes. Useful for multiple lipids and molecules for encapsulation. Best for entrapping membrane-associated proteins	Limited number of useful detergents. Dialysis is very slow process, and some detergent is likely to remain in the sample. Detergent may negatively interact with molecule of interest
	Membrane extrusion	Homogenization of liposomes improved over other methods; rapid preparation of unilamellar vesicles from MLVs	Liposomes must be held above TM to facilitate extrusion. Manual extruders handle only small volumes (1 ml). Solute leakage can occur during extrusion. High salt concentration
Novel methods	Heating method	Absence of potentially toxic solvents and using very low shear forces; applied to large-scale production	Long time to process
	Freeze-drying of double emulsions	Relatively high encapsulation efficiency and excellent stability during long-term storage	Long time to process
	High-pressure homogenization, microfluidization	Can process high lipid concentration (∼150 mg/ml). Lab data is easily inferred to processing plant. High reproducibility. Most useful for large-scale production of liposomes	Solvent ionic strength must be carefully controlled to control liposome size. Complete homogenization is time-consuming in microfluidizer
	Supercritical fluid injection and decompression	Control of particle size, possible in situ sterilization, low organic solvent consumption	High cost, low yield, high pressure up to 350 bar used
	Dual asymmetric centrifugation	Simple method, homogenous liposome production with 60 nm size, high trapping efficiency	Not suitable for bulk production, high pressure, with agitation
	Dense gas techniques	Possible in situ sterilization, producing stable and homogenous iposome, low organic solvent consumption	Need multiple stages to achieve the final size of liposome, high pressure up to 200–300 bar, readily block nozzles

compartment and the lipid moieties. Furthermore, nanoliposomes provide simultaneous encapsulation, release, and delivery of compounds with different polarities [4]. For instance, this possibility has been revealed for α-tocopherol with ascorbic acid and glutathione [141].

Despite mentioned advantageous properties, some drawbacks restrict potential applications of liposomes. They are unstable systems and easily undergo oxidation, hydrolysis, fusion, and aggregation, decreasing loading capacity of bioactive compounds [37, 203]. Also, liposomes exhibit a short release time and inadequate half-life circulation. Using cholesterol, hydrogenated soy phosphatidylcholine, and neutral long-chain saturated phospholipids (e.g., disteroylphosphatidylcholine) can increase the stability and rigidity of bilayers. Phytosterols have been recently introduced as a suitable substitute of cholesterol in liposomal formulations especially for patients with hypercholesterolemia [47, 121]. Moreover, several techniques such as freezing, lyophilization (freeze-drying), supercritical fluid technology, and spray-drying have been evaluated for extending the shelf life of liposome structures [54]. Overall, lyophilization is considered as the best stabilization method for thermolabile substances loaded within liposomes [28]. Furthermore, polymer coating is a suitable and efficient stabilization method for liposomal systems. Coating with chitosan can improve stability, bioavailability, and sustained release of liposomes containing bioactive compounds [37]. Also, it was demonstrated that polyethylene glycol coating could effectively promote circulation half-life, functionality, and stability of bioactives [125].

2.3 Nano-Phytosomes

The term phytosome comes from two words of “phyto” and “some” which mean plant and cell-like, respectively [19, 184]. Phytosome, also called as herbosome and phytolipid delivery system, is resulted from the complex of plant extracts or phytoactive compounds (i.e., flavonoids and terpenoids) with phospholipids, which are formed via hydrogen bonds between their polar moieties [94, 95].

Basically, main components of phytosome formulations include phytochemicals, phospholipids, and solvents. The ratio of phospholipid to phyto-actives plays an important role in phytosome systems, and 1:1 or 2:1 ratios are often preferred. The common phospholipids for producing phytosomes are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. The former is most widely used for producing phytosomes. Aprotic solvents like acetone, ethyl acetate, dioxane, and methylene chloride can be successfully utilized in phytosome systems, but ethanol as a safe solvent is a good candidate for food purposes [59, 64, 205]. In order to separate organic solvents from obtained phytosomes, various methods can be used including spray-drying, freeze-drying, vacuum evaporation, and precipitation via n-hexane as an aliphatic hydrocarbon. Different techniques have been applied to develop phytosomes such as precipitation, anhydrous cosolvent lyophilization, anti-solvent precipitation, and solvent evaporation [95, 206].

The phytosome and liposomes are similar in the terms of structure. However, phytosomes possess a higher ability to enhance stability, absorption, and bioavailability of incorporated ingredients than liposomes which is associated with presence of H-bonds between phospholipids and the loaded compounds in phytosome structures. In contrast, there is no chemical interaction in liposomes, and the bioactives are merely surrounded by phospholipid bilayer. Intensifying bioavailability and absorption of lipid-soluble compounds by phytosomes as a delivery system is more evident, which reduces required amount to exhibit their advantageous effects and boost functionality [134, 188, 193].

It has been demonstrated that antioxidant activity of phenolic compounds in phytosomal systems is more than their free ones. In addition to food applications, there is a growing trend for usage of phytosomes in cosmetics owing to improving skin penetration of bioactive compounds. They can easily pass through the membrane and release bioactives into the cells, prevent degradation of bioactives by gut bacteria as well as digestive secretions, and provide target specificity [59, 134, 188]. Phytosomal products are commercially available in the market [7].

In spite of the advantages, phytosomes are sensitive to pH variations, and it is better to use them in food products with the neutral pH values such as milk [64].

2.4 Lipid Nanoparticles

2.4.1 Solid Lipid Nanoparticles (SLNs)

SLNs are nanoparticles with an organized crystalline structure and can entrap different bioactive components within their lipid network [212]. SLNs are basically derived from O/W emulsions, but they use solid lipids instead of the liquid one (oil), and therefore, they remain in solid form at ambient as well as human body temperatures [211].

Main ingredients utilized within SLN formulations are solid lipids, surface active materials (emulsifiers), and water. Lipid matrices are fabricated using a wide variety of natural or synthetic digestible lipids. Some of the most common lipids include triglycerides (tripalmitin, tristearin, and trimyristin), glyceryl monostearate (Imwitor), glyceryl palmitostearate (Precirol ATO 5), glyceryl behenate (Compritol 888 ATO), fatty acids (palmitic acid, stearic acid, and decanoic acid), cholesterol, waxes (carnauba wax, cetyl palmitate, and beeswax), and different types of hard fats [49, 63, 214]. Phospholipids as neutral surfactants (lecithin), ionic surfactants (sodium lauryl sulfate, sodium deoxycholate, sodium dodecyl sulfate, sodium cholate, sodium taurodeoxycholate, sodium oleate, trimethylammonium bromide, and stearylamine), nonionic surfactants (Poloxamer, Span, Pluronic, Tween, Solutol, Tego Care, and Brij), polyvinyl alcohol, and polyethylene glycol are the most important emulsifiers or surfactants which improve stability of lipid matrices [49, 110, 190]. Furthermore, mono- and diglycerides as solubilizing agents can enhance solubility and loading efficiency of bioactive compounds [54].

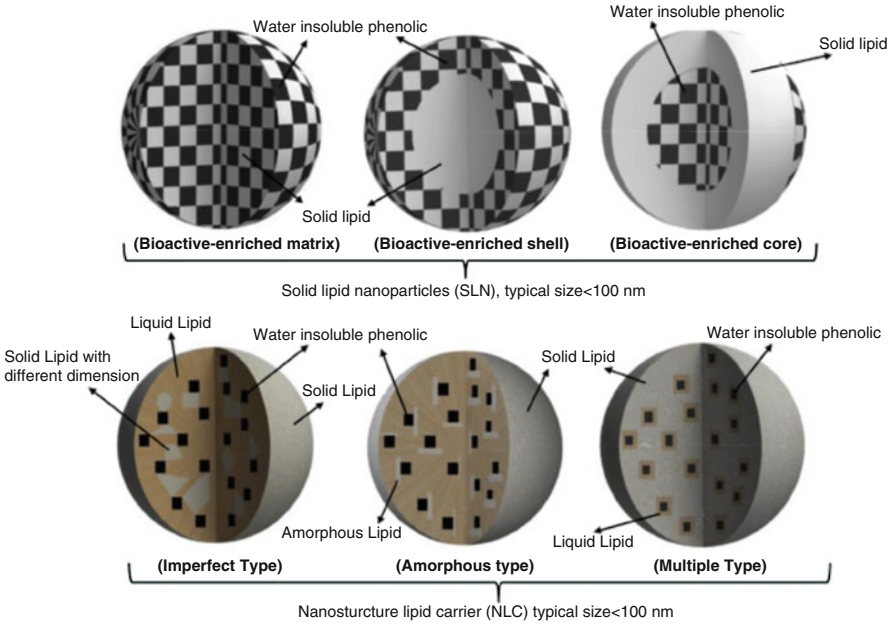


Fig. 2 Classification of SLNs and NLCs. (Reprinted with permission from Ref. [97])

Encapsulation of bioactive compounds within SLNs is usually carried out via three models including homogeneous matrix (solid solution) model, bioactive-enriched shell model, and bioactive-enriched core model (Fig. 2). In homogeneous matrix model, the dispersion of bioactive compounds entirely occurred in the lipid matrix. This type is mainly achieved by cold homogenization technique or when highly lipophilic compounds are entrapped inside SLNs applying hot homogenization. No surfactant is used for fabrication in this model. Bioactive-enriched shell model is formed as high concentration of surfactants is used or when phase separation from the liquid oil droplet takes place during the cooling process. This model has a burst release pattern. When the inverse phenomenon of bioactive-enriched shell model occurs, bioactive-enriched core type can be achieved. In this model, bioactive compounds precipitate first, and subsequently the shell entraps lower amounts of incorporated ingredients. This model results in controlled release of bioactive compounds [60, 97].

SLNs were developed to overcome the defects of other lipid-based nanocarriers such as nanoliposomes and nanoemulsions. They can be prepared without use of organic solvents; provide prolonged release, target specificity, and biocompatibility; protect incorporated compounds against adverse external conditions as well as chemical reactions (e.g., oxidation); improve stability, solubility, dispersibility, and bioavailability; facilitate large-scale production; and be utilized in different food products [148]. However, SLNs have limited space for encapsulating bioactive

compounds. They may also undergo crystallization, gelation, aggregation, and expulsion of core materials during storage period which negatively influences their characteristics and release behavior [40, 60].

2.4.2 Nanostructured Lipid Carriers (NLCs)

To overcome deficiencies of SLNs, NLCs were introduced by [221] as an efficient strategy for nanoencapsulation of bioactive compounds. NLCs are originated from O/W emulsions like SLNs. However, inner core of NLCs is obtained from a mixture of solid lipid (70%) and liquid lipid (30%), while lipid matrix of SLNs entirely consists of solid lipids (100%) [6, 43].

Basically, both saturated oils (e.g., paraffin oil and medium-chain triglycerides) and unsaturated oils (e.g., oleic acid, seed and vegetable oils) are employed for developing NLCs as liquid lipids [49]. Most common type of liquid lipid for producing NLCs is medium-chain triglycerides (MCT) such as Miglyol® 812. However, healthy unsaturated fatty acids (e.g., oleic acid and linoleic acid) can be considered as a substitute for saturated oils [44, 143, 199, 219].

NLCs are structurally divided into three diverse types: imperfect crystal, amorphous, and multiple models by considering type of bioactive compounds, the purpose of nanoencapsulation, preparation techniques, and makeup (Fig. 2). In the first type, NLCs are fabricated by diverse lipids with specific dimensions which increase their capacity for incorporating bioactive compounds. In the second group called amorphous model, noncrystalline structures are produced by a mixture of solid lipids with some other lipids (isopropyl myristate, hydroxyoctacosanyl, hydroxystearate, or MCT). In turn, the mixture can effectively decrease expulsion and release rate by forming an amorphous matrix. This model can prevent degradation of bioactive compounds which are highly soluble within liquid lipids. In the latter group called multiple model, employing certain liquid lipids (e.g., hydroxyl stearate and isopropyl myristate) leads to reduction of crystallization and therefore expulsion. The model is particularly suitable for enclosing and modulated release of bioactive compounds which exhibit great solubility in liquid lipids [60, 97, 143].

Preparation method of NLCs and SLNs are the same. Diverse approaches have been applied to fabricate these nanocarriers as shown in Table 3 which could be categorized into two major groups. The first group includes methods which require large amounts of energy like high-speed homogenization, ultrasound, and high-pressure homogenization, and the second group are methods with low energy consumption like microemulsions, solvent-based methods, and phase inversion temperature [143, 144]. It could be concluded that hot, cold, and the high-pressure homogenization techniques are practically more favorable for making these nanocarriers compared with other methods [6]. NLCs have attracted increasing attention because they possess good capability to optimize penetration, absorption, bioavailability, and release rate of loaded bioactives in food, cosmetic, and pharmaceutical applications [30].

Table 3 Overview of production methods for SLNs and NLCs (Reprinted with permission from Ref. [97])

Technology	Process steps
Hot homogenization technique	1. Melting of the lipid and dissolving/dispersing of the bioactive in the lipid 2. Dispersing of the bioactive-loaded lipid in hot aqueous surfactant mixture 3. Premix using a stirrer to form a coarse pre-emulsion 4. High-pressure homogenization at temperature above lipid melting point 5. Hot O/W nanoemulsion 6. Solidification of the nanoemulsion by cooling down to room temperature
Cold homogenization technique	1. Melting the lipid and dissolving/dispersing of the bioactive in the lipid 2. Solidification of the bioactive-loaded lipid in liquid nitrogen or dry ice 3. Grinding in a powder mill (50e100 mm) 4. Dispersing the powder in an aqueous surfactant dispersion medium (premix) 5. High-pressure homogenization at room temperature or below
High-pressure homogenization	1. The lipid is pushed with high pressure (100e2000 bars) through a very high shear stress 2. Disruption of particles down to the submicrometer or nanometer range
Solvent emulsification-evaporation method	1. Lipids and bioactive compound are dissolved in a water-immiscible organic solvent with low boiling point 2. The solution is then emulsified in the aqueous emulsifier solution 3. Evaporation in a rotary evaporator at 50–60 °C
Solvent emulsification-diffusion technique	1. Both the solvent and water are mutually saturated in order to ensure the initial thermodynamic equilibrium of both liquids 2. Lipid and bioactive are dissolved in water-saturated solvent, and this organic phase is stirred using mechanical stirrer 3. After the formulation of O/W emulsion, water in typical ratio from 1:5 to 1:10 is added to the system in order to allow solvent diffusion into the continuous phase, thus leading to the aggregation of the lipid in the nanoparticles
Microemulsion technique	1. Lipids are melted, and bioactive is incorporated in molten lipid 2. A mixture of water, co-surfactant(s), and the surfactant is heated to the same temperature as the lipids and added under mild stirring to the lipid melt 3. A transparent, thermodynamically stable system is formed when the compounds are mixed in the correct ratios for microemulsion formation. Thus the microemulsion is the basis for the formation of nanoparticles of a requisite size 4. This microemulsion is then dispersed in a cold aqueous medium under mild mechanical mixing of hot microemulsion with water in a ratio in the range 1:25–1:50.

(*continued*)

Table 3 (continued)

Technology	Process steps
Melting dispersion method	1. Bioactive and solid lipid are melted in an organic solvent regarded as oil phase. Simultaneously water phase is also heated to the same temperature as oil phase 2. The oil phase is added to a small volume of water phase, and the resulting emulsion is stirred at high speed for few hours 3. The emulsion is cooled down to room temperature to yield nanoparticles
Ultrasonication technique	1. The core material is melted 2. Addition of phospholipids along with an aqueous medium 3. Dispersing the melted material at increased temperature by ultrasonication
Solvent injection	1. Lipids are dissolved in a water-miscible solvent (e.g., acetone, isopropanol, and methanol) or water-miscible solvent mixture 2. Quickly injected into an aqueous solution of surfactants through an injection needle
Double emulsion technique	1. Bioactive (mainly hydrophilic ones) is dissolved in aqueous solution emulsified in melted lipid 2. The primary emulsion is stabilized by adding stabilizer that is dispersed in aqueous phase containing hydrophilic emulsifier 3. Emulsion is stirred and filtered

3 Nanoencapsulation of Food Bioactive Compounds by Lipid-Based Nanocarriers

3.1 Phenolic Compounds

Phenolic compounds are regarded as a main group of bioactive compounds with many unique features (such as antioxidant, antimicrobial, antiviral, anti-inflammatory, anticancer, antithrombotic, and anti-allergic effects) that make them a qualified choice for food and pharmaceutical sectors [13]. Enrichment/fortification of foods by phenolic compounds not only improves their chemical and microbial stability but also can be effective in prevention and cure of some disease such as cardiovascular and neurodegenerative diseases [145, 148].

In spite of several beneficial properties, employment of phenolic compounds in food matrices has some limitations. Generally, phenolic compounds are readily decomposed in food as well as GIT systems. Nonpolar phenolic compounds are poorly dissolved in aqueous foods due to their lipophilic character. Highly polar ones do not pass through the biological membranes because of low hydrophobicity. Moreover, phenolic compounds with multiple phenolic rings are insufficiently absorbed as a result of forming massive molecular structures [64, 117]. Other problems are related to their bitter and stringent taste, creation of hazy appearance in beverages, and unfavorable effects on sensory attributes. Moreover, phenolic compounds participate in browning reactions as well as oxidation, leading to

produce brown color and undesirable odors along with nutritional losses in fortified foods [145, 148]. Interaction between phenolic compounds and food components not only leads to aggregated and precipitated proteins but also can adversely affect their bioactivity [173].

In contrast to in vitro experiments, phenolic compounds exhibit low bioactivity within body system because they have conditioned solubility, low gastric retention time, and weak penetrability and stability. Thus, higher amounts of these compounds will be required for exerting their biological activities which result in further effects on food products [51].

Lipid-based nanocarriers potentially help to solve these issues. They provide the stability and controlled release for phenolic compounds in food matrices as well as GIT system, consequently causing a longer shelf life and enhancing bioaccessibility. The nanodelivery systems facilitate passage of polar phenolics from aqueous parts toward lipophilic mediums and then bloodstream which enhances their in vivo efficiency and bioavailability [101]. They promote antioxidant and antimicrobial activity of phenolic compounds. Solubility of lipophilic phenolic compounds can be also improved in beverages and aqueous foods without any significant changes in sensorial properties of final products by lipid-based nanocarriers [53].

Many studies have been carried out on incorporation of phenolic compounds into lipid-based nanocarriers as displayed in Table 4. For instance, Ni et al. [147] encapsulated quercetin in nanoemulsions via high-pressure homogenization technology. Quercetin-loaded nanoemulsions displayed good phytochemical properties by considering an average particle size, zeta potential, and encapsulation efficiency. The prepared nanoemulsions exhibited similar antioxidant activity to free quercetin. Furthermore, they found that nanoencapsulation improved release behavior and bioavailability of quercetin in simulated GIT conditions. Also, they added the loaded quercetin into a beverage formulation and observed that it caused no negative effects on texture and appearance of the beverage during storage [147].

Epigallocatechin gallate (EGCG) and catechin loaded in soy lecithin nanoliposomes was prepared by Rashidinejad et al. [174] and then added to ripened low-fat cheese. The results showed that addition of the loaded EGCG and catechin increased antioxidant activity and phenol content of cheese after simulated GIT digestion without any notable effects on pH, chemical composition, and cheese production yield. Moreover, EGCG and catechin completely remained in cheese structure and were not detected in whey [174].

Gaber et al. [58] fabricated SLNs containing myricetin and evaluated effect of some additives, pH variation, and heat treatments on their stability. Based on their results, lipid-soluble antioxidants (vitamin E and BHT) could improve thermal stability of incorporated compounds. Beside antioxidants, presence of Poloxamer 407 and Tween 80 as stabilizer was essential to form stable systems. Loaded myricetin exhibited a lower decomposition rate and longer half-time in buffer solutions compared to its free counterpart. Nanoencapsulation also influenced release pattern of loaded phenolic compounds.

Babazadeh et al. [15] prepared NLCs as carriers of rutin using food-grade components (oleic acid, cacao butter, and Tween) and added nanoencapsulated

Table 4 Some selected studies on incorporation of phenolic compounds into lipid-based nanocarriers

Nanocarrier	Phenolic compound	Results	Reference
Nanoliposomes	Luteolin	Enhancement of bioavailability upon nanoencapsulation	[213]
	Curcumin	- High stability against alkaline pH and metal ions - Good storage stability - Sustained release - Maintenance of cellular antioxidant activity after nanoencapsulation	[31]
	Quercetin and resveratrol	- Higher antioxidant activity than free flavonoids - Suitable stability	[25]
	Curcumin	Nanoliposomes obtained from ethanol injection method were superior to those produced by dry thin-film method regarding encapsulation efficiency and storage stability	[191]
	Quercetin	Better physicochemical and sensory properties along with higher stability of whey drink fortified with whey protein isolate-coated liposomes in comparison with uncoated ones	[57]
	Resveratrol	- Maintenance of antioxidant capability after nanoencapsulation - Effect of preparation method on physiochemical properties of nanoliposomes	[77]
	Resveratrol	Improved stability against UV-B light	[24]
	Catechin and epigallocatechin gallate	- Retention of phenolic compounds in cheese matrix without any significant change in its physiochemical properties - Improved antioxidant activity - Protection against digestion	[174]
	Curcumin	Enhanced bioavailability and plasma antioxidant activity	[198]
	Epigallocatechin gallate	- Increase of stability in simulated intestinal conditions - Retention of antioxidant capacity during in vitro digestion	[220]
	Ellagic acid	Sustained release	[118]
Phytosome	Curcumin	Enhancement of bioavailability and health-promoting properties	([38]; [122])
	Hesperetin	Promoted bioavailability and antioxidant activity	[142]
	Curcumin	Improvement of oral absorption and stability by combining chitosan and phytosome	[217]

(*continued*)

Table 4 (continued)

Nanocarrier	Phenolic compound	Results	Reference
	Rutin	- Maintenance of antioxidant activity upon nanoencapsulation - Good storage stability - Desirable sensory attributes of beverages containing phytosomal formulation	[16]
Nanoemulsion	Curcumin	- Significant effect of emulsifier charge on phytochemical properties, release pattern, and performance of nanoemulsions during digestion	[161]
	Curcumin	- Good stability against ionic strengths, pH, and pasteurization - Gradual release in simulated gastrointestinal condition	[185]
	Quercetin	- Enhanced bioaccessibility - Maintenance of antioxidant activity upon nanoencapsulation - High physical and chemical stability - Good sensorial properties of beverage formulation enriched with nanoencapsulated quercetin	[147]
	Quercetin	Enhanced stability	[92]
	Quercetin	Good physical stability and sustained release	[21]
	Resveratrol	Enhancement of chemical stability against UV light	[42]
	Resveratrol	- Sustained release - Improved stability and bioavailability	[189]
Solid lipid nanoparticles	Resveratrol	Improved oral bioavailability	[153]
	Curcumin	Increase of chemical stability, bioavailability, dispersibility, cellular uptake, and functionality	[197]
	Myricetin	Using stabilizers (tween 80 and Poloxamer 407): prolonged half-life time, decrease of degradation rate of flavonoid and sustained release in buffer solutions	[58]
	Epigallocatechin gallate	Increase of stability and anticancer activity	[170]
	Resveratrol	NLCs had smaller particle size and higher encapsulation efficiency than SLNs	[67]
	Quercetin	NLCs and nanoemulsions had higher bioaccessibility as compared to SLNs and free phenolics	[2]
	Curcumin	Reinforced the permeation under in situ intestinal conditions	[90]

(*continued*)

Table 4 (continued)

Nanocarrier	Phenolic compound	Results	Reference
	Curcumin	- Improved oral bioavailability - Increase of permeation after nanoencapsulation	[178]
	Quercetin	Increase of oral absorption	[107]
Nanostructured lipids carriers	Quercetin	Sustained release and improved bioaccessibility	[112]
	Ferulic acid	- Controlled release profile - Increased the pharmacological activities	[73]
	Hesperetin	- Enhanced stability upon coating with different biopolymers - Better release properties - Improvement of solubility and positive effect on sensory attributes of fortified milk regarding color and taste	[55]
	Quercetin	- Promoted solubility - Sustained release - Good storage and oxidative stability	[75]
	Rutin	- Good stability during processing and storage period - Acceptable sensory attributes of fortified beverages	[15]
	Curcumin and genistein	Increment of solubility	[3]
	Curcumin	- Protective role against gastrointestinal digestion - Suitable release behavior in simulated intestinal fluid	([156]; [213])
	Curcumin	Enhancement in intestinal absorption, solubility, and stability	[227]
	Luteolin	Promotion of oral absorption and bioavailability	[112]

rutin into beverages (orange juice, milk, apple juice). The NLCs had good physiochemical and morphological properties. Fortified beverages not only were physically and thermally stable but also presented an acceptable sensorial quality [15]. These researchers in another study applied thin-film hydration method to incorporate rutin into phytosomal systems produced by soybean phosphatidylcholine [16]. Optimized formulations remained highly stable during storage and generated fine particles. Phytosomes preserved antioxidant activity of rutin, while inverse trend was observed for free one. No evident changes were found in sensory attributes and pH values of fortified beverages with loaded phenolic compounds. Furthermore, some studies have demonstrated high potential of phytosomes for improving absorption, bioavailability, and health-promoting properties of curcumin [38, 122, 217].

3.2 Natural Food Colorants

Colorants are extensively applied in the food industry to increase the product attractiveness and consumer satisfaction. Food colorants can be classified into three main groups: natural, synthetic, and inorganic. Consumption of synthetic colorants negatively affects human health and can cause cancer and allergic reactions. Hence, there has been recently growing tendency to natural types [5, 119]. Among natural food colorants, carotenoids and flavonoids are the most common types for food applications. In addition to exhibiting antioxidant activity, some flavonoids like curcumin, quercetin, luteolin, and anthocyanins can be used as coloring agents [79, 124]. Natural colorants are not only a safe alternative to synthetic ones but also can provide numerous health benefits. They seem to be effective in prevention and reducing the risk of many diseases such as cardiovascular diseases, age-associated diseases, cancer, neurodegenerative disorders, and eye diseases [91, 111, 196]. Nonetheless, most of natural colorants display high sensitivity to oxidation and are degraded when exposed to external factors such as light, pH, and heat. On the other hand, food components, enzymes, and other nutrients affect their stability. They also exhibit low bioavailability and limited solubility. Thus, it is necessary to retain structural integrity of natural food colorants in order to exploit them in food products and formulate novel functional foods [111, 120, 176]. In this regard, lipid-based nanocarriers are suitable options, because they have an excellent ability to enhance solubility, absorption, sustained release properties, effectiveness, and stability of natural colorants [70, 200].

Several reports considering the incorporation of different kinds of natural food colorants into lipid-based carriers have been recently published, and some of them have been reviewed in the study published by Akhavan and Jafari [5]. As an example, Tan et al. [202] investigated efficiency of nanoliposomes achieved by thin-film evaporation technique for incorporating diverse carotenoids including β-carotene, canthaxanthin, lycopene, and lutein. They proved that physiochemical properties, release pattern, and bioaccessibility were directly associated with carotenoid type. Indeed, their results indicated a gradual and prolonged release of β-carotene and lutein under GIT tract conditions. By contrast, release of canthaxanthin and lycopene occurred rapidly. Also, bioaccessibility followed the order of lutein> b-carotene> lycopene> canthaxanthin [202]. This group in another study used chitosan to cover liposomal surfaces through layer self-assembly deposition method [201]. Coated nanovesicles could retain lutein and β-carotene better than canthaxanthin and lycopene. Besides, biopolymer coating modified arranged structure of lipid bilayers and increased their rigidity. At the same time, chitosan positively affected release behavior of carotenoids within simulated GIT fluids. Round shape of liposomes was also kept after coating. These authors suggested that coating can be considered as an efficacious way to generate stable nanoliposomal systems.

In a recent work, Rabelo et al. [169] accommodated Açaí berry extract as a rich source of anthocyanins into W/O nanoemulsions via high-pressure homogenizer to boost their stability and functionality. MCT and CR-310 (tetraglycerin monolaurate condensed ricinoleic acid esters) were main components of nanoemulsions and acted

as the oil phase and emulsifying agent, respectively. Formed nanoemulsions demonstrated a good storage stability and no signs of phase separation observed over time. Nanoencapsulation caused a decrease in droplet size. Besides, all formulations were able to preserve phenolic compounds plus antioxidant potency of extracts when stored for up to 1 month at 4 °C [169]. In another research, Li et al. [108] applied high-pressure homogenization approach to formulate astaxanthin-loaded SLNs with three kinds of solid lipids including glycerin monostearate, glycerol distearates, and stearic acid plus Tween 20 as an efficient emulsifier. SLNs were highly stable to degradation at both 4 and 25 °C, and no significant increase was observed in particle size during storage. Indeed, loaded astaxanthin was released slowly over time into simulated intestinal and gastric fluids [108].

Mehrad et al. [130] investigated protective impact of SLNs consisted of corn oil, palmitic acid, and whey protein isolate (as a stabilizer) on β-carotene. They proved a remarkable enhancement in chemical and oxidative stability of this pigment upon nanoencapsulation. However, SLNs were susceptible to increment of ionic strength, elevated temperatures and acidic conditions [130]. Nazemiyeh et al. [222] found that SLNs enabled a prolonged stability for lycopene and there was no evident decrease in encapsulation efficiency after 3 months storage at 4 °C. In another work, Oliveira et al. [150] fabricated NLCs using a mixture of tristearin and high oleic sunflower oil, and their efficiency in incorporation of β-carotene was compared with tristearin SLNs. All samples showed nanometric sizes and a broad particle size distribution (PDI). High oleic sunflower oil created no significant changes in particle size. However, PDI of samples decreased after enclosing β-carotene. NLCs were superior to SLNs in terms of loading efficiency, although both NLCs and SLNs could equally protect β-carotene from deterioration. Better performance of nanodelivery systems was achieved by increasing high oleic sunflower oil levels. Overall, NLCs were more advantageous than SLNs [150].

3.3 Bioactive Oils and Essential Fatty Acids

Main types of omega-3 fatty acids include eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and α-linolenic acid (ALA). Fish oil is regarded as the best source of EPA and DHA [165]. In addition to fish oil, omega-3 fatty acids are found in algae, krill, and land plants [29, 103]. Omega-3 fatty acids possess several health benefits and could effectively prevent and reduce the risk of different diseases such as cardiovascular diseases, inflammation, diabetes, autoimmune disorders, and cancer [27, 61, 146]. Since, the human body does not have the ability to synthesize omega-3 fatty acids; therefore, they are named as essential fatty acids and must be obtained through foods. As most of diets contain insufficient amounts of omega-3 fatty acids, a great interest has been focused on increase of their intake through fortification of different foods. Nonetheless, oxidative, chemical, and thermal instability, poor bioavailability and water solubility, and unfavorable odor and flavor make direct usage of omega-3 fatty acids in food matrices difficult. Lipid-

based nanocarriers allow to take the advantages of omega-3 fatty acids and also offer a wide range of potential applications for these bioactives in food sector [65, 96].

Many nanoemulsion researches have been centered on optimizing preparation methods, composition (regarding oil type, surfactant, and co-surfactant), and different parameters influencing phytochemical characteristics in order to promote stability and bioavailability of fish oil and omega-3 fatty acids. For instance, Gulotta et al. [69] produced fish oil-loaded nanoemulsions by self-emulsification technique and revealed that various parameters including fish oil concentration, oil composition (lemon oil and medium-chain triglycerides), surfactant-to-oil proportion, and cosolvent type (polypropylene glycol and ethanol and glycerol) have a great impact on their characteristics and stability. Moreover, optimized formulations provided stable nanoemulsion systems, and there were no significant changes in the droplet size after storage for 1 month at refrigerated and room temperatures. Nevertheless, particle size increased at higher storage temperatures [69].

Walker et al. [210] indicated that particle size of droplets significantly influences the oxidative stability of fish oil-loaded nanoemulsions, while physical stability of system was dependent on the surfactant-to-oil ratio. On the other hand, microfluidized nanoemulsions contained larger amounts of secondary oxidation products (TBARS) after storage for 2 weeks at 55 °C as compared to those formed with spontaneous emulsification [210]. According to the study of Lane et al. [105], it is better to apply algal oil and mixture of soy lecithin and Tween 40 instead of flaxseed oil and soy lecithin alone for generation of nanoemulsions with fine droplet sizes [105]. Nejadmansouri et al. [146] demonstrated that using whey protein isolate in nanoemulsion formulations leads to a suitable storage stability regarding particle size, but viscosity is slightly increased during storage for 28 days [146]. Polymer coating has a positive effect on the stability of nanoemulsions bearing fish oil. Esquerdo et al. [223] revealed that using chitosan as a coating agent prevents creaming and breaks down of fish oil-loaded nanoemulsions.

Different approaches have been employed for incorporating essential fatty acids and fish oils into nanoliposomes. Hadian et al. [72] produced nanoliposomes entrapping EPA and DHA by sonication (bath and probe types) and extrusion methods. Nanoliposomes produced by sonication contained higher amounts of secondary oxidation products (heptanal propanal, hexanal, and pentanal) than extruded ones. The highest loading capacity was also related to probe sonicated nanoliposomes [72].

Moreover, some food systems have been fortified with incorporated form of these bioactives. In a recent work, Ojagh and Hasani [149] investigated the effect of adding different concentrations of fish oil incorporated in nanoliposomes on the sensory and technological attributes of fortified bread during storage in the refrigerator for 25 days. They confirmed desirable physiochemical properties of nanovesicles regarding particle size, PDI, and encapsulation efficiency. Indeed, addition of the fish oil-loaded nanoliposomes into breads increased nutritional value and loaf volume of the breads without notable adverse effects on their sensory and texture characteristics [149]. Similarly, bread and milk samples enriched with fish oil-loaded nanoliposomes obtained acceptable sensory scores in contrast to free and

microencapsulated ones [175]. In another study, Ghorbanzade et al. [65] used nanoliposome systems to encapsulate fish oil and fortified yogurt with the loaded fish oil. They stated that the fortifying yogurt with the loaded fish oil led to higher EPA and DHA contents as well as a lower peroxide value, acidity, and syneresis than samples treated with its free form during 21 days of storage at 4 °C. Also, they found that the sensory characteristics of the yogurt fortified with nanoencapsulated fish oil was significantly similar to control sample, containing no fish oil [65].

There are few works on fish oil and essential fatty acids loaded within SLNs. Salminen et al. [181] incorporated fish oil into SLNs composed of tristearin (as the carrier) and quillaja extract alone or blended with lecithin (low or high melting point) as a surfactant. They discovered that all formulations showed statistically constant PDI and particle sizes during storage at a dark place for 51 days except for samples containing quillaja/low-melting lecithin. Moreover, the SLNs containing quillaja/high-melting lecithin had a more chemical stability than those containing quillaja alone or quillaja/low-melting lecithin [181].

NLCs have been also successfully used for loading krill oil. Zhu et al. [218] incorporated krill oil into NLCs by hot homogenization technique and used palm stearin and lecithin as oil and emulsifier, respectively. The smallest particle size and the highest homogeneity were obtained from optimized formulation (65% krill oil and 1.1% lecithin). Prepared NLCs showed protective effect against UV light-induced photooxidation. They also had a suitable physical and long-term stability at low temperatures [218]. Salminen et al. [180] utilized NLCs and nanoemulsion systems with similar formulations (except tristearin applied in production of NLCs) for nanoencapsulation of fish oil. Based on their results, NLCs yielded more stable (in the terms of size and aggregation stability) and smaller particles than nanoemulsions. Furthermore, the NLCs showed higher protective effects on fish oil against lipid oxidation compared to the nanoemulsions [180].

3.4 Vitamins

Vitamins are bioactive compounds with outstanding health-promoting properties which effectively contribute to human growth and development. Vitamin deficiencies play a key role in development of some diseases such as cancer and cardiovascular problems [99]. As human body is not able to produce many vitamins, they must be obtained via diet. However, it is not always possible to meet recommended daily intake of vitamins. Thus, fortification of food products with vitamins may be needed to ensure adequate intake of vitamins [20, 157]. Some issues should be considered for successful fortification. Many vitamins are readily degraded under storage and processing conditions. They also exhibit a low stability in GIT system. Depending on type, vitamins show different extents of sensitivity to oxidation, heat treatment, and pH variations. Poor bioavailability is also regarded as an important limitation in direct addition of vitamins into food formulations. Moreover, unit operations of food processing such as blanching cause a considerable decline in the content of water-soluble vitamins [151, 155]. On the other hand, hydrophobic vitamins are not a

suitable choice for fortification of aqueous-based food products due to their low water solubility and insufficient dispersibility [135].

Lipid-based nanocarriers can be applied as a proper alternative in order to protect vitamins against multiple processes and environmental stresses, improve solubility and absorption, and release them in the determined sites [47, 98]. Many researchers have encapsulated different kinds of vitamins into lipid-based nanocarriers as shown in Table 5. For example, Liu et al. [113] evaluated deposition of chitosan and sodium alginate on the surface of nanoliposomes loaded with vitamin C as core material. These nanocarriers showed a slower release and higher rate of oxidation of vitamin C when stored for 90 days at 4 °C than uncoated ones. They concluded that the shell of chitosan and sodium alginate could effectively cover the surface of anionic nanovesicles and enhance their stability toward hydrolysis and oxidation. They also fortified mandarin juice with coated nanoliposomes and observed that microbial stability of the fortified mandarin juice was higher than samples treated with uncoated carriers with a negligible effect on sensory properties of juice [113].

Bochicchio et al. [20] evaluated incorporation of different vitamins (B_{12}, D_2, and E) into nanoliposomes through an ultrasound-assisted method and the thin-film hydration technique. They reported that SUVs and MLVs had a high encapsulation efficiency. Increasing the hydrophobicity of vitamins led to higher encapsulation efficiencies. Moreover, the entrapment of vitamins into lipid nanovesicles protected them against chemical degradation during 10 days of storage at simulated extracellular environment conditions [20]. In another study, Couto et al. [35] loaded vitamin B_2 into the SLN systems through a modified PGSS process using hydrogenated canola oil as lipid phase, polyethylene glycol as stabilizer, and sodium lauryl sulfate as surfactant. They investigated effects of different parameters such as vitamin and stabilizer concentration, pressure, and molecular weight on loading and encapsulation efficiencies and reported that the optimum SLN preparation conditions with respect to loading and encapsulation efficiency were as follow: vitamin concentration of 2%, pressure of 15 MPa, and 5% polyethylene glycol [35].

Walia et al. [209] prepared a fish oil-in-water nanoemulsion via ultrasonication technique to load vitamin D with a high encapsulation efficiency. Their results revealed that nanoencapsulation could preserve antioxidant capacity and antimicrobial activity of vitamin D during storage. Also, the fish oil-based nanoemulsions improved the vitamin D bioavailability in stimulated GIT conditions [209]. Pinto et al. [163] developed different NLCs by using vegetable oils enriched with α-tocopherol and demonstrated that olive, sunflower, coconut, and sweet almond oils could be successfully applied to prepare free and loaded NLCs with negative surface charges and a nanometric size. Also, they found that the formulated NLCs had a high encapsulation efficiency and released α-tocopherol in a controlled manner. However, the DSC analysis has showed that incorporation of α-tocopherol resulted in reduced crystallinity of NLCs but could improve their antioxidant activity. Accordingly, these authors suggested that the formulated NLCs could be successfully used for preparing long-term stable products [163].

Table 5 Incorporation of different vitamins into lipid-based nanocarriers

Nanocarrier	Vitamin type	Results	Reference
Nanoliposomes	Vitamins E and C	- Maintenance of antioxidant activity of vitamins in orange juice before and after pasteurization - Liposomes provided good microbial stability for orange juice during storage without any significant change in sensory properties	[123]
	Vitamin A	- Suitable characteristics - High concentration of cholesterol led to lower encapsulation efficiency	[160]
	Vitamin C	- Higher chemical and microbial stability of mandarin juice and lower release rate after coating of nanoliposomes with chitosan and sodium alginate - Good protection against oxidation without any significant effect of sensory properties of mandarin juice	[113]
	Vitamin C	- Better storage stability and more encapsulation efficiency in nanoliposomes obtained from the double emulsion-dynamic high-pressure microfluidization method	[109]
	Vitamin B_1	High thermal stability during storage	[56]
	Vitamins E, B_{12}, and D_2	Multilamellar large vesicles resulted in the maximum encapsulation efficiency	[20]
	Vitamin C	Enhanced stability during long-term storage	[215]
	Vitamin A	Decreased degradation of vitamin	[102]
Nanoemulsions	Vitamin E	High bioaccessibility in O/W emulsions produced by long-chain triglycerides	[152]
	Vitamin B_2	Improved stability	[22]
	Vitamin E	- Effective protection during storage - Good physical stability	[74]
	Vitamin E	- High thermal stability - The smallest droplets and highest transparency were observed for formulations containing 30% propylene glycol or 20% ethanol	[179]
	Vitamin E	Increase in release rate of vitamin into the buffer solution caused by micellar solubilization	[140]
	Vitamin D	- Increase in thermal stability by using sodium dodecyl sulfate as co-surfactant - Effect of surfactant type, surfactant-	[71]

(*continued*)

Table 5 (continued)

Nanocarrier	Vitamin type	Results	Reference
		to-oil proportion, and stirring conditions on characteristics of droplets	
	Vitamin K_1	- Good permeation ability - Good storage stability over time under (at) different temperatures	[26]
	Folic acid (Vitamin B_9)	The optimum conditions for preparing maltodextrin-whey protein double emulsions: 3 mg/ml folic acid in the 12% dispersed phase and water to span 80 ratio of 0.9	(Assadpour et al. 2016)
	Vitamin E	- Enhanced antimicrobial effect, shelf life, and bioavailability of vitamin in fruit juice - High encapsulation efficiency by using mustard oil and tween 80	[41]
	Vitamin E	The optimum condition for incorporating vitamin: 1% vitamin E acetate concentration, 6.18% oil contents, 135 MPa homogenization pressure, and 6.39% surfactant concentration	[129]
	Vitamin D	Enhanced bioavailability acquired by fish oil-based nanoemulsion	[209]
	A type of vitamin B: Thiamine dilauryl sulfate (TDS)	Inhibitory effect on the spore germination of *Fusarium oxypansum* f. sp. as well as mycelial growth	[32]
	Vitamin D_3	Good stability against UV irradiation	[106]
	Vitamin E	Production of nanoemulsions bearing vitamin by natural surfactant (Q-Naturale®) for using in food formulations	[216]
Solid lipid nanoparticles (SLNs)	Vitamin B_2	Encapsulation efficiency and loading capacity influenced by different parameters such as molecular weight and concentration of stabilizer, vitamin concentration, and pressure	[35]
	Vitamin B_{12}	Improved anticarcinogenic activity	[62]
	Vitamin D_2	Protective effect against oxygen and light	[158]
	Vitamin A	- Controlled release during 6 h - Higher release rate compared to nanoemulsions in longer storage times	[89]

(*continued*)

Table 5 (continued)

Nanocarrier	Vitamin type	Results	Reference
Nanostructured lipids carriers (NLCs)	Vitamin A	Good storage stability	[159]
	Vitamin E	The highest stability achieved by adding 5% (w/w) of tween 20 to NLCs formulation	[208]
	Vitamin D_3	- Good physiochemical properties and suitable storage stability - Controlled release	[155]
	Vitamin D_3	Improvement of intestinal absorption Production of stable NLCs by using Precirol as solid lipid and Poloxamer 407 as surfactants	[135]
	Vitamin E	- NLC formulations-based vegetable oils (sunflower, sweet almond, olive, and coconut oils) led to good phytochemical properties and high physical stability - Improved scavenging activity	[163]

3.5 Natural Antimicrobial Agents and Essential Oils

Recently, the tendency for utilizing natural antimicrobial agents has been increased due to hazardous and harmful impacts of synthetic additives on human health and emergence of antibiotic-resistant bacteria [46, 126]. Among natural antimicrobial agents, essential oils and bacteriocins especially nisin have attracted much consideration to inhibit microbial growth in food systems. In addition to antimicrobial effect, essential oils possess numerous beneficial and health-promoting properties such as antioxidant, anti-allergic, anticancer, anti-inflammatory, and antiviral activities. Besides, essential oils and their components act as natural flavoring agents in food, pharmaceutical, and cosmetics industries [45]. In spite of remarkable advantages, there are serious challenges in development of food products containing essential oils. They are extremely reactive, volatile, and chemically unstable; readily oxidized when exposed to heat, oxygen, or light; and decomposed during processing stages and storage as well as within GIT conditions that reduces their antimicrobial potency. Furthermore, essential oils display poor solubility and dispersibility in aqueous food systems and influence sensory attributes of food products [10, 43, 204].

Nisin is a polypeptide composed of 34 amino acids obtained from certain strains of *Lactococcus lactis* and found in two main forms, namely, nisin A and nisin Z. Nisin Z shows a higher stability and dispersibility in food formulations than A type [34]. Nisin as the only GRAS (generally recognized as safe) bacteriocin can inhibit growth of several species of pathogenic and nonpathogenic Gram-positive bacteria such as *Listeria monocytogenes*, *Bacillus* spp., and *Staphylococcus aureus*, protect food products from microbial spoilage, prolong their shelf life, and improve food safety [88, 166]. Nevertheless, there are some problems that restrict direct usage of

nisin and cause remarkable losses in its antimicrobial activity such as low stability against adverse environmental factors as well as proteolytic inactivation, unwanted interaction with food ingredients, and inadequate dispersion in food systems [34, 166]. Moreover, utilizing free form of nisin may create some issues within foods. For example, nisin may negatively influence starter culture activity in cheese matrix and consequently decrease consumer acceptance [23].

Lipid-based nanodelivery systems can solve these problems because they provide targeted and prolonged release, inhibit undesirable interactions and degradation, and minimize appearance of resistant strains of bacteria. These carriers also facilitate passage of antimicrobials through cell membrane, enhance bioactivity of essential oils, and thus reduce concentrations required for exerting antimicrobial activity which leads to preserving sensorial properties and quality of fortified foods [34, 100, 116].

Several studies have demonstrated the beneficial effects of lipid-based nanocarriers on antimicrobial activity, stability, and flavor retention of essential oils and their constituents for food applications. For instance, Balta et al. [18] evaluated antimicrobial potential of nanoemulsions containing geraniol and linalool against some foodborne bacteria *(E. coli*, *Listeria innocua*, and *Pseudomonas lundensis*) by a simulated medium of meat. Both loaded compounds could control the microbial growth. However, they showed a lower efficiency for declining *Pseudomonas lundensis* counts compared to others [18]. In another research by Lu et al. [116], citral essential oil-loaded nanoemulsions exhibited an inhibitory effect on bacterial strains (*S. aureus, E. coli, Pseudomonas aeruginosa, Enterococcus faecalis, Salmonella typhimurium*, and *L. monocytogenes*). Nevertheless, they had a similar inhibition zone diameter and antimicrobial efficiency against both Gram-positive and Gram-negative species [116].

In the study conducted by Artiga-Artigas et al. [10], four essential oils obtained from lemongrass, mandarin, thyme, and oregano oil were incorporated into nanoemulsion systems via microfluidization with the aid of Tween 80 and pectin as emulsifying agents [10]. Chuesiang et al. [33] produced nanoemulsions loaded with cinnamon essential oils by phase inversion temperature technique using MCT and Tween 80. They revealed that droplet size and stability of nanoemulsions were affected by concentration of essential oils, oil phase, and surfactant [33]. In another work, Mendes et al. [133] fabricated nanoemulsion systems for loading *Eugenia brejoensis Mazine* essential oil as an antimicrobial agent by homogenization. Based on their results, nanoemulsions had good characteristics, and physical stability resulted from appropriate emulsifiers and increasing rotational speed. Besides, bactericidal activity was dependent on droplet size, and nanoemulsions with smaller particle sizes were more efficient against *Pseudomonas fluorescens*. When loaded essential oils were also applied in sliced ham, their antimicrobial capability was reduced [133].

The dual incorporation of nisin and garlic extract into nanoliposomes was carried out by Pinilla and Brandelli [162], and antimicrobial effectiveness of loaded nanoliposomes was investigated against some bacterial species (*Salmonella enteritidis*, *L. monocytogenes*, *and S. aureus* and *E. coli*) in whole milk when stored at 37 °C.

Nanoliposomes were equally effective on bacteria, while free form of nisin and garlic extract could not individually inhibit microbial growth. Natural antimicrobial agents also presented a higher ability to reduce *L. monocytogenes* counts [162]. Cui et al. [36] employed soy lecithin-derived nanoliposomes for boosting the stability and antimicrobial activity of clove oil against *E. coli* and *S. aureus*. Loaded essential oils could prevent growth of bacteria. However, as *S. aureus* increased the release rate of clove oil by secreting pore-forming toxins, nanoliposomes showed a higher antimicrobial effect. Moreover, adding loaded nanoliposomes into tofu formulation led to an efficacious inhibitory activity toward *S. aureus* [36].

Sebaaly et al. [187] found that nanoliposomes prepared by ethanol injection method promoted stability of eugenol against UV radiation. Additionally, antioxidant activity of eugenol was preserved after nanoencapsulation [187]. Tian et al. [204] fabricated SLNs bearing citral through high-pressure homogenization approach by a blend of Span 80 and Tween 80 (1:1 ratio) and glycerol monostearate as surfactant and solid lipid, respectively. According to their results, larger amounts of incorporated citral were retained during storage at 37 °C for 12 days in comparison with its free counterpart [204]. Zhao et al. [224] revealed that SLNs were able to promote bioavailability and sustained release properties of Yuxingcao essential oil.

Prombutara et al. [166] nanoencapsulated nisin within SLNs via high-pressure homogenization technique by Imwitor 900 as solid lipid with aid of surfactant and co-surfactant that were Poloxamer 188 and sodium deoxycholate, respectively. Nisin concentration significantly influenced physiochemical properties of SLNs. Release rate of nisin was dependent on medium conditions in terms of pH and salt concentration. Nanoencapsulation of nisin led to a prolonged antimicrobial activity toward *Lactobacillus plantarum* and *L. monocytogenes* [166]. Keivani Nahr et al. [100] entrapped cardamom essential oil into NLCs produced by food-grade ingredients including cocoa butter and Tween 80 and assessed impact of nanoencapsulation on its antimicrobial capacity. Obtained NLCs displayed desirable physiochemical characteristics and a good stability over 30 days of storage. Loaded essential oil was also superior to free form regarding antimicrobial potentials [100]. Lewies et al. [225] proved the efficient bactericidal activity of nisin-loaded NLCs against *S. aureus* and *S. epidermidis*.

3.6 Phytosterols

Phytosterols are one of the most popular bioactive compounds with a high hydrophobicity and can be divided into two main groups including stanols and plant sterols (i.e., β-sitosterol, campesterol, and stigmasterol). Phytosterols possess numerous health benefits such as anticancer, inflammatory, antioxidant, and antidiabetic activities. They can also prevent cardiovascular diseases and arteriosclerosis by decreasing low-density lipoprotein (LDL) cholesterol in serum [17, 39, 195].

Phytosterols especially β-sitosterol have recently attracted great attention in order to develop novel functional foods [154], although low bioavailability, high melting point, and poor aqueous solubility make it difficult to incorporate β-sitosterol

into food systems without a suitable carrier. Besides, they show a high oxidation and crystallization tendency, reducing their functionality. Phytosterols are often applied in esterified forms which can enhance their solubility in fat-rich foods such as spreads without any adverse influence on other food matrices [79, 195]. Nanoencapsulation presents an efficient solution for these obstacles. There are few studies on incorporation of phytosterols into lipid-based nanocarriers and their applications in the food industry. Bagherpour et al. [17] fortified butter with NLCs bearing β-sitosterol prepared by hot homogenization approach, and physicochemical characteristics plus oxidative stability of samples were assessed. Obtained NLCs had a nanometric size, negative surface charge, desirable homogeneity, and high encapsulation efficiency. Also, they were physically stable during long-term storage. No evident change was observed at peroxide and acid values of butter samples containing NLCs and they showed a good oxidative stability. Antioxidant activity of loaded β-sitosterol was maintained in butter matrix over 3 months of storage at refrigerated temperature, while its free form experienced a significant loss [17].

Alexander et al. [8] added plant sterols instead of cholesterol into liposomal formulations. Characteristics of nanoliposomes are influenced by phospholipid and sterol concentrations. Their results revealed that plant sterols significantly increased the particle size and encapsulation efficiency, although nanoliposomes exhibited a low physical stability in presence of plant sterols and undergo phase separation during storage. These authors suggested that optimization of plant sterol to phospholipid ratios and selection of suitable preparation methods can improve storage stability of nanoliposomes containing plant sterols [8]. Panpipat et al. [154] employed different sterol to phospholipid ratios plus various types of β-sitosteryl fatty acid esters with the aim of producing nanodispersions. On the basis of their results, β-sitosterol was less effective in producing homogeneity of nanodispersions compared to its esterified forms. Moreover, nanoparticles obtained from esters of β-sitosterol were smaller and more stable. On the other hand, β-sitosteryl unsaturated fatty acid esters preferred to make nanoemulsions with desirable characteristics instead of nanodispersions which can be satisfactory for pharmaceutical and food sectors [154].

Ribeiro et al. [177] formulated and characterized novel lipid-based colloidal nanodispersions for enclosing phytosterols using various types of triacylglycerols and Tween 20. They found that supercooled emulsions and amorphous particles were created as a result of applying Myritol and trilaurin as liquid and solid triacylglycerols, respectively. These systems could entirely prevent the crystallization of phytosterols as well as lipids. Furthermore, they presented good stability during long-term storage [177]. In a recent study by Soleimanian et al. [195], β-sitosterol-loaded NLCs were fabricated by three different types of lipids including glyceryl behenate, pomegranate seed oil, and propolis wax. They demonstrated a notable impact of type and content of lipids on physiochemical properties of NLCs. Increase in phytosterol content and decrease of lipid content led to larger size and lower values for encapsulation efficiency. Crystallinity of β-sitosterol also diminished upon nanoencapsulation [195].

In the study of Lacatusu et al. [104], a mixture of natural oils including fish oil, grape seed oil, and squalene was used to make NLCs comprising β-sitosterol alone and simultaneously β-sitosterol and green tea extract. Obtained NLCs had a great encapsulation efficiency and surface charge less than −30 mV, indicating stable systems. Dual nanoencapsulation of β-sitosterol and green tea extract not only caused an evident decrease in particle size but also promoted antioxidant efficiency. Loaded β-sitosterol was able to entrap free radicals better than unencapsulated sample. Additionally, NLCs enabled gradual and controlled release of β-sitosterol [104].

3.7 Enzymes

Among the lipid-based nanocarriers, nanoliposomes are the most commonly used system for delivering enzymes in food matrices particularly cheese to shorten the ripening time and reduce production costs. High amount of enzymes are lost by whey when directly utilized in milk to accelerate ripening and consequently raise manufacturing cost of cheese. Moreover, adding free form of enzymes leads to low cheese yield, uneven distribution in curd, and undesirable sensory attributes of final product [47, 85].

Nanoliposomes enable gradual release, more stability, appropriate partitioning into food matrix, and enhanced performance for enzymes and also positively influence taste, flavor, and texture of cheese [121, 136]. Flavourzyme is a mixture of fungal proteases which has desirable effects on flavor and ripening of cheese. Jahadi et al. [86] prepared nanoliposomal formulations for accommodating Flavourzyme by heating method. Then, nanoencapsualted form of Flavourzyme was added into cow milk and produced Iranian white brined cheese. Nanoliposomes did not significantly affect the curd and whey composition as well as cheese yield as compared to control [86]. These researchers in another study found that ripening period had the greatest effect on protein decomposition of cheese samples followed by concentration of loaded Flavourzyme and brine treatment time (brining time). Moreover, 0.3% w/w of loaded enzyme, 1 month of ripening, and brining time for 8 h processed samples with the most proteolytic activities and the highest level of acceptance in sensory attributes. Also, the optimum concentration of enzyme-loaded nanoliposome led to defeat drawbacks related to texture and taste in cheese samples treated with free form [87].

On the other hand, stability and functionality of enzymes dramatically are affected by temperature and pH values within food products, and they are easily denatured and inactivated under harsh environmental conditions. Karami et al. [93] fabricated SLNs entrapping superoxide dismutase as an antioxidant enzyme by cold homogenization technique. Loaded SLNs had desirable properties concerning particle size and encapsulation efficiency. Moreover, nanoencapsulation provided remarkable improvement in stability, enzymatic potency, release behavior, and permeability of superoxide dismutase [93]. In the study conducted by Qi et al. [226], catalase as a hydrogen peroxide-scavenging enzyme was nanoencapsulated

into SLNs. Main components of SLNs were Poloxamer 188, tripalmitin, and soybean phosphatidylcholine which acted as surfactant, oil phase, and stabilizers, respectively. SLNs were highly stable owing to bear a surface charge above −30 mV. Indeed, nanodelivery systems demonstrated protective effects against proteolysis and released catalase sustainably [168].

4 Conclusion and Future Trends

Lipid-formulation nanoencapsulation technologies could be considered as an efficient alternative to overcome the limitations related to direct usage of bioactive compounds especially hydrophobic ones within food systems and also applied to design novel functional foods. They can be prepared without use of organic solvents; provide prolonged release, target specificity, and biocompatibility; protect incorporated compounds against adverse external conditions, chemical reactions, and digestion; improve their stability, solubility, dispersibility, efficiency, and bioavailability which consequently facilitate intestinal absorption; and enhance biological activity of bioactives. However, there are still some challenges in development of nanoformulations regarding toxicological safety, effects on human body, and commercialization. Moreover, it is essential to fabricate lipid-based nanocarriers by using food-grade materials and healthy lipids for food and pharmaceutical practices. Besides, more investigations need to be carried out on stability, release behavior and added level of nanoparticles to preserve sensory attributes especially appearance in food products, promote functionality of bioactive compounds, and ensure safety for in vivo applications. Therefore, these issues must be taken into account for applying nanoencapsulated bioactive compounds in food, pharmaceutical, and cosmetics industries.

References

1. Abaee A, Mohammadian M, Jafari SM (2017) Whey and soy protein-based hydrogels and nano-hydrogels as bioactive delivery systems. Trends Food Sci Technol 70:69–81
2. Aditya N, Macedo AS, Doktorovova S, Souto EB, Kim S, Chang P-S, Ko S (2014) Development and evaluation of lipid nanocarriers for quercetin delivery: a comparative study of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid nanoemulsions (LNE). LWT Food Sci Technol 59:115–121
3. Aditya N, Shim M, Lee I, Lee Y, Im M-H, Ko S (2013) Curcumin and genistein coloaded nanostructured lipid carriers: in vitro digestion and antiprostate cancer activity. J Agric Food Chem 61:1878–1883
4. Akbarzadeh A et al (2013) Liposome: classification, preparation, and applications. Nanoscale Res Lett 8:102
5. Akhavan S, Jafari SM (2017) Chapter 6: Nanoencapsulation of natural food colorants. In: Nanoencapsulation of food bioactive ingredients. Academic Press, London, pp 223–260

6. Akhavan S, Assadpour E, Katouzian I, Jafari SM (2018) Lipid nano scale cargos for the protection and delivery of food bioactive ingredients and nutraceuticals. Trends Food Sci Technol 74:132–146
7. Alexander A, Patel RJ, Saraf S, Saraf S (2016) Recent expansion of pharmaceutical nanotechnologies and targeting strategies in the field of phytopharmaceuticals for the delivery of herbal extracts and bioactives. J Control Release 241:110–124
8. Alexander M, Lopez AA, Fang Y, Corredig M (2012) Incorporation of phytosterols in soy phospholipids nanoliposomes: encapsulation efficiency and stability. LWT Food Sci Technol 47:427–436
9. Anton N, Benoit J-P, Saulnier P (2008) Design and production of nanoparticles formulated from nano-emulsion templates – a review. J Control Release 128:185–199
10. Artiga-Artigas M, Guerra-Rosas M, Morales-Castro J, Salvia-Trujillo L, Martín-Belloso O (2018) Influence of essential oils and pectin on nanoemulsion formulation: a ternary phase experimental approach. Food Hydrocoll 81:209–219
11. Assadpour E, Maghsoudlou Y, Jafari S-M, Ghorbani M, Aalami M (2016a) Evaluation of folic acid Nano-encapsulation by double emulsions. Food Bioprocess Technol 9:2024–2032
12. Assadpour E, Maghsoudlou Y, Jafari S-M, Ghorbani M, Aalami M (2016b) Optimization of folic acid nano-emulsification and encapsulation by maltodextrin-whey protein double emulsions. Int J Biol Macromol 86:197–207
13. Assadpour E, Jafari SM, Esfanjani AF (2017) Protection of phenolic compounds within nanocarriers. CAB Rev 12:1–8
14. Assadpour E, Jafari SM (2018) A systematic review on nanoencapsulation of food bioactive ingredients and nutraceuticals by various nanocarriers. Crit Rev Food Sci Nutr:1–47. https://doi.org/10.1080/10408398.2018.1484687
15. Babazadeh A, Ghanbarzadeh B, Hamishehkar H (2016) Novel nanostructured lipid carriers as a promising food grade delivery system for rutin. J Funct Foods 26:167–175
16. Babazadeh A, Ghanbarzadeh B, Hamishehkar H (2017) Phosphatidylcholine-rutin complex as a potential nanocarrier for food applications. J Funct Foods 33:134–141
17. Bagherpour S, Alizadeh A, Ghanbarzadeh S, Mohammadi M, Hamishehkar H (2017) Preparation and characterization of Betasitosterol-loaded nanostructured lipid carriers for butter enrichment. Food Biosci 20:51–55
18. Balta I, Brinzan L, Stratakos AC, Linton M, Kelly C, Pinkerton L, Corcionivoschi N (2017) Geraniol and linalool loaded Nanoemulsions and their antimicrobial activity bulletin UASVM. Animal Sci Biotechnol 74:2
19. Bhosale AP, Patil A, Swami M (2015) Herbosomes as a novel drug delivery system for absorption enhancement. World J Pharmacy Pharmaceut Sci 5:345–355
20. Bochicchio S, Barba AA, Grassi G, Lamberti G (2016) Vitamin delivery: carriers based on nanoliposomes produced via ultrasonic irradiation. LWT Food Sci Technol 69:9–16
21. Bose S, Du Y, Takhistov P, Michniak-Kohn B (2013) Formulation optimization and topical delivery of quercetin from solid lipid based nanosystems. Int J Pharm 441:56–66
22. Bou R, Cofrades S, Jiménez-Colmenero F (2014) Physicochemical properties and riboflavin encapsulation in double emulsions with different lipid sources. LWT Food Sci Technol 59:621–628
23. Bouksaim M, Lacroix C, Audet P, Simard R (2000) Effects of mixed starter composition on nisin Z production by Lactococcus lactis subsp. lactis biovar. Diacetylactis UL 719 during production and ripening of gouda cheese. Int J Food Microbiol 59:141–156
24. Caddeo C, Teskač K, Sinico C, Kristl J (2008) Effect of resveratrol incorporated in liposomes on proliferation and UV-B protection of cells. Int J Pharm 363:183–191
25. Cadena PG et al (2013) Nanoencapsulation of quercetin and resveratrol into elastic liposomes. Biochimica et Biophysica Acta (BBA)-Biomembranes 1828:309–316
26. Campani V, Biondi M, Mayol L, Cilurzo F, Pitaro M, De Rosa G (2016) Development of nanoemulsions for topical delivery of vitamin K1. Int J Pharm 511:170–177

27. Cavazos-Garduño A, Flores AO, Serrano-Niño J, Martínez-Sanchez C, Beristain C, García H (2015) Preparation of betulinic acid nanoemulsions stabilized by ω-3 enriched phosphatidylcholine. Ultrason Sonochem 24:204–213
28. Chen C, Han D, Cai C, Tang X (2010) An overview of liposome lyophilization and its future potential. J Control Release 142:299–311
29. Chen F, Liang L, Zhang Z, Deng Z, Decker EA, McClements DJ (2017) Inhibition of lipid oxidation in nanoemulsions and filled microgels fortified with omega-3 fatty acids using casein as a natural antioxidant. Food Hydrocoll 63:240–248
30. Chen H, Weiss J, Shahidi F (2006) Nanotechnology in nutraceuticals and functional foods. Food Technol 03.06(3):30–36
31. Chen X, Zou L-Q, Niu J, Liu W, Peng S-F, Liu C-M (2015) The stability, sustained release and cellular antioxidant activity of curcumin nanoliposomes. Molecules 20:14293–14311
32. Cho JS, Seo YC, Yim TB, Lee HY (2013) Effect of nanoencapsulated vitamin B1 derivative on inhibition of both mycelial growth and spore germination of fusarium oxysporum f. Sp. raphani. Int J Mol Sci 14:4283–4297
33. Chuesiang P, Siripatrawan U, Sanguandeekul R, McLandsborough L, McClements DJ (2018) Optimization of cinnamon oil nanoemulsions using phase inversion temperature method: impact of oil phase composition and surfactant concentration. J Colloid Interface Sci 514:208–216
34. Colas J-C, Shi W, Rao VM, Omri A, Mozafari MR, Singh H (2007) Microscopical investigations of nisin-loaded nanoliposomes prepared by Mozafari method and their bacterial targeting. Micron 38:841–847
35. Couto R, Alvarez V, Temelli F (2017) Encapsulation of vitamin B2 in solid lipid nanoparticles using supercritical CO2. J Supercrit Fluids 120:432–442
36. Cui H, Zhao C, Lin L (2015) The specific antibacterial activity of liposome-encapsulated clove oil and its application in tofu. Food Control 56:128–134
37. Cuomo F, Cofelice M, Venditti F, Ceglie A, Miguel M, Lindman B, Lopez F (2018) In-vitro digestion of curcumin loaded chitosan-coated liposomes. Colloids Surf B: Biointerfaces 168:29–34
38. Cuomo J et al (2011) Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. J Nat Prod 74:664–669
39. da Silva BV, Barreira JC, Oliveira MBP (2016) Natural phytochemicals and probiotics as bioactive ingredients for functional foods: extraction, biochemistry and protected-delivery technologies. Trends Food Sci Technol 50:144–158
40. Das S, Chaudhury A (2011) Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. AAPS PharmSciTech 12:62–76
41. Dasgupta N, Ranjan S, Mundra S, Ramalingam C, Kumar A (2016) Fabrication of food grade vitamin E nanoemulsion by low energy approach, characterization and its application. Int J Food Prop 19:700–708
42. Davidov-Pardo G, McClements DJ (2015) Nutraceutical delivery systems: resveratrol encapsulation in grape seed oil nanoemulsions formed by spontaneous emulsification. Food Chem 167:205–212
43. de Souza Simões L, Madalena DA, Pinheiro AC, Teixeira JA, Vicente AA, Ramos ÓL (2017) Micro-and nano bio-based delivery systems for food applications: in vitro behavior. Adv Colloid Interf Sci 243:23–45
44. DiNicolantonio JJ, Lucan SC, O'Keefe JH (2016) The evidence for saturated fat and for sugar related to coronary heart disease. Prog Cardiovasc Dis 58:464–472
45. Donsì F, Ferrari G (2016) Essential oil nanoemulsions as antimicrobial agents in food. J Biotechnol 233:106–120
46. Donsì F, Sessa M, Mediouni H, Mgaidi A, Ferrari G (2011) Encapsulation of bioactive compounds in nanoemulsion-based delivery systems. Procedia Food Sci 1:1666–1671
47. Emami S, Azadmard-Damirchi S, Peighambardoust SH, Valizadeh H, Hesari J (2016) Liposomes as carrier vehicles for functional compounds in food sector. J Exp Nanosci 11:737–759

48. Ezhilarasi P, Karthik P, Chhanwal N, Anandharamakrishnan C (2013) Nanoencapsulation techniques for food bioactive components: a review. Food Bioprocess Technol 6:628–647
49. Fang C-L, Al-Suwayeh S, Fang J-Y (2013) Nanostructured lipid carriers (NLCs) for drug delivery and targeting. Recent Pat Nanotechnol 7:41–55
50. Fang J-Y, Fang C-L, Liu C-H, Su Y-H (2008) Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). Eur J Pharm Biopharm 70:633–640
51. Fang Z, Bhandari B (2010) Encapsulation of polyphenols–a review. Trends Food Sci Technol 21:510–523
52. Faridi Esfanjani A, Jafari SM (2016) Biopolymer nano-particles and natural nano-carriers for nano-encapsulation of phenolic compounds. Colloids Surf B: Biointerfaces 146:532–543
53. Faridi Esfanjani A, Assadpour E, Jafari SM (2018) Improving the bioavailability of phenolic compounds by loading them within lipid-based nanocarriers. Trends Food Sci Technol 76:56–66
54. Fathi M, Mozafari M-R, Mohebbi M (2012) Nanoencapsulation of food ingredients using lipid based delivery systems. Trends Food Sci Technol 23:13–27
55. Fathi M, Varshosaz J (2013) Novel hesperetin loaded nanocarriers for food fortification: production and characterization. J Funct Foods 5:1382–1391
56. Fathima SJ, Fathima I, Abhishek V, Khanum F (2016) Phosphatidylcholine, an edible carrier for nanoencapsulation of unstable thiamine. Food Chem 197:562–570
57. Frenzel M, Krolak E, Wagner A, Steffen-Heins A (2015) Physicochemical properties of WPI coated liposomes serving as stable transporters in a real food matrix. LWT Food Sci Technol 63:527–534
58. Gaber DM, Nafee N, Abdallah OY (2017) Myricetin solid lipid nanoparticles: stability assurance from system preparation to site of action European. J Pharm Sci 109:569–580
59. Gandhi A, Dutta A, Pal A, Bakshi P (2012) Recent trends of phytosomes for delivering herbal extract with improved bioavailability. J Pharmacog Phytochem 1:6–14
60. Ganesan P, Narayanasamy D (2017) Lipid nanoparticles: different preparation techniques, characterization, hurdles, and strategies for the production of solid lipid nanoparticles and nanostructured lipid carriers for oral drug delivery. Sustain Chem Pharmacy 6:37–56
61. García-Márquez E, Higuera-Ciapara I, Espinosa-Andrews H (2017) Design of fish oil-in-water nanoemulsion by microfluidization. Innovative Food Sci Emerg Technol 40:87–91
62. Genç L, Kutlu HM, Güney G (2015) Vitamin B12-loaded solid lipid nanoparticles as a drug carrier in cancer therapy. Pharm Dev Technol 20:337–344
63. Geszke-Moritz M, Moritz M (2016) Solid lipid nanoparticles as attractive drug vehicles: composition, properties and therapeutic strategies. Mater Sci Eng C 68:982–994
64. Ghanbarzadeh B, Babazadeh A, Hamishehkar H (2016) Nano-phytosome as a potential food-grade delivery system. Food Biosci 15:126–135
65. Ghorbanzade T, Jafari SM, Akhavan S, Hadavi R (2017) Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt. Food Chem 216:146–152
66. Gleeson JP, Ryan SM, Brayden DJ (2016) Oral delivery strategies for nutraceuticals: delivery vehicles and absorption enhancers. Trends Food Sci Technol 53:90–101
67. Gokce EH, Korkmaz E, Dellera E, Sandri G, Bonferoni MC, Ozer O (2012) Resveratrol-loaded solid lipid nanoparticles versus nanostructured lipid carriers: evaluation of antioxidant potential for dermal applications. Int J Nanomedicine 7:1841
68. Gómez-Hens A, Fernández-Romero JM (2005) The role of liposomes in analytical processes. TrAC Trends Anal Chem 24:9–19
69. Gulotta A, Saberi AH, Nicoli MC, McClements DJ (2014) Nanoemulsion-based delivery systems for polyunsaturated (ω-3) oils: formation using a spontaneous emulsification method. J Agric Food Chem 62:1720–1725
70. Gutiérrez FJ et al (2013) Methods for the nanoencapsulation of β-carotene in the food sector. Trends Food Sci Technol 32:73–83

71. Guttoff M, Saberi AH, McClements DJ (2015) Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: factors affecting particle size and stability. Food Chem 171:117–122
72. Hadian Z, Sahari MA, Moghimi HR, Barzegar M (2014) Formulation, characterization and optimization of liposomes containing eicosapentaenoic and docosahexaenoic acids; a methodology approach. IJPR 13:393
73. Hassanzadeh P, Arbabi E, Atyabi F, Dinarvand R (2018) Ferulic acid-loaded nanostructured lipid carriers: a promising nanoformulation against the ischemic neural injuries. Life Sci 193:64–76
74. Hategekimana J, Chamba MV, Shoemaker CF, Majeed H, Zhong F (2015) Vitamin E nanoemulsions by emulsion phase inversion: effect of environmental stress and long-term storage on stability and degradation in different carrier oil types. Colloids Surf A Physicochem Eng Asp 483:70–80
75. Huang J, Wang Q, Li T, Xia N, Xia Q (2017) Nanostructured lipid carrier (NLC) as a strategy for encapsulation of quercetin and linseed oil: preparation and in vitro characterization studies. J Food Eng 215:1–12
76. Huang Z, Li X, Zhang T, Song Y, She Z, Li J, Deng Y (2014) Progress involving new techniques for liposome preparation. Asian J Pharmaceut Sci 9:176–182
77. Isailović BD, Kostić IT, Zvonar A, Đorđević VB, Gašperlin M, Nedović VA, Bugarski BM (2013) Resveratrol loaded liposomes produced by different techniques. Innovative Food Sci Emerg Technol 19:181–189
78. Jafari S, McClements D (2017) Nanotechnology approaches for increasing nutrient bioavailability. In: Advances in food and nutrition research, vol 81. Elsevier, Cambridge, MA, pp 1–30
79. Jafari SM (2017) Nanoencapsulation of food bioactive ingredients: principles and applications. Academic Press, San Diego
80. Jafari SM, Assadpoor E, He Y, Bhandari B (2008) Re-coalescence of emulsion droplets during high-energy emulsification. Food Hydrocoll 22:1191–1202
81. Jafari SM, He Y, Bhandari B (2007a) Production of sub-micron emulsions by ultrasound and microfluidization techniques. J Food Eng 82:478–488
82. Jafari SM, He Y, Bhandari B (2007b) Optimization of nano-emulsions production by microfluidization. Eur Food Res Technol 225:733–741. https://doi.org/10.1007/s00217-006-0476-9
83. Jafari SM, Paximada P, Mandala I, Assadpour E, Mehrnia MA (2017) Chapter 2: encapsulation by nanoemulsions. In: Nanoencapsulation technologies for the food and nutraceutical industries. Academic Press, London, pp 36–73
84. Jafari SM, McClements DJ (2018) Nanoemulsions. Academic Press
85. Jahadi M, Khosravi-Darani K (2017) Liposomal encapsulation enzymes: from medical applications to kinetic characteristics. Mini Rev Med Chem 17:366–370
86. Jahadi M, Khosravi-Darani K, Ehsani M-R, Saboury A, Zoghi A, Egbaltab K, Mozafari M-R (2015) Effect of protease-loaded Nanoliposome produced by heating method on yield and composition of whey and curd during the production of Iranian brined cheese. Nut Food Sci Res 2:49–53
87. Jahadi M, Khosravi-Darani K, Ehsani MR, Mozafari MR, Saboury AA, Zoghi A, Mohammadi M (2016) Modelling of proteolysis in Iranian brined cheese using proteinase-loaded nanoliposome. Int J Dairy Technol 69:57–62
88. Jay JM, Loessner M, Golden D (2005) Modern food microbiology, 7th edn. Springer, New York
89. Jenning V, Gysler A, Schäfer-Korting M, Gohla SH (2000) Vitamin a loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. Eur J Pharm Biopharm 49:211–218
90. Ji H, Tang J, Li M, Ren J, Zheng N, Wu L (2016) Curcumin-loaded solid lipid nanoparticles with Brij78 and TPGS improved in vivo oral bioavailability and in situ intestinal absorption of curcumin. Drug Deliv 23:459–470
91. Johnson EJ (2002) The role of carotenoids in human health. Nutr Clin Care 5:56–65

92. Karadag A, Yang X, Ozcelik B, Huang Q (2013) Optimization of preparation conditions for quercetin nanoemulsions using response surface methodology. J Agric Food Chem 61: 2130–2139
93. Karami MA, Zadeh BSM, Koochak M, Moghimipur E (2016) Superoxide dismutase-loaded solid lipid nanoparticles prepared by cold homogenization method: characterization and permeation study through burned rat skin. Jundishapur J Nat Pharmaceutical Product 11: e33968 (https://doi.org/10.17795/jjnpp-33968)
94. Kareparamban JA, Nikam PH, Jadhav AP, Kadam VJ (2012) Phytosome: a novel revolution in herbal drugs. IJRPC 2:299–310
95. Karimi N, Ghanbarzadeh B, Hamishehkar H, Keivani F, Pezeshki A, Gholian MM (2015) Phytosome and liposome: the beneficial encapsulation systems in drug delivery and food application. Applied Food Biotechnol 2:17–27
96. Karthik P, Anandharamakrishnan C (2016) Enhancing omega-3 fatty acids nanoemulsion stability and in-vitro digestibility through emulsifiers. J Food Eng 187:92–105
97. Katouzian I, Esfanjani AF, Jafari SM, Akhavan S (2017) Formulation and application of a new generation of lipid nano-carriers for the food bioactive ingredients. Trends Food Sci Technol 68:14–25
98. Katouzian I, Jafari SM (2016) Nano-encapsulation as a promising approach for targeted delivery and controlled release of vitamins. Trends Food Sci Technol 53:34–48
99. Katouzian I, Jafari SM (2017) Chapter 4: Nanoencapsulation of vitamins. In: Nano-encapsulation of food bioactive ingredients. Academic Press, London, pp 145–181
100. Keivani Nahr F, Ghanbarzadeh B, Hamishehkar H, Kafil HS (2018) Food grade nanostructured lipid carrier for cardamom essential oil: preparation, characterization and antimicrobial activity. J Funct Foods 40:1–8
101. Khan J, Alexander A, Saraf S, Saraf S (2013) Recent advances and future prospects of phyto-phospholipid complexation technique for improving pharmacokinetic profile of plant actives. J Control Release 168:50–60
102. Ko S, Lee S-C (2010) Effect of nanoliposomes on the stabilization of incorporated retinol. Afr J Biotechnol 9:6158–6161
103. Komaiko J, Sastrosubroto A, McClements DJ (2016) Encapsulation of ω-3 fatty acids in nanoemulsion-based delivery systems fabricated from natural emulsifiers: sunflower phospholipids. Food Chem 203:331–339
104. Lacatusu I, Badea N, Stan R, Meghea A (2012) Novel bio-active lipid nanocarriers for the stabilization and sustained release of sitosterol. Nanotechnology 23:455702
105. Lane KE, Li W, Smith CJ, Derbyshire EJ (2016) The development of vegetarian omega-3 oil in water nanoemulsions suitable for integration into functional food products. J Funct Foods 23: 306–314
106. Lee H, Yildiz G, Dos Santos L, Jiang S, Andrade J, Engeseth N, Feng H (2016) Soy protein nano-aggregates with improved functional properties prepared by sequential pH treatment and ultrasonication. Food Hydrocoll 55:200–209
107. Li H, Zhao X, Ma Y, Zhai G, Li L, Lou H (2009) Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. J Control Release 133:238–244
108. Li M, Zahi MR, Yuan Q, Tian F, Liang H (2016) Preparation and stability of astaxanthin solid lipid nanoparticles based on stearic acid. Eur J Lipid Sci Technol 118:592–602
109. Li T et al (2015) Preparation and characterization of nanoscale complex liposomes containing medium-chain fatty acids and vitamin C. Int J Food Prop 18:113–124
110. Lin C-H, Chen C-H, Lin Z-C, Fang J-Y (2017) Recent advances in oral delivery of drugs and bioactive natural products using solid lipid nanoparticles as the carriers. J Food Drug Anal 25: 219–234
111. Lin Q, Liang R, Williams PA, Zhong F (2018) Factors affecting the bioaccessibility of β-carotene in lipid-based microcapsules: digestive conditions, the composition, structure and physical state of microcapsules. Food Hydrocoll 77:187–203

112. Liu L et al (2014) Characterization and biodistribution in vivo of quercetin-loaded cationic nanostructured lipid carriers colloids and surfaces B. Biointerfaces 115:125–131
113. Liu W, Tian M, Kong Y, Lu J, Li N, Han J (2017) Multilayered vitamin C nanoliposomes by self-assembly of alginate and chitosan: long-term stability and feasibility application in mandarin juice. LWT Food Sci Technol 75:608–615
114. Livney YD (2015) Nanostructured delivery systems in food: latest developments and potential future directions. Curr Opin Food Sci 3:125–135
115. López-Rubio A, Lagaron JM (2012) Whey protein capsules obtained through electro-spraying for the encapsulation of bioactives. Innovative Food Sci Emerg Technol 13: 200–206
116. Lu W-C, Huang D-W, Wang C-C, Yeh C-H, Tsai J-C, Huang Y-T, Li P-H (2018) Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. J Food Drug Anal 26(1):82–89
117. Lu W, Kelly AL, Miao S (2016) Emulsion-based encapsulation and delivery systems for polyphenols. Trends Food Sci Technol 47:1–9
118. Madrigal-Carballo S, Lim S, Rodriguez G, Vila AO, Krueger CG, Gunasekaran S, Reed JD (2010) Biopolymer coating of soybean lecithin liposomes via layer-by-layer self-assembly as novel delivery system for ellagic acid. J Funct Foods 2:99–106
119. Mahdavi SA, Jafari SM, Ghorbani M, Assadpoor E (2014) Spray-drying microencapsulation of anthocyanins by natural biopolymers: a review. Dry Technol 32:509–518
120. Mahdavee Khazaei K, Jafari SM, Ghorbani M, Hemmati Kakhki A (2014) Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's anthocyanins and evaluating their storage stability and color. Carbohydr Polym 105:57–62
121. Maherani B, Arab-Tehrany E, Mozafari MR, Gaiani C, Linder M (2011) Liposomes: a review of manufacturing techniques and targeting strategies. Curr Nanosci 7:436–452
122. Marczylo TH, Verschoyle RD, Cooke DN, Morazzoni P, Steward WP, Gescher AJ (2007) Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. Cancer Chemother Pharmacol 60:171–177
123. Marsanasco M, Márquez AL, Wagner JR, Alonso SV, Chiaramoni NS (2011) Liposomes as vehicles for vitamins E and C: an alternative to fortify orange juice and offer vitamin C protection after heat treatment. Food Res Int 44:3039–3046
124. Martins N, Roriz CL, Morales P, Barros L, Ferreira IC (2016) Food colorants: challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. Trends Food Sci Technol 52:1–15
125. Maruyama K et al (2004) Intracellular targeting of sodium mercaptoundecahydrododecaborate (BSH) to solid tumors by transferrin-PEG liposomes, for boron neutron-capture therapy (BNCT). J Control Release 98:195–207
126. Maté J, Periago PM, Palop A (2016) Combined effect of a nanoemulsion of D-limonene and nisin on Listeria monocytogenes growth and viability in culture media and foods. Food Sci Technol Int 22:146–152
127. McClements DJ (2012) Nanoemulsions versus microemulsions: terminology, differences, and similarities. Soft Matter 8:1719–1729
128. McClements DJ, Rao J (2011) Food-grade Nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit Rev Food Sci Nutr 51:285–330. https://doi.org/10.1080/10408398.2011.559558
129. Mehmood T (2015) Optimization of the canola oil based vitamin E nanoemulsions stabilized by food grade mixed surfactants using response surface methodology. Food Chem 183:1–7
130. Mehrad B, Ravanfar R, Licker J, Regenstein JM, Abbaspourrad A (2018) Enhancing the physicochemical stability of β-carotene solid lipid nanoparticle (SLNP) using whey protein isolate. Food Res Int 105:962–969
131. Mehrnia MA, Jafari SM, Makhmal-Zadeh BS, Maghsoudlou Y (2016) Crocin loaded nano-emulsions: factors affecting emulsion properties in spontaneous emulsification. Int J Biol Macromol 84:261–267

132. Mehrnia M-A, Jafari S-M, Makhmal-Zadeh BS, Maghsoudlou Y (2017) Rheological and release properties of double nano-emulsions containing crocin prepared with Angum gum, Arabic gum and whey protein. Food Hydrocoll 66:259–267
133. Mendes J et al (2018) Chemical composition and antibacterial activity of Eugenia brejoensis essential oil nanoemulsions against Pseudomonas fluorescens. LWT 93:659–664
134. Mirzaei H, Shakeri A, Rashidi B, Jalili A, Banikazemi Z, Sahebkar A (2017) Phytosomal curcumin: a review of pharmacokinetic, experimental and clinical studies. Biomed Pharmacother 85:102–112
135. Mohammadi M, Pezeshki A, Abbasi MM, Ghanbarzadeh B, Hamishehkar H (2017) Vitamin D3-loaded nanostructured lipid carriers as a potential approach for fortifying food beverages; in vitro and in vivo evaluation. Adv Pharmaceutical Bulletin 7:61
136. Mohammadi R, Mahmoudzade M, Atefi M, Khosravi-Darani K, Mozafari M (2015) Applications of nanoliposomes in cheese technology. Int J Dairy Technol 68:11–23
137. Mohammadi A, Jafari SM, Esfanjani AF, Akhavan S (2016a) Application of nano-encapsulated olive leaf extract in controlling the oxidative stability of soybean oil. Food Chem 190:513–519
138. Mohammadi A, Jafari SM, Assadpour E, Faridi Esfanjani A (2016b) Nano-encapsulation of olive leaf phenolic compounds through WPC-pectin complexes and evaluating their release rate. Int J Biol Macromol 82:816–822
139. Mokhtari S, Jafari SM, Assadpour E (2017) Development of a nutraceutical nano-delivery system through emulsification/internal gelation of alginate. Food Chem 229:286–295
140. Morais JM, Burgess DJ (2014) In vitro release testing methods for vitamin E nanoemulsions. Int J Pharm 475:393–400
141. Mozafari RM (2005) Nanoliposomes: from fundamentals to recent developments. Trafford
142. Mukherjee K, Maiti K, Venkatesh M, Mukherjee P (2008) Phytosome of hesperetin, a value added formulation with phytomolecules. In: 60th Indian Pharmaceutical Congress
143. Müller R, Radtke M, Wissing S (2002a) Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm 242:121–128
144. Müller RH, Radtke M, Wissing SA (2002b) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 54:S131–S155
145. Munin A, Edwards-Lévy F (2011) Encapsulation of natural polyphenolic compounds: a review. Pharmaceutics 3:793–829
146. Nejadmansouri M, Hosseini SMH, Niakosari M, Yousefi GH, Golmakani MT (2016) Physicochemical properties and storage stability of ultrasound-mediated WPI-stabilized fish oil nanoemulsions. Food Hydrocoll 61:801–811
147. Ni S, Hu C, Sun R, Zhao G, Xia Q (2017) Nanoemulsions-based delivery Systems for Encapsulation of quercetin: preparation, characterization, and cytotoxicity studies. J Food Process Eng 40:e12374 (https://doi.org/10.1111/jfpe.12374)
148. Nunes S, Madureira AR, Campos D, Sarmento B, Gomes AM, Pintado M, Reis F (2017) Solid lipid nanoparticles as oral delivery systems of phenolic compounds: overcoming pharmacokinetic limitations for nutraceutical applications. Crit Rev Food Sci Nutr 57:1863–1873
149. Ojagh SM, Hasani S (2018) Characteristics and oxidative stability of fish oil nano-liposomes and its application in functional bread. J Food Measur Characterization 12(2):1084–1092
150. Oliveira DRB, Michelon M, de Figueiredo FG, Sinigaglia-Coimbra R, Cunha RL (2016) β-Carotene-loaded nanostructured lipid carriers produced by solvent displacement method. Food Res Int 90:139–146
151. Öztürk B (2017) Nanoemulsions for food fortification with lipophilic vitamins: production challenges, stability, and bioavailability. Eur J Lipid Sci Technol 119:1500539 (https://doi.org/10.1002/ejlt.201500539)
152. Ozturk B, Argin S, Ozilgen M, McClements DJ (2014) Formation and stabilization of nanoemulsion-based vitamin E delivery systems using natural surfactants: Quillaja saponin and lecithin. J Food Eng 142:57–63

153. Pandita D, Kumar S, Poonia N, Lather V (2014) Solid lipid nanoparticles enhance oral bioavailability of resveratrol, a natural polyphenol. Food Res Int 62:1165–1174
154. Panpipat W, Dong M, Xu X, Guo Z (2013) Thermal properties and nanodispersion behavior of synthesized β-sitosteryl acyl esters: a structure–activity relationship study. J Colloid Interface Sci 407:177–186
155. Park SJ, Garcia CV, Shin GH, Kim JT (2017) Development of nanostructured lipid carriers for the encapsulation and controlled release of vitamin D3. Food Chem 225:213–219
156. Park SJ, Garcia CV, Shin GH, Kim JT (2018) Improvement of curcuminoid bioaccessbility from turmeric by a nanostructured lipid carrier system. Food Chem 251(15):51–57
157. Patel AR, Bhandari B (2014) Nano-and microencapsulation of vitamins. In: Nano-and micro-encapsulation for foods. Wiley, Chichester, pp 223–248
158. Patel MR, Martin-Gonzalez S, Fernanda M (2012) Characterization of ergocalciferol loaded solid lipid nanoparticles. J Food Sci 77(1):N8–13
159. Pezeshki A, Ghanbarzadeh B, Mohammadi M, Fathollahi I, Hamishehkar H (2014) Encapsulation of vitamin a palmitate in nanostructured lipid carrier (NLC)-effect of surfactant concentration on the formulation properties. Adv Pharmaceutical Bulletin 4:563
160. Pezeshky A, Ghanbarzadeh B, Hamishehkar H, Moghadam M, Babazadeh A (2016) Vitamin A palmitate-bearing nanoliposomes: preparation and characterization. Food Biosci 13:49–55
161. Pinheiro AC, Coimbra MA, Vicente AA (2016) In vitro behaviour of curcumin nanoemulsions stabilized by biopolymer emulsifiers–effect of interfacial composition. Food Hydrocoll 52:460–467
162. Pinilla CMB, Brandelli A (2016) Antimicrobial activity of nanoliposomes co-encapsulating nisin and garlic extract against gram-positive and gram-negative bacteria in milk. Innovative Food Sci Emerg Technol 36:287–293
163. Pinto F, de Barros DP, Fonseca LP (2018) Design of multifunctional nanostructured lipid carriers enriched with α-tocopherol using vegetable oils. Ind Crop Prod 118:149–159
164. Porter CJ, Trevaskis NL, Charman WN (2007) Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov 6:231
165. Pourashouri P, Shabanpour B, Razavi SH, Jafari SM, Shabani A, Aubourg SP (2014) Impact of wall materials on physicochemical properties of microencapsulated fish oil by spray drying. Food Bioprocess Technol 7:2354–2365
166. Prombutara P, Kulwatthanasal Y, Supaka N, Sramala I, Chareonpornwattana S (2012) Production of nisin-loaded solid lipid nanoparticles for sustained antimicrobial activity. Food Control 24:184–190
167. Pyo S-M, Müller RH, Keck CM (2017) Encapsulation by nanostructured lipid carriers. In: Nanoencapsulation technologies for the food and nutraceutical industries. Elsevier, London, pp 114–137
168. Qi C, Chen Y, Huang JH, Jin QZ, Wang XG (2012) Preparation and characterization of catalase-loaded solid lipid nanoparticles based on soybean phosphatidylcholine. J Sci Food Agric 92:787–793
169. Rabelo CA, Taarji N, Khalid N, Kobayashi I, Nakajima M, Neves MA (2018) Formulation and characterization of water-in-oil nanoemulsions loaded with açaí berry anthocyanins: insights of degradation kinetics and stability evaluation of anthocyanins and nanoemulsions. Food Res Int 106:542–548
170. Radhakrishnan R, Kulhari H, Pooja D, Gudem S, Bhargava S, Shukla R, Sistla R (2016) Encapsulation of biophenolic phytochemical EGCG within lipid nanoparticles enhances its stability and cytotoxicity against cancer. Chem Phys Lipids 198:51–60
171. Rafiee Z, Barzegar M, Sahari MA, Maherani B (2017) Nanoliposomal carriers for improvement the bioavailability of high–valued phenolic compounds of pistachio green hull extract. Food Chem 220:115–122
172. Rao J, McClements DJ (2011) Food-grade microemulsions, nanoemulsions and emulsions: fabrication from sucrose monopalmitate & lemon oil. Food Hydrocoll 25:1413–1423

173. Rashidinejad A, Birch EJ, Sun-Waterhouse D, Everett DW (2014) Delivery of green tea catechin and epigallocatechin gallate in liposomes incorporated into low-fat hard cheese. Food Chem 156:176–183
174. Rashidinejad A, Birch EJ, Sun-Waterhouse D, Everett DW (2016) Effect of liposomal encapsulation on the recovery and antioxidant properties of green tea catechins incorporated into a hard low-fat cheese following in vitro simulated gastrointestinal digestion. Food Bioprod Process 100:238–245
175. Rasti B, Erfanian A, Selamat J (2017) Novel nanoliposomal encapsulated omega-3 fatty acids and their applications in food. Food Chem 230:690–696
176. Ravanfar R, Tamaddon AM, Niakousari M, Moein MR (2016) Preservation of anthocyanins in solid lipid nanoparticles: optimization of a microemulsion dilution method using the placket–Burman and box–Behnken designs. Food Chem 199:573–580
177. Ribeiro H, Gupta R, Smith K, van Malssen K, Popp A, Velikov K (2016) Super-cooled and amorphous lipid-based colloidal dispersions for the delivery of phytosterols. Soft Matter 12:5835–5846
178. Righeschi C, Bergonzi MC, Isacchi B, Bazzicalupi C, Gratteri P, Bilia AR (2016) Enhanced curcumin permeability by SLN formulation: the PAMPA approach. LWT Food Sci Technol 66:475–483
179. Saberi AH, Fang Y, McClements DJ (2013) Fabrication of vitamin E-enriched nanoemulsions by spontaneous emulsification: effect of propylene glycol and ethanol on formation, stability, and properties. Food Res Int 54:812–820
180. Salminen H, Aulbach S, Leuenberger BH, Tedeschi C, Weiss J (2014) Influence of surfactant composition on physical and oxidative stability of Quillaja saponin-stabilized lipid particles with encapsulated ω-3 fish oil. Colloids Surf B: Biointerfaces 122:46–55
181. Salminen H, Gömmel C, Leuenberger BH, Weiss J (2016) Influence of encapsulated functional lipids on crystal structure and chemical stability in solid lipid nanoparticles: towards bioactive-based design of delivery systems. Food Chem 190:928–937
182. Salvia-Trujillo L, Soliva-Fortuny R, Rojas-Graü MA, McClements DJ, Martín-Belloso O (2017) Edible nanoemulsions as carriers of active ingredients: a review. Annu Rev Food Sci Technol 8:439–466
183. Santana R, Perrechil F, Cunha R (2013) High-and low-energy emulsifications for food applications: a focus on process parameters. Food Eng Rev 5:107–122
184. Saraf S (2010) Applications of novel drug delivery system for herbal formulations. Fitoterapia 81:680–689
185. Sari TP, Mann B, Kumar R, Singh RRB, Sharma R, Bhardwaj M, Athira S (2015) Preparation and characterization of nanoemulsion encapsulating curcumin. Food Hydrocoll 43:540–546
186. Saxena V, Hasan A, Sharma S, Pandey LM (2018) Edible oil nanoemulsion: an organic nanoantibiotic as a potential biomolecule delivery vehicle. Int J Polym Mater Polym Biomater 67:410–419
187. Sebaaly C, Jraij A, Fessi H, Charcosset C, Greige-Gerges H (2015) Preparation and characterization of clove essential oil-loaded liposomes. Food Chem 178:52–62
188. Semalty A, Semalty M, Rawat MSM, Franceschi F (2010) Supramolecular phospholipids–polyphenolics interactions: the PHYTOSOME® strategy to improve the bioavailability of phytochemicals. Fitoterapia 81:306–314
189. Sessa M et al (2014) Bioavailability of encapsulated resveratrol into nanoemulsion-based delivery systems. Food Chem 147:42–50
190. Shah R, Eldridge D, Palombo E, Harding I (2015) Lipid nanoparticles: production, characterization and stability. Springer, Berlin
191. Shin GH, Chung SK, Kim JT, Joung HJ, Park HJ (2013) Preparation of chitosan-coated nanoliposomes for improving the mucoadhesive property of curcumin using the ethanol injection method. J Agric Food Chem 61:11119–11126
192. Shin GH, Kim JT, Park HJ (2015) Recent developments in nanoformulations of lipophilic functional foods. Trends Food Sci Technol 46:144–157

193. Sindhumol P, Thomas M, Mohanachandran P (2010) Phytosomes: a novel dosage form for enhancement of bioavailability of botanicals and neutraceuticals. Int J Pharm Pharm Sci 2:10–14
194. Solans C, Izquierdo P, Nolla J, Azemar N, Garcia-Celma M (2005) Nano-emulsions. Curr Opin Colloid Interface Sci 10:102–110
195. Soleimanian Y, Goli SAH, Varshosaz J, Sahafi SM (2018) Formulation and characterization of novel nanostructured lipid carriers made from beeswax, propolis wax and pomegranate seed oil. Food Chem 244:83–92
196. Soukoulis C, Bohn T (2018) A comprehensive overview on the micro-and nano-technological encapsulation advances for enhancing the chemical stability and bioavailability of carotenoids. Crit Rev Food Sci Nutr 58:1–36
197. Sun J, Bi C, Chan HM, Sun S, Zhang Q, Zheng Y (2013) Curcumin-loaded solid lipid nanoparticles have prolonged in vitro antitumour activity, cellular uptake and improved in vivo bioavailability. Colloids Surf B: Biointerfaces 111:367–375
198. Takahashi M, Uechi S, Takara K, Asikin Y, Wada K (2009) Evaluation of an oral carrier system in rats: bioavailability and antioxidant properties of liposome-encapsulated curcumin. J Agric Food Chem 57:9141–9146
199. Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A (2013) Nanostructured lipid carriers (NLC): a potential delivery system for bioactive food molecules. Innovative Food Sci Emerg Technol 19:29–43
200. Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A (2014) Design and characterization of astaxanthin-loaded nanostructured lipid carriers. Innovative Food Sci Emerg Technol 26:366–374
201. Tan C, Feng B, Zhang X, Xia W, Xia S (2016) Biopolymer-coated liposomes by electrostatic adsorption of chitosan (chitosomes) as novel delivery systems for carotenoids. Food Hydrocoll 52:774–784
202. Tan C, Zhang Y, Abbas S, Feng B, Zhang X, Xia S (2014) Modulation of the carotenoid bioaccessibility through liposomal encapsulation. Colloids Surf B: Biointerfaces 123:692–700
203. Taylor TM, Weiss J, Davidson PM, Bruce BD (2005) Liposomal nanocapsules in food science and agriculture. Crit Rev Food Sci Nutr 45:587–605
204. Tian H, Lu Z, Li D, Hu J (2018) Preparation and characterization of citral-loaded solid lipid nanoparticles. Food Chem 248:78–85
205. Ting Y, Jiang Y, Ho C-T, Huang Q (2014) Common delivery systems for enhancing in vivo bioavailability and biological efficacy of nutraceuticals. J Funct Foods 7:112–128
206. Tripathy S, Patel DK, Barob L, Naira SK (2013) A review on phytosomes, their characterization, advancement & potential for transdermal application. J Drug Del Therapeutics 3:147–152
207. Tsai W-C, Rizvi SS (2016) Liposomal microencapsulation using the conventional methods and novel supercritical fluid processes. Trends Food Sci Technol 55:61–71
208. Uraiwan K, Satirapipathkul C (2016) The entrapment of vitamin E in nanostructured lipid carriers of rambutan seed fat for cosmeceutical uses. In: Key engineering materials. Trans Tech, Pfaffikon, pp 77–80
209. Walia N, Dasgupta N, Ranjan S, Chen L, Ramalingam C (2017) Fish oil based vitamin D nanoencapsulation by ultrasonication and bioaccessibility analysis in simulated gastro-intestinal tract. Ultrason Sonochem 39:623–635
210. Walker RM, Decker EA, McClements DJ (2015) Physical and oxidative stability of fish oil nanoemulsions produced by spontaneous emulsification: effect of surfactant concentration and particle size. J Food Eng 164:10–20
211. Wang JL et al (2014) Preparation and characterization of novel lipid carriers containing microalgae oil for food applications. J Food Sci 79(2):E169–177
212. Weiss J, Decker EA, McClements DJ, Kristbergsson K, Helgason T, Awad T (2008) Solid lipid nanoparticles as delivery systems for bioactive food components. Food Biophysics 3:146–154

213. Wu G, Li J, Yue J, Zhang S, Yunusi K (2018) Liposome encapsulated luteolin showed enhanced antitumor efficacy to colorectal carcinoma. Mol Med Rep 17:2456–2464
214. Yadav P, Soni G, Mahor A, Alok S, Singh PP, Verma A (2014) Solid lipid nanoparticles: an effective and promising drug delivery system-a review. Int J Pharm Sci Res 5:1152
215. Yang S, Liu W, Liu C, Liu W, Tong G, Zheng H, Zhou W (2012) Characterization and bioavailability of vitamin C nanoliposomes prepared by film evaporation-dynamic high pressure microfluidization. J Dispers Sci Technol 33:1608–1614
216. Yang Y, McClements DJ (2013) Encapsulation of vitamin E in edible emulsions fabricated using a natural surfactant. Food Hydrocoll 30:712–720
217. Zhang J, Tang Q, Xu X, Li N (2013) Development and evaluation of a novel phytosome-loaded chitosan microsphere system for curcumin delivery. Int J Pharm 448:168–174
218. Zhu J, Zhuang P, Luan L, Sun Q, Cao F (2015) Preparation and characterization of novel nanocarriers containing krill oil for food application. J Funct Foods 19:902–912
219. Zhuang C-Y et al (2010) Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. Int J Pharm 394:179–185
220. Zou L-q et al (2014) Improved in vitro digestion stability of (−)-epigallocatechin gallate through nanoliposome encapsulation. Food Res Int 64:492–499
221. Radtke M, Müller, RH (2001) Nanostructured lipid drug carriers. New Drugs 2:48–52
222. Nazemiyeh E, Eskandani M, Sheikhloie H, Nazemiyeh H (2016) Formulation and physico-chemical characterization of lycopene-loaded solid lipid nanoparticles. Adv Pharm Bull 6 (2):235–241
223. Esquerdo V, Dotto G, Pinto L (2015) Preparation of nanoemulsions containing unsaturated fatty acid concentrate–chitosan capsules. J Colloid Interf Sci 445:137–142
224. Zhao Y, Chang Y-X, Hu X, Liu C-Y, Quan L-H, Liao Y-H (2017) Solid lipid nanoparticles for sustained pulmonary delivery of Yuxingcao essential oil: preparation, characterization and in vivo evaluation. Int J Pharmaceut 516:364–371
225. Lewies A, Wentzel JF, Jordaan A, Bezuidenhout C, Du Plessis LH (2017) Interactions of the antimicrobial peptide nisin Z with conventional antibiotics and the use of nanostructured lipid carriers to enhance antimicrobial activity. Int J Pharmaceut 526:244–253
226. Qi C, Chen Y, Huang JH, Jin QZ, Wang XG (2012) Preparation and characterization of catalase-loaded solid lipid nanoparticles based on soybean phosphatidylcholine. J Sci Food Agric. 92:787–793
227. Chanburee S, Tiyaboonchai W (2018) Enhanced intestinal absorption of curcumin in Caco-2 cell monolayer using mucoadhesive nanostructured lipid carriers. J Biomed Mater Res B Appl Biomater. 106(2):734–741

CLAs in Animal Source Foods: Healthy Benefits for Consumers

23

Paolo Polidori, Silvia Vincenzetti, Stefania Pucciarelli, and Valeria Polzonetti

Contents

P. Polidori (✉)
School of Pharmacy, University of Camerino, Camerino (MC), Italy
e-mail: paolo.polidori@unicam.it

S. Vincenzetti · S. Pucciarelli · V. Polzonetti
School of Biosciences and Veterinary Medicine, University of Camerino, Camerino (MC), Italy
e-mail: silvia.vincenzetti@unicam.it; stefania.pucciarelli@unicam.it; valeria.polzonetti@unicam.it

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_51

Abstract

Conjugated linoleic acid (CLA) is a group of polyunsaturated fatty acids that exist as positional and stereo-isomers of octadecadienoate (18:2). Among these isomers, the most studied two isomers are cis 9, trans 11-CLA and trans 10, cis 12-CLA due to their biological effects. CLA can be naturally synthesized in the rumen of ruminant animals by bacteria *Butyrivibrio fibrisolvens* via the Δ-9-desaturase of trans 11 octadecanoic acid pathway. The major dietary sources of CLA are represented by meat and milk from ruminant animals. Although references to CLA can be traced back to the 1950s, current interest in the health benefits of CLA started in the late 1980s, after it was identified as the anti-carcinogenic component present in fried ground beef. Since then, an extensive literature has documented the anticarcinogenic effects of CLA. In addition, there is some evidence that CLA is also anti-atherosclerotic, has beneficial effects on type 2 diabetes, and may play a key role in helping to regulate body fat. The fact that the richest natural sources of CLA, meat and dairy products, are consumed by people worldwide has very interesting implications for public health.

Keywords

Conjugated linoleic acid · Fatty acids · Animal source foods · CLA and cancer · CLA and human diet · CLA and human health

List of Abbreviations

CLA	Conjugated Linoleic Acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAME	Fatty Acid Methyl Esters
MUFA	Monounsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
SFA	Saturated Fatty Acids
TFA	*Trans* Fatty Acids

1 Introduction

Anthropology has previously recognized the importance of food and diet variations among time periods. It is possible to identify the profile of meat consumption during human evolution in four periods: the first could be characterized by an opportunist hunting; while in the second, hunting had grown to a bigger scale and lasted 2 to 3 million years; in the third period, men started to domesticate animals and plants, which had began 10,000 years ago; during the fourth and last period studies determined that meat contained compounds which could increase disease risk [1]. Anthropological data have also suggested an important influence of meat consumption in human erect posture. Bipedalism is probably the first and most important characteristic which distinguished humans from their ancestors as it allowed a more efficient locomotion and load carrying, which are important advantages in hunting [2]. Cranial–dental

changes are quite visible when analyzing hominids fossils. Molar teeth size has decreased and the jaws and front teeth have become stronger. Also shearing crests have grown. These changes could be explained through the urgent need of tearing and chewing meat rather than grinding leaves, fruits, seeds, and cereals [3].

Gastrointestinal tract features can also aid in determining dietary preferences considering that the gut of herbivores and pure carnivores suffered different physiological and metabolic adaptations. On one hand, plant-based diets are associated with a sacculated stomach and well-developed caecum and colon, which increased with plant fiber content. On the other hand, a carnivore's stomach is well developed and acidic with a large small intestine. Humans are omnivorous thus fitting in neither category. They have a simple stomach and a relatively long small intestine but also a reduced caecum and colon [4]. The fact that the small intestine is the most prominent organ in the human gastrointestinal tract is due to the need for adaptation to a varied diet, including nutritionally dense foods, with great volume and conducive to being digested in the small intestine.

Meat is an important source of high-value animal protein in many regions of the world. Around the globe, the diets of relatively more urbanized populations are characterized by a higher content of meat, poultry, and other animal products than the less diversified diets of rural communities [5]. Contrary to popular perception, average red meat intakes appear to be moderate and in line with current recommendations in developed countries [6]. Red meat intakes in these countries range from the lowest consumption in Greece at 31 g per day for women and 55 g per day for men to the highest consumption in The Netherlands at 79 g per day for women and 136 g per day for men (see Table 1).

Essentially, meat is basically composed of water, protein, lipids, minerals, and carbohydrates. Lean muscle tissue contains approximately 72–74% moisture, 20–22% protein, 3–5% fat, 1% ash, and 0.5% of carbohydrate. These proportions are largely variable, especially in the lipid content, which depends on species, amount of fattening, inclusion of the adipose tissue, and so on. There is an inverse relationship between the percentages of protein and moisture and the percentage of fat so that meats with high content of fat have lower content of moisture and proteins [7].

Skeletal muscle contains a variable amount of lipids, between 1% and 13%. Lipid content mainly depends on the degree of fattening and the amount of adipose tissue. Lipids can be found within the muscle (intramuscular), between muscles

Table 1 Mean daily intake (g/d) of total red meat (fresh and processed) in selected countries

Countries	Women	Men
Spain	67	127
Australia	55	110
Canada	55	101
Italy	60	91
United Kingdom	47	78
Greece	31	55

Source: Adapted from [6]

(intermuscular), and in adipose tissue. Intramuscular lipids are mainly composed of triacylglycerols, which are stored in fat cells, and phospholipids, which are located in cell membranes. The amount of cholesterol in lean meat is around 50–70 mg/100 g. Intermuscular and adipose tissue lipids are mainly composed of triacylglycerols and small amounts of cholesterol, around 40–60 mg/100 g [8].

Triacylglycerols are the major constituents of fat. The fatty acid content mainly depends on age, production system, type of feed, and environment [7]. Monogastric animals such as swine and poultry tend to reflect the fatty acid composition of the feed in their fat. In the case of ruminants, the nutrients and fatty acid composition are somehow standardized due to biohydrogenation by the microbial population of the rumen [9]. The properties of the fat will depend on its fatty acid composition. A great percentage of the triacylglycerols are esterified to saturated and monounsaturated fatty acids. When triacylglycerols are rich in polyunsaturated fatty acids (PUFA) such as linoleic and linolenic acids, fats tend to be softer and prone to oxidation. These fats may even have an oily appearance when kept at room temperature. Phospholipids are present in cell membranes, and although present in minor amounts, they have a strong relevance to flavor development due to their relatively high proportion of PUFA. Major constituents are phosphatidylcholine (lecithin) and phosphatidylethanolamine. The phospholipid content may vary depending on the genetic type of the animal and the anatomical location of the muscle [10]. For instance, red oxidative muscles have a higher amount of phospholipids than white glycolytic muscles.

2 Lipids in Meat

Lipid contributes substantially to the caloric content of meat. It also has marked effects on mouth-feel and flavor of meat. There are three sources of lipid in meat: the muscle fibers, subcutaneous adipose tissue, and intramuscular (interfascicular, marbling) adipose tissue [11]. Once subcutaneous adipose tissue has been removed, the primary contributor to lipid content of meat is intramuscular adipose tissue.

Pioneering researchers from the seventeenth and eighteenth century such as Robert Boyle, Poulletier de la Salle, Antoine François de Fourcroy, and others began the study of modern lipid chemistry. During the nineteenth century the chemist, Chevreul, identified several fatty acids, suggested the name “cholesterine” for the fatty substance in gallstones, coined the word “glycerine” and showed that fats are comprised of glycerol and fatty acids [12]. During the twentieth century many advances have been made in terms of the understanding of lipid structure and function. Lipids include waxes, oils, fats, steroids, and related compounds ranging from soaps to petrochemicals [13]. Triacylglycerol, which is a neutral lipid, are made up of three fatty acids attached to a molecule of glycerol, and they vary in their physical properties according to the chemical structure of the fatty acid. A phospholipid is a lipid containing phosphoric acid as mono- or diester and is the main building block for cell membranes. Fatty acids are “amphiphilic,” i.e., has a carboxyl group (hydrophilic) at the polar end and a hydrocarbon chain at the nonpolar tail (hydrophobic). Fatty acids consisting of only single bonds are termed

"saturated." If there are carbon–carbon double bonds in a fatty acid chain, the fatty acid is termed "unsaturated." Oleic acid (C18:1) contains one double bond and is termed" monounsaturated." A fatty acid with more than one double bond is termed "polyunsaturated." The polyunsaturated fatty acids (PUFA) predominate in vegetable oils. The two determinants of the consistency of a lipid is the length of the predominant fatty acid chains and the presence or the absence of double bonds [13]. Fatty acids with twelve or more carbon atoms are referred to as long-chain fatty acids and are typical of fats from animal origin.

Meat fat comprises mostly monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA). The most ubiquitous fatty acids are oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids. Poultry and pork contain somewhat more unsaturated fatty acids (10–15% of total fatty acids) than beef and lamb, and also a notable amount of PUFAs. Linoleic acid (C18:2) is the predominant PUFA (0.5–7%), followed by alpha-linolenic acid (up to 0.5%) [14]. *Trans*-fatty acids (TFA) comprise about 1–2% of total fatty acids across all types of meat; in ruminant meats they represent 2–4%. Pigs have much higher proportions of the major polyunsaturated fatty acid (PUFA) linoleic acid (18:2 n-6) than cattle and sheep [15]. Linoleic acid is derived entirely from the diet. It passes through the pig's stomach unchanged and is then absorbed into the blood stream in the small intestine and incorporated from there into tissues. In ruminants, the fatty acid, which is at high levels in concentrate feedstuffs (grains and oilseeds), is degraded into monounsaturated and saturated fatty acids in the rumen by microbial biohydrogenation and only a small proportion, around 10% of dietary 18:2 n-6, is available for incorporation into tissue lipids.

The second most important PUFA is α-linolenic acid (18:3 n-3), which is present in many concentrate feed ingredients but at lower levels than 18:2 n-6. This is a major dietary fatty acid for ruminants since it constitutes over 50% of total fatty acids in grass and grass products. Again, a high proportion is biohydrogenated to saturated fatty acids in the rumen. A variable proportion of dietary 18:3 n-3 is biohydrogenated (85–100%) but this is more than for 18:2 n-6 (70–95%), so less is available for incorporation into tissues [16]. As with 18:2n-6, proportions in ruminants are higher in muscle than adipose tissue. Muscle contains significant proportions of long chain (C20–22) PUFA which are formed from 18:2 n-6 and 18:3 n-3 by the action of Δ5 and Δ6 desaturase and elongase enzymes. Important products are arachidonic acid (20:4 n-6) and eicosapentaenoic acid (EPA, 20:5 n-3) which have various metabolic roles including eicosanoid production.

The n-3 and n-6 fatty acids are required in the diets of humans as well as other monogastric mammals as they cannot be synthesized de novo. These fatty acids function as carriers of the fat-soluble vitamins (Vitamin A, D, E and K) and play a crucial role in the immune response of both man and animal. Essential fatty acids with 18-carbon molecules include linolenic acid (9-cis-, 12-cis-,15-cis-octadecatrienoic acid; C18:3 n-3) and linoleic acid (9-cis, 12-cis-octadecadienoic acid; C18:2n-6). The two most important 20-carbon essential fatty acids are arachidonic acid (C20:4 n-6), which is formed by desaturation and elongation of linoleic acid, and EPA (C20:5 n-3), which is formed by desaturation and elongation of α-linolenic acid [17]. Meat, fish, and fish oil are the only significant dietary sources of C20:4 n-6

(arachidonic acid) and C22:6 n-3 docosahexaenoic acid (DHA). Meat contains lower concentrations of these polyunsaturated fatty acids when compared with oily fish [18]. The two most readily found n-3 fish oil fatty acids are EPA and DHA.

Cholesterol in meat exists in two forms: as free cholesterol and as cholesterol ester [19]. Free cholesterol is associated primarily with cellular and subcellular membranes of muscle and intramuscular adipocytes. Cholesterol ester, located within the triacylglycerol-rich central lipid vacuole, comprises about 75% of the total cholesterol in adipose tissue. Muscle fibres, which are rich in membranes but contain comparatively little lipid, have approximately 75% of their total cholesterol associated with membranes and the other 25% associated with their neutral lipids. There are two reasons for the minimal effect of intramuscular adipose tissue on the concentration of cholesterol in meat:

1. Each gram of intramuscular adipose tissue contributes only 1.2 mg of cholesterol.
2. Any increase in intramuscular adipose tissue replaces an equal volume of muscle, which contributes as much as 0.65 mg of cholesterol per gram.

Thus, any contribution of intramuscular adipose tissue to total cholesterol is diluted by the amount of muscle it displaces. For this reason, different cuts of meat may vary substantially in the number of calories from fat but will differ only slightly in their cholesterol content [19].

3 Effects of Animal Nutrition on Meat Lipidic Profile

A great research effort has been exerted since the 1980s for the manipulation of the fatty acid composition of meat, to achieve nutritional recommendations, especially an increase in the ratio between PUFA and saturated fatty acids (SFA). More recently, nutritionists recommend that PUFA composition should be manipulated toward a lower *n*-6:*n*-3 ratio. Fats with a higher content of PUFA have lower melting points that affect the fat firmness. Softer fats may raise important problems during processing if the integrity of the muscle is disrupted by any mechanical treatment (chopping, mincing, stuffing, etc.). The major troubles are related to oxidation and generation of off-flavors (rancid aromas) and color deterioration, specifically a trend toward yellowness in the fat [8].

Pigs and poultry are monogastric animals that incorporate part of the dietary fatty acids practically unchanged into the adipose tissue and cellular membranes, where desaturation and chain elongation processes may occur [7]. The extent of incorporation may vary depending on the specific fatty acid and the type of feed. Different types of cereals as well as dietary oils and their effects on the proportions in fatty acid composition have been studied. The use of canola or linseed oils produces a substantial increase in the content of linolenic acid (C 18:3), which is an n-3 fatty acid. In this way, the n-6:n-3 ratio can be reduced from 9 to 5 [20]. Other dietary oils such as soy, peanut, corn, and sunflower increase the content of linoleic acid (C18:2), an n-6 fatty acid. Although it increases the total PUFA content, this fatty acid does

not contribute to decrease the n-6:n-3 ratio, just the reverse. A similar trend is observed in the case of poultry, where the feeds with a high content of linoleic acid such as grain, corn, plant seeds, or oils also increase the n-6/n-3 ratio. As in the case of pork, the use of feeds containing fish oils or algae, enriched in *n*-3 fatty acids such as EPA (C 22:5 n-3) and DHA (C 22:6 n-3) acids, can enrich the poultry meat in n-3 fatty acids and reduce the n-6/n-3 ratio from around 8.4 to 1.7 [15]. The main problem arises from oxidation during heating, because some volatile compounds such as hexanal are typically generated, producing rancid aromas. The rate and extent of oxidation of muscle foods mainly depends on the level of PUFA, but they are also influenced by early postmortem events such as pH drop, carcass temperature, aging, and other factors. Feeds rich in saturated fats such as tallow yield the highest levels of palmitic, palmitoleic, stearic, and oleic acids in pork loin [21]. Linoleic and linolenic acid content may vary as much as 40% between the leanest and the fattest animals [22]. The PUFA content is especially high in phospholipids, located in subcellular membranes such as mitochondria, microsomes, and so on, making them vulnerable to peroxidation because of the proximity of a range of prooxidants such as myoglobin, cytochromes, nonheme iron, and trace elements [23]. Muscle contains several antioxidant systems, for example, those of superoxide dismutase and glutathione peroxidase, and ceruloplasmin and transferrin, although they are weakened during postmortem storage.

The fatty acid profile in ruminants is more saturated than in pigs, and thus the fat is firmer [14]. The manipulation of fatty acids in beef is more difficult due to the rumen biohydrogenation. More than 90% of the PUFA are hydrogenated, leaving a low margin for action to increase the PUFA/SFA ratio above 0.1. However, meats from ruminants are rich in conjugated linoleic acid (CLA), mainly 9-*cis,*11-*trans*-octadecadienoic acid, which exerts important health-promoting biological activity [24]. In general, a good level of nutrition increases the amount of intramuscular fat. On the other hand, food deprivation may result in an induced lipolysis that can be rapidly detected (in just 72 h) through a higher content of free fatty acids and monoacylglycerols, especially in glycolytic muscles [25].

4 Conjugated Linoleic Acid (CLA)

During the 1930s, biological chemists began to realize that the cow could convert dietary nonconjugated fatty acids to a conjugated component. However, the mechanism for this transformation and the location at which it was effected were unknown. By 1950, it was established that there was a species-to-species difference in the unsaturated fatty acid content of body fat from pasture-fed animals. Linolenic acid is the predominant pasture fatty acid. When ruminant animals such as cows and sheep consume this acid, only trace amounts appear in body tissues or milk. On the other hand, the horse, a nonruminant, transfers a considerable proportion of dietary linolenate to its depot fat [26].

Observations that rumen contents could hydrogenate polyunsaturated fatty acids to produce acids with *trans*-unsaturation provided a biological explanation for the

presence of *trans*-fatty acids in the depot fat of sheep and oxen, noted as early as 1928 [27]. Later, scientists from New Zealand [28] examined the *trans*-unsaturated fatty acid content of fat from a selection of ruminant and nonruminant animals. As expected, the depot fats from ruminants (Sambur, fallow deer, ox, and sheep) contained conjugated diene at around the 0.5% level. An unexpected finding was the presence of considerable conjugated diene in the body fat of the nonruminant marsupials, wallaby (3.5%), and quokka (2.9%). Depot fat *trans*-monounsaturated fatty acid content from these marsupials was also exceptionally high, 19% and 21%, respectively. The high conjugated diene and transmonoene content is explained by their possession of a ruminant-like digestion.

The isomer *cis*-9,*trans*-11-18:2 was identified in the depot fat of pasture-fed lambs using Gas Liquid Chromatography analysis [29]. Trivial names for the natural *cis*-9, *trans*-11-isomer have been suggested: firstly [30] was proposed bovinic acid, but this name was considered too restrictive because the isomer is also produced in the rumen of a number of other species of commercial importance. For this reason was later suggested rumenic acid, a name that is now accepted and used [31].

During the early 1980s, Michael Pariza and his colleagues at the University of Wisconsin found that an isolate from grilled minced beef could inhibit carcinogenesis. The anticarcinogenic isolate was shown to consist of isomers of conjugated octadecadienoic acid in which the constituent double bonds are separated by a single carbon-to-carbon bond instead of a methylene group. The isomers were referred to collectively as conjugated linoleic acid for which the acronym CLA is now used [27].

Conjugated linoleic acid (CLA) refers to a group of polyunsaturated fatty acids that exist as positional and stereo-isomers of conjugated dienoic octadecadienoate (18:2). The predominant geometric isomer in foods is the c9t11-CLA isomer, also called "rumenic acid" [24], followed by t7,c9-CLA, 11,13-CLA (c/t), 8,10-CLA (c/t), and the t10c12-CLA isomer. The three-dimensional stereo-isomeric configuration of CLA may be in combinations of cis and/or *trans* configurations (Fig. 1).

CLA is found in foods such as beef and lamb, as well as dairy foods derived from these ruminant sources [32]. Numerous physiological properties have been attributed to CLA including action as an antiadipogenic, antidiabetogenic, anticarcinogenic, and antiatherosclerotic agent (Table 2). In addition, CLA has effects on bone

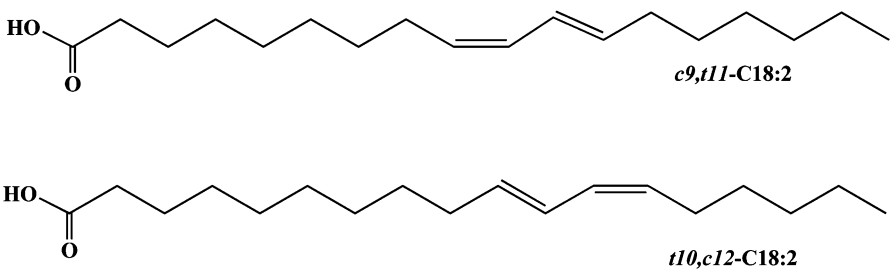


Fig. 1 Main isomers conjugated linoleic acid. Top: C18:2Δ9c,11 t (rumenic acid). Bottom: C18:2Δ10t,12c

Table 2 Physiological properties of CLA

Major function	Physiological model
Body composition	Decrease adiposity in chicks, mice, rats;
	Decrease adiposity in human subjects.
Diabetes	Decrease the onset of diabetes in male rats;
	Aids in the management of metabolic parameters in human subjects with type 2 diabetes;
	Decrease insulin sensitivity in mice.
Carcinogenesis	Decrease chemically induced mammary carcinogenesis in rats;
	Decrease growth of transplantable breast cancer tumor cells in nude mice;
	Decrease growth of transplantable prostate cancer tumor cells in nude mice;
	Decrease chemically induced colon carcinogenesis in rats;
	Decrease chemically induced forestomach.
Atherosclerosis	Decrease atherosclerotic plaque formation in hamsters.
Immune system	Decrease eicosanoid and histamine production;
	Increase onset of lupus in mouse model.

Source: Adapted from [24]

formation and the immune system as well as fatty acid and lipid metabolism and gene expression in numerous tissues [24].

The primary dietary sources of CLA for humans are food products derived from ruminant animals, mostly cattle, including meat fat, milk, cheese, yoghurt, and butter [33]. There are two biosynthetic processes responsible for the formation of CLA. These processes are carried out primarily in ruminant animals but also to a lesser extent in nonruminant animals. The first process is the incomplete biohydrogenation of linoleic acid and linolenic acid in the rumen; the second biosynthetic process is the endogenous conversion of transvaccenic acid, an intermediate of biohydrogenation, to CLA in tissues [34]. The main dietary source of linoleic acid for ruminant animals is concentrated feed consisting mainly of grains and seed oils, whereas the main dietary source of linolenic acid is pasture grasses [35].

4.1 Biosynthesis of CLA

Because potential health benefits have been associated with dietary consumption of CLA, enhancement of CLA concentrations in meat and milk has become an important objective in animal nutrition research. It is generally accepted that CLA in ruminant meat and milk originates from incomplete biohydrogenation of linoleic acid in the rumen [36]. A study performed in 1951 [37] noticed that the body fats of ruminants possessed less linolenic acid than horses on the same high linolenic acid diet and suspected something to have happened in the rumen. Linolenic acid was incubated in rumen fluid, and it was demonstrated the formation of TFA in the rumen. Utilizing rumen contents from fistulated sheep grazing pasture [38], it was confirmed the existence of TFA as a result of rumen biohydrogenation. Later [39] it

was also demonstrated that conjugated dienoic acids accumulated when linoleic acid was incubated with rumen contents, but not when linolenic acid was incubated with it.

4.2 CLA Synthesis in the Rumen

A diverse range of rumen bacterial species have been isolated; they demonstrate a capacity to isomerize *cis*-double bonds of unsaturated fatty acids to form conjugated *cis*/*trans* double bond systems and further hydrogenate these conjugated acids [36]. A study performed in 1967 [40] showed the production of *c*-9, *t*-11 CLA from linoleic acid by *Butyrivibrio fibrisolvens*. When the same bacteria were incubated using linolenic acid as the substrate, *c*-9, *t*-11, *c*-15 C18:3 were produced, in which 18:3 later was found to be hydrogenated to trans vaccenic acid [41]. No *c*-9, *t*-11 CLA was formed from linolenic acid.

The biohydrogenation of linoleic acid and linolenic acid in the rumen occurs in a similar manner. The first reaction in linoleic acid biohydrogenation is the isomerization where the double bond at carbon-12 position is transferred to carbon-11 position forming *c*-9, *t*-11 CLA. It is followed by the rapid hydrogenation of *cis*-9 bond leaving *trans* vaccenic acid. Both these steps are carried out by group A bacteria, while the last step of biohydrogenation of oleic to stearic acid is carried out by group B bacteria [42]. The enzyme responsible for the conjugation of *cis*-9, *cis*-12 double bonds was identified as linoleic acid isomerase (EC 5.3.1.5). It is a particulate enzyme bound to the bacterial cell membrane [36] and demonstrates an absolute substrate requirement for a *cis*-9, *cis*-12 diene system and a free carboxyl group [43], found in both linoleic acid and linolenic acid. Similarly, the linolenic acid is first isomerized at *cis*-12 position to form *c*-9, *t*-11, *c*-15 C18:3, which were then reduced at both the *cis* bonds to produce *trans* vaccenic acid. The final step is similar to that of linoleic acid. A common intermediate during the biohydrogenation of linoleic acid and linolenic acid was found to be *trans* vaccenic acid [42]. Its reduction appears to be rate limiting in the complete biohydrogenation of unsaturated C18 fatty acids resulting in the accumulation of trans vaccenic acid in the rumen [44]. This is the predominant pathway of rumen biohydrogenation of linoleic acid and linolenic acid. Subsequently, a product precursor relationship between *trans* vaccenic acid and CLA was observed both in vitro and in vivo (sheep) with increasing concentrations of LA in the diet [45].

With a low-fiber diet, a change in *trans* octadecenoic acid profile of milk occurred and *trans*-10 octadecenoic acid became the predominant *trans* octadecenoic acid in milk fat [46]. This suggested the hypothesis of another pathway for the ruminal synthesis of *t*-10, *c*-12 CLA involving bacterial *c*-9, *t*-10 isomerase with the formation of a *t*-10, *c*-12 double bond as the first step in the process [36]. The *c*-12, *t*-11 isomerase from *Butyrivibrio fibrisolvens* can hydrogenate *t*-10, *c*-12 octadecadienoic acid [41], thus producing *t*-10 octadecenoic acid. It has been shown that more than 50% of the linoleic acid was converted to *t*-10, *c*-12 isomer of CLA and only 10% was converted to *t*-10 octadecenoic acid by anaerobic *Propionibacterium* isolated from mouse cecum [47]. Another rumen bacteria *Megasphaera elsdenii* YJ-4 have

also been shown to produce *t*-10, *c*-12 isomer of CLA [48]. The *t*-10, *c*-12 isomer was formed from linoleic acid but not from either of the linolenic acid as was the case with *c*-9, *t*-11 isomer of CLA. It is not clear whether *t*-10 octadecenoic acid is desaturated at *cis*-12 position to produce *t*-10, *c*-12 isomer in the rumen or in some other tissues endogenously.

Rumen pH has an important role in maintaining a viable rumen environment suitable for *Butyrivibrio fibrisolvens* involved in the biohydrogenation of linoleic acid and linolenic acid. It has been shown that ruminal pH at 6.0 or above has a positive effect on *trans* vaccenic acid and CLA contents in rumen cultures [49]. It is of higher importance in high yielding dairy and beef animal diets where large amounts of grain are included in the diet and thus decrease the rumen pH below 6.0. Other than its positive effects on *Butyrivibrio fibrisolvens,* how rumen pH affects overall biohydrogenation of unsaturated fatty acids of 18, 20, or 22 carbons in the rumen in relation to CLA and *trans* vaccenic acid has not been investigated in detail. It has been shown that CLA could be increased by supplementing diets with feed sources such as fish oil or marine algae (*Schizochytrium* sp.), which are rich in 20- or 22-carbon fatty acids [50] that do not yield either CLA or *trans* vaccenic acid during biohydrogenation in the rumen. The mechanism by which supplementation of fish oil or marine algae increases concentration of milk fat CLA and *trans* vaccenic acid is not clear. It has been proposed that the longer chain polyunsaturated fatty acids from fish oil inhibit the complete biohydrogenation of C in the rumen by inhibiting the 18:2 growth of bacteria responsible for hydrogenating *trans* vaccenic acid or through the inhibition of their hydrogenases [36] leading to an increased escape of *trans* vaccenic acid from the rumen. Further research is needed on the pathway for rumen biohydrogenation of longer chain poly-unsaturated fatty acids from fish oils and other fatty acids sources of marine origin to clearly define the mechanisms involved in enhancing CLA and *trans* vaccenic acid contents in food products from ruminants.

4.3 CLA Endogenous Synthesis

While the origin of CLA from linoleic acid by *Butyrivibrio fibrisolvens* in the rumen was accepted, it was not sufficient to account for all the CLA present in milk or meat. The levels of CLA in meat and milk from ruminants compared with nonruminants suggest a close association between rumen function and CLA levels in tissue and milk fat.

A study performed in 1996 [51] found that lactating sheep grazing pastures with no supplemental linoleic acid produced a high level of *c*-9, *t*-11 CLA in milk fat. The puzzle as to why the milk fat showed greatly increased absorption in the ultraviolet region when cows were turned out to pasture, even though the pastures are high in LNA and not LA, continued to intrigue the scientists. Two years later another study demonstrated that supplementation of fish oil, which is high in PUFA of 20 or more carbons did not produce *c*-9, *t*-11 CLA or *trans* vaccenic acid as the intermediate during biohydrogenation, and also increased *c*-9, *t*-11 CLA content in the milk of

cows [52]. Basing their research on these preliminary results, it was possible to get to the conclusion that ruminal synthesis of CLA was only marginal and could not account for the amount of CLA present in milk and meat from ruminants [36]. Overall these findings suggested that the CLA formed during the biohydrogenation of linoleic acid in the rumen was not the only source and that another source needed to be explored.

Initially it was proposed that CLA could be synthesized endogenously from *trans* vaccenic acid by Δ9-desaturase. A recent study [53] provides several lines of evidence that, by analogy with rumenic acid, trans-11,cis-13 CLA may originate both from ruminal biohydrogenation and from direct Δ13-desaturation of vaccenic acid in mammary tissue.

An experiment [54] was conducted to examine whether increased CLA in milk of dairy cows fed fresh pasture compared with alfalfa and corn silages was because of ruminal or endogenous synthesis. Eight Holsteins were fed a total mixed ration using alfalfa and corn silages as the forage source in confinement or grazed in a replicated crossover design. The proportion of total fatty acids as CLA (primarily c9, t11-18:2) in g/100 g was 0.44 v. 0.28 in ruminal digesta, 0.89 v. 0.53 in omasal digesta and 0.71 v. 1.06 in milk during confinement feeding and grazing, respectively. Blood plasma CLA was 0.54 v. 1.05 mg/l for the two treatments, respectively. The increased concentration of CLA in milk with grazing likely resulted from increased synthesis through desaturation of t11-18:1 in the mammary gland. Other authors [55] estimated rumen output of CLA in nonlactating cows and then extrapolated the results to lactating cows on the basis of feed intake. They estimated the endogenous synthesis of CLA to be >80% of the total. Another study [56] even estimated 100% of CLA to be derived from endogenous synthesis. It is not surprising, considering the fact that the amount of CLA detected in the blood serum of cows is none or very small [57].

It is possible that a higher proportion of CLA is synthesized endogenously in cows fed all pasture diets compared with cows fed grains, oil seeds, or oils, because LNA, which is high in fresh pastures, does not produce CLA as the intermediate product during its biohydrogenation in the rumen. However, all these studies used an indirect approach to estimate the CLA synthesized endogenously and it may be over- or underestimated under different feeding regimens. A direct approach, which may be difficult, is probably needed to measure the actual uptake of CLA and its incorporation into milk fat by the mammary gland to estimate the mammary synthesis of CLA more accurately.

In other ruminants, information on the proportion of rumen and endogenous origin of CLA is limited. However, the fact that increased CLA content in lamb meat [58] and goat milk fat [59] was associated with high TVA contents indicates that post ruminal synthesis could be the predominant one. A very high correlation ($r = 0.99$) of CLA with TVA in goat milk fat [59] suggests that mammary synthesis predominates over rumen synthesis of CLA.

Several other *trans*-C 18:2 isomers are found in rumen digesta, and milk and tissue lipids [41]. Unlike *c*-9, *t*-11 CLA, no detailed studies have been conducted to study the endogenous synthesis of other isomers of CLA, including *t*-10, *c*-12,

probably because many of them contribute 0.05% or less in milk or meat fat and their biological significance has not been established.

5 CLA in Foods

CLAs are widely distributed in various foods, primarily in dairy products and meat from ruminants. In general, ruminant products have the highest amounts of CLA, while vegetable products and some seafood contain only trace amounts [60].

The wide variation of the CLA content in dairy products reflects the differences in CLA contents of the milk fat. The CLA content in milk fat is markedly influenced by the cows' diet [46], the PUFA content of the feed [61], dietary regimen [62], and the presence of ionophores [63]. The influences of the processing parameters and starter cultures on the preparation of cheese are contradictory [64], and further studies are necessary to evaluate whether the conditions increase the CLA content and change the isomer distribution in cheese. CLA contents in long-ripened propionic-acid-fermented cheeses (e.g., old Emmenthal, or yogurt) with added probiotic cultures appear to be slightly elevated [60]. The content of CLA in dairy products ranged from 0.63% in condensed milk to 1.16% in cow's milk, and from 0.40% in Gouda to 1.70% in Jurassic cheese [60]. These values should not be viewed as representative of one product. The wide variation of the CLA content in dairy products reflects the differences in CLA contents of the milk fat.

Meat from animals such as pigs contains low amounts (0.12%) of CLA because such animals lack a rumen. The CLA in pork may originate from feedstuffs that contain CLA such as meat meal or tallow.

5.1 CLA in Meats

Food sources originating from ruminants are known to have markedly higher CLA concentration than those from monogastric animals. Table 3 shows the CLA concentration of meat from different animal species usually used in the human diet. The

Table 3 CLA content in different meat and meat products (mg/g FAME)

Lamb	5.6
Beef	2.9–4.3
Pork	0.6
Chicken	0.9
Turkey	2.5
Salami	4.2
Cooked ham	2.7
Smoked bacon	0.8–2.7
Smoked ham	2.9

Source: Adapted by [36]

highest CLA concentrations were found in lamb (4.3–19.0 mg/g lipid) and with slightly lower concentrations in beef (1.2–10.0 mg/g lipid). The CLA content of pork, chicken, and meat from horses is usually lower than 1 mg/g lipid. Interestingly, turkey seems to have a relatively high CLA content (2–2.5 mg/g lipid), but reasons for this are unclear [32]. Some data on CLA meat content of animals less common in human diets like meat from elk (1.3–2.1 mg CLA per gram fatty acids), bison (2.9–4.8 mg/g fatty acids), water buffalo (1.83 mg/g fatty acids), and zebu-type cattle (1.47 mg/g fatty acids) are also available [65]. The highest CLA concentration (38 mg/g fatty acids) of all animals was found in adipose tissue of kangaroos [66]. Large variations in the CLA content are not only reported between animal species but also within muscles of the same species.

The CLA concentrations reported in various studies may be underestimated because only the c9,t11–18:2 isomer was determined, and not the total CLA content [67]; however, this isomer accounts for more than 80% of the total CLA, even if a study found that between 76% and 92% (depending on the species) of the total CLA was c9,t11–18:2, while other studies [68] determined a value of 78% in lamb rib loins, and a value of about 59% in beef [69].

A trial performed with the aim to evaluate the effect of the slaughter weight on the CLA isomer content of intramuscular fat in three different kind of lamb muscles, specifically *Longissimus dorsi*, *Triceps brachii*, and *Semimembranosus*, showed that the body weight at slaughter significantly affected the amount of rumenic acid in intramuscular fat of *Semimembranosus* and *Triceps brachii* in Massese lambs, but not in the *Longissimus dorsi*. In particular, the heaviest animals showed the highest amount of rumenic acid [70]. The different behavior shown by LD muscle did not seem to be related either to differences in the stearoyl Co-A desaturase enzyme activity, or to differences in the rumen activity, or to differences in fat deposition. Thus, further studies are needed to verify whether this different behavior among muscles is related to their different metabolism and/or to a different tissue utilization of fatty acids [71].

Grilling rib-eye, sirloin, T-bone, and ground beef to an internal temperature of 80 °C increases both the c9,t11-isomer (mg/g fat) and total CLA (mg/g fat) content of these meats [69], but a significant increase was observed only in the case of the grilled T-bone steak. Results obtained in the same study [69] showed that frying, grilling, microwaving, and baking ground beef patties did not produce any major changes in the CLA content. With the exception of baking, the higher internal temperature (80 °C) generally resulted in higher CLA concentrations. During refrigerated storage of ground beef, oxidative damage occurs but this does not influence the CLA content. Results obtained till now demonstrated that cooking and storage of meat does not negatively alter its CLA content [72].

CLA can be produced with very limited amount by gastric bacterial biohydrogenation in pig resulting in low amount of CLA in pork [73]. However, pork is an ideal candidate for CLA enrichment by feeding chemically synthesized CLA because CLA cannot be further saturated and can be deposited in tissues with relatively high efficiency [73]. The cis 9, trans 11 isomer of CLA could be incorporated by 46.4% in subcutaneous adipose tissue and the cis 11 and trans 13 was

incorporated by 0.74% in intramuscular fat. Feeding pigs with 1% CLA for 47 days significantly increased the CLA content including the cis 9, trans 11 and the trans 10, cis 12 in belly fat [74]. However, results on pig growth performance in response to CLA supplementation are not homogeneous, as reported by [75]. This may be due to a deficit of nutrients in diets containing CLA isomers, as these fatty acids act as repartitioning agents, promoting lean tissue deposition. The high lean tissue deposition and the low body fat deposition of CLA-treated pigs [76] may require an increase in dietary protein content and/or protein quality to maintain protein synthesis and growth in finishing swine. In fact, the utilization of substances with repartitioning effects such as ractopamine required an increase in lysine content in finishing pigs [77]. Consequently dietary CLA supplementation might induce an insufficient nutritional level of an essential amino acid like lysine, the first limiting amino acid in pig diet used for protein deposition.

In a study performed on rabbit meat, it was demonstrated that dietary CLA improved the oxidative stability of rabbit muscle, increasing shelf life, decreasing lipogenic enzyme activity, and reducing plasma triglycerides and total cholesterol [78]. The same authors also found that addition of CLA isomers to rabbit diet modified lipid content and fatty acid composition and reduced lipid oxidation in LL [79]. The increase in CLA content, from about 1.3 to 10.4 mg/100 g of edible meat, suggests that the nutritional quality of rabbit meat for human consumption may be improved.

CLA content very similar to those found in ruminants has been determined in meat obtained from foals slaughtered at 24 months of age [80]; equid meat is a typical red meat, even if it is produced by a monogastric hind-gut fermenter [81]. However, considering another equid such as donkey, in one of the few studies recently performed on donkey meat lipid profile characterization, it was not possible to determine CLA [82].

5.2 CLA in Chicken, Turkey, and Eggs

The content of CLA may be increased by 40 times in breast and thigh meat, by feeding CLA enriched diets to broilers [83]. Despite this high increase in the CLA content in breast and thigh meat, the recommended daily intake for CLA in humans may only be covered by roughly 10% [84]. Furthermore, CLA enriched poultry meat shows deviations in meat quality, increasing toughness and showing also a darker color [85]. This is also confirmed by sensorial evaluation: color and flavor of CLA enriched meat received positive results from the test panel, whereas, texture and juiciness were negatively scored. The toughness of the meat may be explained by the higher proportion of SFA in CLA enriched muscle tissues and the consequent decrease in UFA, which increase the fat melting point [85].

In egg yolk lipids, CLA was not even detected when laying hens were fed a normal concentrate diet [35]. The effects of dietary CLA supplementation on eggs fat profile have been recently reviewed [86]; an interesting result showed that the egg yolk surface from hens fed CLA diets sometimes had relatively dark color with light

spots. Dietary CLA increased the firmness of hard-cooked egg yolk. The texture of yolks from hard-cooked CLA eggs was rubbery and elastic, and the yolks were more difficult to break. It was speculated that the quality changes of CLA eggs were related to the increase of yolk water content, the movement of ions between yolk and albumen through yolk membrane, and the changes of egg yolk pH during storage.

Dietary supplementation of CLA significantly influenced fatty acid profile in egg yolk lipids: myristic, palmitic, stearic, CLA (9-cis, 11-trans CLA and 10-trans, 12-cis CLA) were increased by dietary CLA, while palmitoleic, oleic, linoleic, linolenic, arachidonic, and DHA acids were decreased. Total CLA concentration observed when 5% CLA was fed ranged between 8.5 to 8.6% (Ahn et al. 1999). The decrease in the concentrations of linoleic and linolenic acids in yolk lipids of hens fed CLA probably reflects the relatively low concentration of these fatty acids in the CLA source as compared with soybean oil. Decreases in arachidonic acid and DHA acid in yolk lipids from hens fed CLA also could be related to the low concentration of dietary linoleic and linolenic acids, which serve as precursors to the formation of arachidonic and DHA acids. Another possibility is that CLA may compete with linoleic or linolenic acid for Δ6-desaturase, the rate-limiting step for the conversion of these fatty acids into arachidonic acids or DHA acid in liver microsomes [87].

5.3 CLA in Fish

Several studies demonstrated the cardiac benefits of a regular intake of fish, a source of the long-chain n-3 fatty acids EPA and DHA [88]. A sharp demarcation in the linoleic acid content of fish showed that most marine fish typically had only 1–2% of linoleic acid. This content is so low as to continue to preclude speculation concerning whether the fatty acid of the muscle of this food would or would not contain CLA [89]. The situation changes completely when considering the new developments in farmed fish that accumulate fatty acids from a variety of dietary ingredients in their growth period. The reason is that the diets represent a balance between the cost and efficacy of the ingredients. The need is to provide good quality protein for growth and meet minimal specific needs for “essential” fatty acids, especially in salmonids, plus vitamins and minerals. For salmonids, replacement of the most common dietary fat, fish oil, with vegetable oils has become common lately.

Seafoods are not considered to be important sources of CLA, either, but the term seafood is so loosely used that it is often difficult to learn from brief references whether the source of most of the fat or lipid in the reference is from cold-water or warm-water fish, or from the fish alone [89].

5.4 CLA in Milk and Dairy Products

The CLA content in dairy cows milk fat can be affected by a cow’s diet, breed, age, non-nutritive feed additives, such as ionophores, and by the use of synthetic mixtures

of CLA supplements [90]. Among these factors, the diet is known to strongly influence the CLA content of milk and includes feedstuffs such as pasture, conserved forages, plant seed oils, cereal grains, and animal fat.

The positive effect of pasture-based diets on the CLA content of milk fat has been demonstrated in several experiments [90]. Cows grazing pasture had 500% higher CLA content in milk fat (2.21% of total fatty acids) compared to cows fed a diet containing 50% conserved forage (hay and silages) and 50% grain (0.38% of total fatty acids). Other researchers have also demonstrated that the CLA content of milk increased linearly as the proportion of fresh grass from pasture in the diet was increased.

About 48% to 56% of the total fatty acids in fresh forages consist of C18:3. Fresh grass supplies C18:3 as a substrate for ruminal biohydrogenation. However, the abundant supply of C18:3 from fresh grass only partly explains the large increases in CLA and *trans* vaccenic acid contents of milk fat from pasture-fed cows. Besides this, the high concentrations of soluble fiber and fermentable sugars present in fresh grass may create an environment in the rumen without lowering the ruminal pH that is favorable to the growth of the microbes responsible for CLA and *trans* vaccenic acid production. Ruminal pH is generally relatively high in cows grazing pasture compared to cows fed a combination of conserved forage and grain.

The effects of several feeding strategies on CLA concentration in dairy milk have been recently described; most experiments were conducted on Holstein or Friesian cows. The most common results indicated that CLA in milk is related to the availability of its precursors (oleic acid, linoleic acid and linolenic acid) from ruminal andendogenous synthesis [91]. The average difference in CLA content of milk fat among Brown Swiss, Holstein-Friesian, and Jersey breeds is 15% to 20% when fed similar diets. Brown Swiss cows have inherently higher CLA in milk fat, followed by the Holstein-Friesian and Jersey breeds; data on other breeds are too limited to form any firm conclusions [90].

Microbial fermentation can contribute to increases in CLA content in dairy products through isomerase and reductase reactions in the biohydrogenation pathway. Moreover, the process temperature can also influence the CLA content in dairy products. Dairy products, such as cheese, are the richest sources of CLA, and CLAs are formed in cheese during processing and storage [92]. When the animals were pasture fed, the contents of CLA in milk fat were much higher than that when a fodder mixture was supplied to cows. These data indicate that linoleic acid-enriched feed can increase the CLA contents in milk and cheese through fermentation by ruminal bacteria. The influence of cheese processing, e.g., changes in temperature, microbial fermentation, or ageing, on CLA production are proportional [93]. Existing literature suggests that CLA in milk fat is a stable compound under normal processing and storage conditions, while processing dairy products at >80 °C may slightly elevate the CLA content.

Lactic acid bacteria, bifidobacteria, and some propionibacteria are used as starter cultures in cheese and are able to efficiently convert linoleic acid to CLA. Thus, CLA-enriched cheeses typically originate from CLA enriched milk, and starter cultures also have a great influence on the CLA content [93].

6 Human Dietary Intake of CLA

It is not easy to determine how much CLA humans are currently consuming, and how much should be consumed in order to obtain the health benefits and anticancer effects that have been observed from adding proper levels of CLA to the diet. Rigorous documentation of dietary CLA intake in any population is not available currently. However, several investigators have utilized indirect methodologies to estimate both typical and extreme intakes of CLA in a limited number of populations. Using 3-d dietary records in conjunction with published CLA contents of foods, typical CLA intake has been estimated to be between 52 and 137 mg/day for young men and women in the United States and 430 and 350 mg/day for German men and women, respectively [30]. The use of a semiquantitative food-frequency questionnaire to estimate typical CLA intake in lactating women suggests that CLA intake in this population is 227 mg/day [30].

The CLA and TFA dietary intake in Germany is shown in Table 4; the estimated daily CLA intake was found to be 0.36 g/day for women and 0.44 g/day for men. The CLA intake compares to approximately one-fifth the daily intake of TFA and may reach physiologically active levels.

To date, statements about health promoting effects of CLA are mainly based on animal trials and remain to be proven in humans [36]. In human trials synthetic CLA supplements are usually used and these do not reflect natural isomer composition in foodstuffs. Whether natural CLA sources (meat and milk from ruminants) have a similar impact on human health warrants further research.

Furthermore, estimations of the dietary intakes of CLA isomeric forms by infants, children, and adolescents have not been documented. The dietary CLA intake reflects individual, dietary, and ethnic consumption habits. Finally, examination of the relationships among human dietary intake of CLA isomers, their concentrations in adipose tissue and plasma, and risk of various chronic degenerative diseases (e.g., cancer, diabetes, and obesity) is essential for scientists to better understand the importance of dietary CLA in human health. Thus, enhancing our knowledge concerning CLA intake in various populations must remain a primary focus for research in this area.

Table 4 Estimated CLA and TFA intake in Germany

	Women		Men	
	CLA (g/d)	TFA (g/d)	CLA (g/d)	TFA (g/d)
Milk and dairy products	0.24	0.90	0.28	1.1
Meat and meat products	0.08	0.2	0.11	0.3
Fish	<0.01	<0.01	<0.01	<0.01
Margarines	<0.01	0.1	<0.01	0.2
Cakes and pastries	0.03	0.2	0.03	0.2
Chocolate and sweets	<0.01	0.1	0.01	0.1

Source: Adapted by [60]

7 Cancer and CLA Consumption

The initial observations arousing interest in the biological effects of CLA showed their ability to reduce chemically induced cancers in animal models. In contrast to the many hundreds of phytochemicals that possess varying degrees of anti-carcinogenic activity, CLA is unique because it is a group of fatty acids isomers found in highest amounts in animal products [94]. Furthermore, CLA was found to possess anticarcinogenic activity that turned out be quite potent. The following sections present the published experimental literature that indicates an effect of CLA on cancer inhibition, primarily in animal models of chemically induced cancer.

7.1 Colorectal Cancer (CRC)

The publication of the World Cancer Research Fund/American Institute for Cancer Research [95] raised considerable alarms about the cancer risks associated with red and processed meats, in concluding that they are a convincing cause of colorectal cancer (CRC). There are a number of possible mechanisms for a link between meat consumption and CRC. These include the promotion of carcinogenesis by high-fat intake, the production of carcinogenic heterocyclic amines (HCAs), and/or polycyclic aromatic hydrocarbons (PAHs) during cooking, the formation of carcinogenic N-nitroso compounds (NOCs) either within meat per se or as a result of endogenous processes, and the promotion of carcinogenesis by hem iron [96]. It is also suggested that the high energy density of meat increases the likelihood of obesity, itself a major risk factor for cancer [95]. For each of the mechanisms implicated in cancer formation, there is an approach to reducing any cancer threat. The selection of meat will affect its fat content. It seems that wild animals are overall lower in fat, with a lower proportion of saturated fatty acids and higher proportion of polyunsaturated fatty acids as compared with farmed animals. In general, the selection of animals according to genotype, and diet they are fed on will, profoundly influence not only the total fat concentration in the meat but also the nature of the fats. Obvious modulations include dietary fat intake, but controlling the intake of other nutrients such as vitamin A will also have an influence.

Meat and meat products vary greatly in their fat content according to the animal species, age of the animal, and part of the carcass used. The fat content and fat composition is also affected by animal feeding, a fact that is exploited for modification of the meat fatty acid composition, with the relatively best results in single-stomached pigs and poultry [97]. Data on the average fat content and fatty acid composition of meats and meat products are published as part of food composition tables throughout the world. The fat content of meat has decreased in recent years, with new breeds coming onto the market, and different trimming processes. Of the nutrients considered to have detrimental effects, saturated fatty acids (SFA) provide around 50% of the fatty acids found in meat, while *trans* fatty acids (TFAs) make up only a small component. The rest is mainly made up of monounsaturated fats, with

small amounts of polyunsaturated fatty acids (PUFA), including small amounts of n-3 PUFA, which are likely to be beneficial in cancer prevention [95].

7.2 Breast Cancer

Breast cancer is the clinical expression of the development of tumor tissue initially within a breast, then within different other sites during the metastatic evolution period. This tumor tissue results from a multistep carcinogenic process that probably extends over several decades. This process consists of an accumulation of genetic alterations acquired during a woman's life span, which leads to the neoplastic transformation of normal epithelial cells. Dietary lipids can modulate mammary carcinogenesis at several stages; some of them may contribute to the prevention of acquired genetic alterations through their antimutagenic activity and therefore act at the very early stages of the initiation process. Lipids can also influence tumor promotion [98]. Fatty acids, the main components of dietary lipids, have several properties that make them potential targets for use in a dietary prevention of breast cancer. For a few years, growing interest has focused on particular fatty acids, geometrical and positional isomers of linoleic acid, i.e., conjugated linoleic acids (CLA). These dietary conjugated dienes present interesting anticarcinogenic properties in animal studies of mammary carcinogenesis; furthermore, these properties are unique in that CLA can act at very low concentration, close to that observed in the diet [99].

Whether CLA may be considered as potential targets for use in a nutritional prevention of breast cancer remains an attractive issue. The amount of CLA needed, the duration of intervention, as well as the proper stage in life for such an intervention are not known at present. In humans, only epidemiological studies have been conducted and results are inconclusive. Only 1 study found a negative association between the intake of CLA and risk of breast cancer in postmenopausal women, while other studies reported no effect of CLA intake on protection against breast cancer [100].

7.3 Skin Cancer

Although not nearly equal in extent to the investigations on mammary cancer inhibition, the effect of CLA on skin carcinogenesis has received a fair amount of examination and was the tumorigenic site of the first study to demonstrate an anticancer effect of CLA [94]. From the many hundreds of chemicals in a partially purified extract from fried ground beef, a substance that exhibited anticarcinogenic activity has been identified [101]. Using the classic two-stage mouse epidermal carcinogenesis model, this substance was identified as CLA. In this experiment, synthetically prepared CLA was topically applied 7 d (20 ng/mouse), 3 d (20 ng/mouse), and 5 min (10 ng/mouse) before dermal treatment with 50 nmol DMBA per mouse ($n = 20$/group). One week later, the female CD-1 mice received twice weekly

topical treatments with 6 μg 12-*O*-tetradecanoyle-13-acetate (TPA) until study termination at 16 weeks post-DMBA. Compared with control mice ($n = 30$), CLA-treated mice had an ~15% lower papilloma incidence and 50% fewer papillomas per mouse.

Some years later, results obtained by other researchers [102] demonstrated that CLA has less biopotency against mouse skin carcinogenesis and perhaps a less steep dose-response curve than for mammary cancer inhibition.

7.4 Prostate Cancer

In contrast to the research on breast cancer cell lines, the investigation of CLA on prostate cell lines is limited. A study was carried out in order to examine the antiproliferative effects of different concentrations of a commercial preparation of conjugated linoleic acids (CLA) mixture of isomers [cis -9, trans -11 CLA (c9,t11 CLA): trans -10, cis -12 CLA (50:50)] and their constituent isomers on PC-3, a human prostatic carcinoma cell line, and to study their effects on gene expression (mRNA and protein levels) of different enzymes and oncoproteins involved in oncogenesis and progression of prostate cancer [103]. The trans -10, cis -12 CLA was the most effective isomer (55% inhibition); this isomer seems to work preferentially through modulation of apoptosis and cell cycle control, while c9,t11 CLA isomer affects arachidonic acid metabolism.

8 CLA and Cardiovascular Function

The intake of fat, particularly of saturated fat, is directly associated with the concentration of cholesterol in the blood, and the cholesterol in the blood is directly associated with the risk of ischemic heart disease [104]. A little more than two-thirds of the cholesterol is transported by low-density lipoprotein (LDL) and one-third by high-density lipoprotein (HDL). High concentrations of total cholesterol in the blood will almost always mean that LDL cholesterol is high. High concentrations of LDL cholesterol and low concentrations of HDL cholesterol increase the risk of arteriosclerosis and ischemic heart disease. Many epidemiological studies have found that a high content of meat in the diet is associated with increased risk of ischemic heart disease. The higher risk of ischemic heart disease has early on been ascribed to the contribution of SFA from the meat. PUFA increase HDL cholesterol; if SFA in the diet are replaced by MUFA or PUFA, a fall in total cholesterol and in LDL cholesterol is seen. SFA with a carbon chain length of 12–16 increase total cholesterol and LDL cholesterol compared to the monounsaturated fatty acid oleic acid (C18:1), while stearic acid (C18:0) has a neutral effect on concentrations of total cholesterol and LDL cholesterol [105].

Some studies [106] investigated on the effects of dietary CLA on atherosclerosis. In a study, rabbits were fed semisynthetic atherogenic diets (14% fat and 1% cholesterol) with or without CLA (0.5 g/day per rabbit) for 22 weeks. CLA feeding

was associated with significant reductions in total cholesterol, LDL-cholesterol, and plasma triacylglycerol concentrations. CLA did not affect HDL-cholesterol concentrations per se; accordingly the decrease in LDL-cholesterol resulted in a significant reduction in the LDL-cholesterol/HDL-cholesterol ratio.

Feeding 1% CLA as part of a semipurified atherogenic diet (0.2% cholesterol) had a significant beneficial impact on both the progression and regression of atherosclerosis [107]. The effects on atherosclerosis were observed, even though plasma lipids were unaffected. All levels of CLA significantly reduced total cholesterol and VLDL plus LDL-cholesterol concentrations, whereas HDL-cholesterol was not affected.

Different studies found that CLA reduces atherosclerosis, is hypocholesterolemic, reduces adipose tissue mass; however, epidemiological studies and controlled trials in humans are inconclusive and the methodology used is often questionable [108]. The studies evaluating CLA and atherogenesis, carried out till today in different animal models, are thus inconclusive in predicting how CLA will behave in man; there is at present no evidence in support of the anti-atherogenic effect of CLA. In no species has a consistent, reproducible, dose-dependent effect of CLA been established. Until such data are published, the debate on the involvement of CLA in cardiovascular function and blood lipids will continue.

9 CLA and Obesity

Meat fat has a relatively high content of stearic acid and, hence, is less cholesterol-increasing compared with fats with a lower ratio between stearic acid and SFA with a lower carbon chain length (e.g., dairy fat). The high fat content in the diets of industrialized populations, in connection with low level of physical activity, is directly related to the high and increasing occurrence of obesity and ischemic heart disease [104]. Prevention and treatment of obesity, therefore, is based on a reduced fat intake by exchanging high-fat meat with lean meat. There is some evidence that lean meat can be advantageous in a slimming diet, primarily since a high protein intake seems to increase satiety. Consequently, meat, especially lean meat, does not increase the risk of ischemic heart disease or obesity.

CLA is marketed in the United States as a dietary supplement. The focus of marketing efforts is directed toward the implications that CLA will reduce fat mass and increase lean body mass in humans. The basis for these clinical impressions arises from animal studies that demonstrate remarkable alterations in body fat and lean body mass [109]. There are numerous theories on the mechanisms of alterations in body composition seen in animals ingesting CLA, but few studies have been carried out to address this question. Table 5 lists some of the potential mechanisms by which CLA may reduce fat mass and/or increase lean body mass. Alteration of membrane structure is an overarching potential mechanism that might affect a number of activities such as enzyme and hormonal response, permeability, membrane fluidity, and receptor number and/or function. CLA has a different spatial configuration than the parent linoleic acid molecule, which could produce an altered

Table 5 Potential mechanisms of action of CLA to alter body composition

1. Alter membrane structure or function
2. Alter eicosanoid, arachidonic, and/or prostaglandin metabolism
3. Alter cytokine production or response
4. Alter peroxisome proliferator–activated receptor (PPAR) activity
5. Alter sympathetic nervous system activity
6. Alter growth hormone or growth factors

Source: [109]

membrane structure. CLA may be metabolized to different compounds in the eicosanoid pathway, thus affecting the amounts of arachidonic acid and prostaglandins.

Although there is evidence in animals of the ability of trans-10,cis-12 CLA to reduce adiposity and increase lean mass, such unequivocal data supporting the efficacy of either of the CLA isomers in humans are not available [110]. Before CLA supplementation is recommended, further research on human effectiveness and safety of the different isomers of CLA is required.

CLA is known to alter cytokines [111] and tumor necrosis factor (TNF)-a and other cytokines may play major roles in fat metabolism and the etiology of at least some types of obesity. Increased amounts of CLA in the diet reduce fat mass and increase lean body mass. This is the picture seen with administration of β-3 adrenergic agonists to animals [112] and is postulated to occur in humans [113]. β-3 agonists increase both the metabolic rate and the proportion of energy derived from fat oxidation. CLA has been shown to increase metabolic rate and specifically to increase fat oxidation. Finally, it is possible that CLA might alter the secretion or action of growth hormone, insulin-like growth factor, or other growth factors. Administration of growth hormone results in an increase in accumulation of lean body mass and a decrease in fat mass due to increased lipolysis [114].

A recent study [115] demonstrated that dietary supplementation with CLA isomers to dairy cows resulted in a higher plasma IGF-I and leptin level, but in the conclusions section it was stathed that further investigations are necessary to define the exact mode of action of CLA as well as to understand its role in the regulation of the somatotropic axis regulating liver IGF-I secretion.

The first study performed in humans to evaluate body composition used 20 subjects who were of normal weight to mildly overweight [109]. This was a randomized, double-blind, placebo-controlled trial of CLA, 1.8 g/day, versus placebo for a 3-month period. Body composition measurements were performed using a near infrared apparatus. The initial body weights and percentage body fat measurements for the CLA and placebo subjects were 70.9 kg versus 71.8 kg and 21.3% versus 22.0%, respectively. After 3 months, the placebo group had a body weight of 72.4 kg and body fat of 22.4%. The CLA group had a nonsignificant decrease in body weight to 70.2 kg, but the percentage of body fat significantly decreased by 4.3–17.0%.

Another study [116] evaluated 23 experienced resistance-trained males who were matched into two groups for body weight and total training volume. In a randomized

study design, the CLA group was given six capsules per day of a 60% pure CLA preparation and the placebo group was given olive oil capsules for a total period of 28 days. Subjects also were tested for strength performance with bench press and leg press exercises at the beginning and end of the 28-day period. There were no significant differences in body mass, percentage of body fat, or lean body mass between placebo and CLA subjects. Also, there were no significant changes in strength performance on the exercise tests. However, the trends for both strength exercises favored an improved performance in the CLA group. The length of time that the CLA preparation was given was short compared with the time it was administered in animals, and the doses of CLA given to the humans were much lower on a per kilogram basis.

In a clinical trial performed with the aim to test CLA for the treatment of obesity [109], 80 obese subjects were randomized to CLA versus placebo in a double-blind fashion over 6 months. All subjects were asked to follow a standardized diet and exercise regimen with a modest reduction in calories and a modest increase in exercise. Subjects took 2.7 g of CLA daily and body composition was assessed by underwater weights. Mean body weight at baseline in the CLA and placebo groups was 97.1 kg and 96.9 kg, respectively. The percentage of body fat was 38.8% and 35.7%, respectively. There were no significant changes in either body weight or body fat after 6 months. Both groups lost ~2.5 kg of weight and ~1 kg of body fat. *Post-hoc* analyses of the data revealed a subpopulation of individuals who gained lean body mass. There were twice as many subjects in the CLA group than in the placebo group who gained lean body mass, but the CLA subjects lost body fat on average, whereas the placebo group gained. The difference in body fat change was statistically significant. There were no major side effects or adverse events noted in either group, with the exception of one person in the CLA group who exhibited significant edema and weight gain for a period of several weeks. This resolved when CLA was discontinued, but the subject restarted CLA without the knowledge of the investigators and had no further problems over the last 3 months of the study.

CLA has been demonstrated to affect a wide variety of enzymes and hormones in the body, multiple mechanisms may come into play. Priority areas for future research include attention to the changes CLA produces in membrane structure and function, the effects on prostaglandin and cytokine function, and the effects on sympathetic nervous system activity. Human studies of CLA did not demonstrate a clear effect of CLA on body composition. Because of the contradictory results, raise concern about the possibility of the deleterious effects of trans-10,cis-12 CLA on the lipid profile, glucose metabolism and insulin sensitivity, it is advisable to consider with caution the use of CLA supplements containing high quantities of trans-10,cis-12 CLA, especially in obese patients with type 2 diabetes or metabolic syndrome, which constitutes a considerable proportion of the whole overweight/obese population [117].

However, uncertainty about the appropriate dose for humans, coupled with the possibility that the effects may be best demonstrated during a weight accumulation phase, suggests that dose-response studies are critical. Studies of the usefulness of CLA in the prevention of weight gain would be of major interest. In addition, studies

in growing children may be considered in the future when questions of dose are answered and more information on safety is available.

10 CLA and Diabetes

Diabetes mellitus type 2 is the most prevalent form of diabetes, comprising 90–95% of the diagnosed cases [118]. Individuals who have insulin resistance and a relative insulin deficiency exhibit the elevated postprandial blood glucose levels that typify this disease. Insulin resistance may be improved with weight reduction, dietary management, and/or pharmacological treatments. There are numerous risk factors for the development of type 2 diabetes including the presence of impaired glucose tolerance, ethnicity, age, gender, and genetics; central to all of these risk factors is obesity.

Based on the fact that CLA can reduce adiposity in experimental animals, a study to elucidate the role of CLA in the development of type 2 diabetes in male rats has been performed [24]. Male rats were fed semipurified diets containing no CLA (control), 1.5% CLA, or the antidiabetic thiazolidinedione drug, troglitazone (0.02%) for 2 weeks. Rats fed the CLA or thiazolidinedione diet exhibited significantly reduced ($p < 0.05$) fasting glucose, insulinemia, triglyceridemia, free fatty acid levels, and leptinemia compared with control rats.

Whereas CLA reduces fasting insulin in diabetic animals, it modestly increases fasting serum insulin in nondiabetic swine [119], mice [120], and humans [121]. Because fasting insulin may be used as a surrogate marker for insulin resistance, these data suggest that CLA reduces insulin sensitivity under a normoglycemic state. In agreement, after long-term feeding (8 months) of a CLA-diet, an induction of insulin resistance was observed in male mice; CLA-induced insulin resistance was associated with lipodystrophy. The impact and significance of CLA for reducing insulin sensitivity and/or altering lipodystrophy for people who are normoglycemic is unknown.

Because CLA was able to delay the onset of diabetes in the rat model, CLA as an aid in the management of type 2 diabetes in humans was examined [24]. A double blind, randomized study to determine the effect of daily supplementation with CLA or placebo (safflower oil) on metabolic parameters of diabetes was conducted. Subjects with type 2 diabetes were provided with supplements with CLA or placebo, instructed to maintain a healthy diet using the Food Guide Pyramid and asked not to change their diet or activity habits for the 8-week intervention period. CLA supplementation (6.0 g CLA/day) significantly decreased fasting blood glucose, plasma leptin, body mass index, and weight. Low density lipoprotein levels significantly increased, but less in the CLA-supplemented group than in the placebo group. In addition, body fat (%) was modestly decreased ($p < 0.08$) in subjects supplemented with CLA. Fasting insulin, triglycerides, cholesterol, and high-density lipoprotein were not significantly affected by CLA. According to 3-day diet records, energy intake was not significantly different between groups at baseline or throughout the study. Supplementation with CLA for 8 weeks could be associated with favorable

alterations of several metabolic parameters of subjects with type 2 diabetes. Further work is needed to determine the therapeutic potential of CLA in the management of type 2 diabetes; basing on the available results, it is possible to say that CLA may represent an important agent for the treatment of type 2 diabetes.

11 Conclusions

It is well known that meat contains a large amount of proteins with high biological value. However, in recent years, the bioactivities of certain compounds that are present in meat, even though in minor amounts, have received increased attention because they may have an important nutritional role. CLA is a group of isomers of octadecadienoic acid that is abundant in the fat of ruminants like cattle and sheep. The content may change depending on the breed, feed, and age. CLA has been reported to reduce the risk for certain types of cancer like the colorectal cancer as well as having other activities like antiartherioesclerotic, antioxidative, and also playing certain role in controlling obesity. It has also been found to favorably modulate immune function in humans. Although the consumption of ruminant products contributes to blood concentrations of CLA in humans, it is largely unknown whether these physiological doses might have biological effects in humans.

Because small amounts of CLA (0.5% of diet) have been shown to alter the expression of genes and impact conditions such as carcinogenesis, obesity, diabetes, and atherosclerosis in experimental animals, it is possible that small amounts consumed over a prolonged period of time may exert similar beneficial effects in human beings. Understanding the role of CLA in modulating events associated with macronutrient metabolism suggests that CLA may be a healthy dietary component with the potential for impacting human health in the areas of cancer, obesity, diabetes, and cardiovascular disease. However, more work is needed to fully elucidate the safety and efficacy of isomers and doses that are required for exerting this breadth of potential beneficial effects. It is hoped that with improved understanding of the doses and isomers required, improvements in recommendations may be made to people regarding the intakes of CLA to improve health.

In this context, the role of dietary CLA in human cancer risk is probably the most interesting one. There are some data showing a favorable effect of CLA on human cancer cells. More extensive evidence from human studies is generally lacking, although there are a few epidemiologic studies that have focused on evaluating the role of dairy products in relation to various cancers, and at least one has found an inverse relationship between milk intake and breast cancer.

Among anticarcinogens, CLA is unique for the following reasons:

- It is an animal-derived product with its highest levels found in ruminant meat and dairy products.
- It exerts a potent action through dietary intervention under a wide range of conditions.
- It possesses multiple modes of action.

The time has come to conduct clinical studies with CLA in order to judge its potential as a chemopreventative agent for humans.

References

1. de Castro Cardoso Pereira PM, dos Reis Baltazar Vicente AF (2013) Meat nutritional composition and nutritive role in the human diet. Meat Sci 93:586–592
2. Wang WJ, Crompton RH (2004) The role of load-carrying in the evolution of modern body proportions. J Anat 204:417–430
3. Domínguez-Rodrigo M, Bunn HT, Mabulla AZP, Baquedano E, Uribelarrea D, Pérez-González A, Gidna A, Yravedra J, Diez-Martin F, Egeland CP, Barba R, Arriaza MC, Organista E, Ansón M (2014) On meat eating and human evolution: A taphonomic analysis of BK4b (Upper Bed II, Olduvai Gorge, Tanzania), and its bearing on hominin megafaunal consumption. Quaternary Intern 322–323:129–152
4. Mann N (2007) Meat in the human diet: an anthropological perspective. Nutr Dietet 64(s4): S102–S107
5. Valsta LM, Tapanainen H, Männistö S (2005) Meat fats in nutrition. Meat Sci 70:525–530
6. Binnie MA, Barlow K, Johnson V, Harrison C (2014) Red meats: time for a paradigm shift in dietary advice. Meat Sci 98:445–451
7. Toldrá F, Reig M (2012) Biochemistry of raw meat and poultry. In: Simpson BK, Nollet LML, Toldrá F, Benjakul S, Paliyath G, Hui YH (eds) Food biochemistry and food processing, 2nd edn. Wiley, New York
8. Toldrá F, Flores M (2004) Analysis of meat quality. In: Nollet LML (ed) Handbook of food analysis. Marcel Dekker, New York
9. K.J.Shingfield KJ, Reynolds CK, Hervás G, Griinari JM, Grandison AS, Beever DE (2006) Examination of the Persistency of Milk Fatty Acid Composition Responses to Fish Oil and Sunflower Oil in the Diet of Dairy Cows. J Dairy Sci 89:714–732
10. Nishimura T (2010) The role of intramuscular connective tissue in meat texture. Anim Sci J 81:21–27
11. Galica S, Oakhilla JS, Steinberga GR (2010) Adipose tissue as an endocrine organ. Molec Cell Endocrin 316:129–139
12. Webb EC, O'Neill HA (2008) The animal fat paradox and meat quality. Meat Sci 80:28–36
13. IUPAC-IUB Commission on Biochemical Nomenclature (1978) The nomenclature of lipids. J Lipid Res 19:114–129
14. Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI, Whittington FM (2008) Fat deposition, fatty acid composition and meat quality: a review. Meat Sci 78:343–358
15. Teye GA, Sheard PR, Whittington FM, Nute GR, Stewart A, Wood JD (2006) Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. Meat Sci 73:157–165
16. De Smet S, Raes K, Demeyer D (2004) Meat fatty acid composition as affected by fatness and genetic factors: a review. Anim Res 53:81–98
17. Smith WL (2007) Nutritionally essential fatty acids and biologically indispensable cyclooxygenases. Trends Biochem Sci 33(1):27–37
18. Kouba M, Mourot J (2011) A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. Biochimie 93:13–17
19. Smith SB, Smith DR, Lunt DK (2004) Adipose tissue. In: Jensen WK, Devine C, Dikeman M (eds) Encyclopedia of meat sciences. Elsevier Academic Press, Oxford, UK
20. Woods VB, Fearon AM (2009) Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A review. Livestock Sci 126:1–20

21. Lauridsen C, Mu H, Henckel P (2005) Influence of dietary conjugated linoleic acid (CLA) and age at slaughtering on performance, slaughter- and meat quality, lipoproteins, and tissue deposition of CLA in barrows. Meat Sci 69:393–399
22. Huuskonen A, Jansson S, Honkavaara M, Tuomisto L, Kauppinen R, Joki-Tokola E (2010) Meat colour, fatty acid profile and carcass characteristics of Hereford bulls finished on grazed pasture or grass silage-based diets with similar concentrate allowance. Livestock Sci 131:125–129
23. Alfaia CPM, Alves SP, Martins SIV, Costa ASH, Fontes CMGA, Lemos JPC, Bessa RJB, Prates JAM (2009) Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. Food Chem 114:939–946
24. Belury MA (2002) Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Ann Rev Nutr 22:505–531
25. Martin D, Muriel E, Gonzalez E, Viguera J, Ruiz J (2008) Effect of dietary conjugated linoleic acid and monounsaturated fatty acids on productive, carcass and meat quality traits of pigs. Livestock Sci 117:155–164
26. Belaunzaran X, Bessa RJB, LavÚn P, Mantecón AR, Kramer JKG, Aldai N (2015) Horse-meat for human consumption – Current research and future opportunities. Meat Sci 108:74–81.
27. Parodi PW (1999) Conjugated linoleic acid: the early years. In: Yurawecz MP, Mossoba MM, Kramer JKG, Pariza MW, Nelson GJ (eds) Advances in conjugated linoleic acid research, vol 1. AOCS Press, Champaign
28. Hartman L, Shorland FB, McDonald IRC (1955) The *trans*-unsaturated acid contents of fats of ruminants and non-ruminants. Biochem J 61:603–607
29. Hansen RP, Czochanska Z (1976) Fatty acid composition of the subcutaneous and perinephric fats of lambs grazed on pastures in New Zealand. N Z J Sci 19:413–419
30. McGuire M, McGuire MA, Ritzenthalera K, Shultz TD (1999) Dietary sources and intakes of conjugated linoleic acid intake in humans. In: Yurawecz MP, Mossoba MM, Kramer JKG, Pariza MW, Nelson GJ (eds) Advances in conjugated linoleic acid research, vol 1. AOCS Press, Champaign
31. Kramer JKG, Parodi PW, Jensen RG, Mossoba MM, Yurawecz MP, Adlof RO (1998) Rumenic acid: a proposed common name for the major conjugated linoleic acid isomer found in natural products. Lipids 33:835
32. Olmedilla-Alonso B, Jiménez-Colmenero F, Sánchez-Muniz FJ (2013) Development and assessment of healthy properties of meat and meat products designed as functional foods. Meat Sci 95:919–930
33. Crumb DJ (2011) Conjugated linoleic acid – an overview. Int J Appl Res Nat Prod 4:12–18
34. Palmquist DL, Lock AL, Shingfield KJ, Bauman DE (2005) Biosynthesis of Conjugated Linoleic Acid in Ruminants and Humans. Adv Food Nutr Res 50:179–217
35. Khanal RC, Olson KC (2004) Factors affecting conjugated linoleic acid (CLA) content in milk, meat, and egg: a review. Pakistan J Nutr 3:82–98
36. Schmid A, Collomb M, Sieber R, Bee G (2006) Conjugated linoleic acid in meat and meat products: A review. Meat Sci 73:29–41
37. Reiser R (1951) Hydrogenation of polyunsaturated fatty acids by the ruminant. Fed Proc 10:236
38. Shorland FB, Weenink RO, Johns AT (1955) Effect of the rumen on dietary fat. Nature 175:1129–1130
39. Shorland FB, Weenink RO, Johns AT, McDonald IRC (1957) The effect of shee-rumen contents on unsaturated fatty acids. Biochem J 67:328–333
40. Kepler CR, Tove SB (1967) Biohydrogenation of unsaturated fatty acids. J Biol Chem 242:5686–5692
41. Khanal RC, Dhiman TR (2004) Biosynthesis of conjugated linoleic acid: a review. Pakistan J Nutr 3:72–81
42. De Beni Arrigoni M, Martins CL, Factori MA (2016) Lipid metabolism in the rumen. In: Millen DD (ed) Rumenology. Springer International Publishing, Switzerland

43. Lourenco M, Ramos-Morales E, Wallace RJ (2010) The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. Animal 4:1008–1023
44. Buccioni A, Decandia M, Minieri S, Molle G, Cabiddu A (2012) Lipid metabolism in the rumen: New insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. Anim Feed Sci Technol 174:1–25
45. Park Y (2009) Conjugated linoleic acid (CLA): Good or bad trans fat? J Food Comp Anal 225: S4–S12
46. Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J, Doreau M (2007) Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur J Lipid Sci Technol 109:828–855
47. Liavonchanka A, Feussner I (2008) Biochemistry of PUFA double bond isomerases producing Conjugated Linoleic Acid. ChemBioChem 9:1867–1872
48. Kim YJ, Liu RH, Bond DR, Russell JB (2000) Effect of linoleic acid concentration on conjugated linoleic acid by Butyrivibrio fibrisolvens A38. Appl Environ Microbiol 66:5226–5230
49. Troegeler-Meynadir A, Nicot MC, Bayourthe C, Moncoulon R, Enjalbert F (2003) Effects of pH and concentrations of linoleic acids on extent and intermediates of ruminal biohydrogenation in vitro. J Dairy Sci 86:4054–4063
50. Shingfield K, Ahvenjȍrvi S, Toivonen V, árlð A, Nurmela K, Huhtanen P, Griinari J (2003). Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Animal Sci 77:165–179.
51. Banni S, Carta C, Contini MS, Angioni E, Deiana M, Dessi MA, Melis MP, Corongiu FP (1996) Characterization of conjugated diene fatty acids in milk, dairy products and lamb tissues. J Nutr Biochem 7:150–155
52. Secchiari P, Antongiovanni M, Mele M, Serra A, Buccioni A, Ferruzzi G, Paoletti F, Petacchi F (2003) Effect of kind of dietary fat on the quality of milk fat from Italian Friesian cows. Livest Prod Sci 83:43–52
53. Garcia C, Duby C, Catheline D, Toral PG, Bernard L, Legrand P, Rioux V (2017) Synthesis of the suspected trans-11,cis-13 conjugated linoleic acid isomer in ruminant mammary tissue by FADS3-catalyzed Δ13-desaturation of vaccenic acid. J Dairy Sci 100:783–796
54. Lahlou M, Kanneganti R, Massingill L, Broderick G, Park Y, Pariza M, Ferguson JD, Wu Z (2014) Grazing increases the concentration of CLA in dairy cow milk. Animal, 8:1191–1200
55. Lock AL, Garnsworthy PC (2002) Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk. Anim Sci 74:163–176
56. Kay JK, Mackle TR, Auldist MJ, Thompson NA, Bauman DE (2002) Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in pasture-fed dairy cows. J Dairy Sci 85(suppl 1):176
57. Khanal RC, Dhiman TR, McMahon DJ, Boman RL (2002) Influence of diet on conjugated linoleic acid content of milk, cheese and blood serum. J Dairy Sci 85(Suppl. 1):142
58. Bolte MR, Hess BW, Means WJ, Moss GE, Rule DC (2002) Feeding lambs high-oleate or high linoleate safflower seeds differentially influences carcass fatty acid composition. J Anim Sci 80:609–616
59. Chilliard Y, Ferlay A, Rouel J, Lamberet G (2003) A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J Dairy Sci 86:1751–1770
60. Fritsche J, Rickert R, Steinhart H, Yurawecz MP, Mossoba MM, Sehat N, Roach JAG, Kramer JKG, Ku Y (1999) Conjugated linoleic acid (CLA) isomers: formation, analysis, amounts in foods, and dietary intake. Fett-Lipid 101:272–276
61. De Marchi FE, Santos GT, Petit HV, Benchaar C (2017) Oxidative status of dairy cows fed flax meal and infused with sunflower oil in the abomasum. Anim Feed Sci Technol 228:115–122
62. Lerch S, Shingfield KJ, Ferlay A,Vanhatalo A, Chilliard Y (2012) Rapeseed or linseed in grass-based diets: Effects on conjugated linoleic and conjugated linolenic acid isomers in milk fat from Holstein cows over 2 consecutive lactations. J Dairy Sci 95:7269–7287
63. Tsai CY, Rezamand P, Loucks WI, Scholte CM, Doumit ME (2017) The effect of dietary fat on fatty acid composition, gene expression and vitamin status in pre-ruminant calves. Anim Feed Sci Technol 229:32–42

64. Buccioni A, Rapaccini S, Antongiovanni M, Minieri S, Conte G, Mele M (2010) Conjugated linoleic acid and C18:1 isomers content in milk fat of sheep and their transfer to Pecorino Toscano cheese. Int Dairy J 20:190–194
65. Rule DC, Broughton KS, Shellito SM, Maiorano G (2002) Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. J Anim Sci 80:1202–1211
66. Engelke CF, Siebert BD, Gregg K, Wright ADG, Vercoe PE (2004) Kangaroo adipose tissue has higher concentrations of cis 9, trans 11-conjugated linoleic acid than lamb adipose tissue. J Anim Feed Sci 13:689–692
67. Poulson CS, Dhiman TR, Ure AL, Cornforth D, Olson KC (2004) Conjugated linoleic acid content of beef from cattle fed diets containing high grain, CLA, or raised on forages. Livest Prod Sci 91:117–128
68. Badiani A, Montellato L, Bochicchio D, Anfossi P, Zanardi E, Maranesi M (2004) Selected nutrient contents, fatty acid composition, including conjugated linoleic acid, and retention values in separable lean from lamb rib loins as affected by external fat and cooking method. J Agric Food Chem 52:5187–5194
69. Dannenberger D, Nuernberg K, Nuernberg G, Scollan N, Steinhart H, Ender K (2005) Effect of pasture vs. concentrate diet on CLA isomer distribution in different tissue lipids of beef cattle. Lipids 40:589–598
70. Zhang W, Xiao S, Samaraweera H, Lee EJ, Ahn DU (2010) Improving functional value of meat products. Meat Sci 86:15–31
71. Serra A, Mele M, La Comba F, Conte G, Buccioni A, Secchiari P (2009) Conjugated linoleic acid (CLA) content of meat from three muscles of Massese suckling lambs slaughtered at different weights. Meat Sci 81:396–404
72. Mulvihill B (2001) Ruminant meat as a source of conjugated linoleic acid (CLA). Nutr Bull 26:295–299
73. Dugan MER, Aalhus JL, Kramer JKG (2004) Conjugated linoleic acid pork research. Amer J Clin Nutr 79:1212–1216
74. Gatlin LA, See MT, Larick DK, Lin X, Odle J (2002) Conjugated linoleic acid in combination with supplemental dietary fat alters pork fat quality. J Nutr 132:3105–3112
75. Corino C, Pastorelli G, Douard V, Rossi R, Musella M, Mourot J (2006) L'acide linoléique conjugué en nutrition porcine. INRA Prod Anim 19:39–46
76. Raes K, De Smet S, Demeyer D (2004) Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. Anim Feed Sci Technol 113:199–221
77. Corino C, Musella M, Pastorelli G, Rossi R, Paolone K, Costanza L, Manchisi A, Maiorano G (2008) Influences of dietary conjugated linoleic acid (CLA) and total lysine content on growth, carcass characteristics and meat quality of heavy pigs. Meat Sci 79:307–316
78. Corino C, Filetti F, Gambacorta M, Manchisi A, Magni S, Pastorelli G, Rossi R, Maiorano G (2003) Influence of dietary conjugated linoleic acids (CLA) and age at slaughtering on meat quality and intramuscular collagen in rabbits. Meat Sci 66:97–103
79. Corino C, Lo Fiego DP, Macchioni P, Pastorelli G, Di Giancamillo A, Domeneghini C, Rossi R (2007) Influence of dietary conjugated linoleic acids and vitamin E on meat quality, and adipose tissue in rabbits. Meat Sci 76:19–28
80. Juárez M, Polvillo O, Gómez MD, Alcalde MJ, Romero F, Valera M (2009) Breed effect on carcass and meat quality of foals slaughtered at 24 months of age. Meat Sci 83:224–228
81. Lorenzo JM, Sarriés MV, Tateo A, Polidori P, Franco D, Lanza M (2014) Carcass characteristics, meat quality and nutritional value of horsemeat: a review. Meat Sci 96:1478–1488
82. Polidori P, Pucciarelli S, Ariani A, Polzonetti V, Vincenzetti S (2015) A comparison of the carcass and meat quality of Martina Franca donkey foals aged 8 or 12 months. Meat Sci 206:6–10
83. Sirri F, Tallarico N, Meluzzi A, Franchini A (2003) Fatty acid composition and productive traits of broiler fed diets containing conjugated linoleic acid. Poult Sci 82:1356–1361

84. Grashorn MA (2005) Enrichment of eggs and poultry meat with biologically active substances by feed modifications and effects on the final quality of the product. Pol J Food Nutr Sci 14:15–20
85. Du M, Ahn DU (2002) Effect of dietary conjugated linoleic acid on the growth rate of live birds and on the abdominal fat content and quality of broiler meat. Poultry Sci 81:428–433
86. Hur SJ, Kim HS, Bahk YY, Park Y (2017) Overview of conjugated linoleic acid formation and accumulation in animal products. Livest Sci 195:105–111
87. Qi X, Xu S, Zhan H, Yue H, Xu S, Ji F, Qi G (2011) Effects of dietary conjugated linoleic acids on lipid metabolism and antioxidant capacity in laying hens. Arch Anim Nutr 65:345–365
88. Kris-Etherton PM, Harris WS, Appel LJ (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106:2747–2757
89. Valente LMP, Bandarra NM, Figueiredo-Silva AC, Rema P, Vaz-Pires P, Martins S, Prates JAM, Nunes ML (2007) Conjugated linoleic acid in diets for large-size rainbow trout (*Oncorhynchus mykiss*): effects on growth, chemical composition and sensory attributes. Br J Nutr 97:289–297
90. Dhiman TR, Nam SH, Ure AL (2009) Factors affecting conjugated linoleic acid content in milk and meat. Crit Rev Food Sci Nutr 45:463–482
91. Siurana A, Calsamiglia S (2016) A metaanalysis of feeding strategies to increase the content of conjugated linoleic acid (CLA) in dairy cattle milk and the impact on daily human consumption. Anim Feed Sci Technol 217:13–26
92. Abd El-Salam MH, Hippen AR, Assem FM, El-Shafel K, Tawfik NF, El-Aassar M (2011) Preparation and properties of probiotic cheese high in conjugated linoleicacid content. Int J Dairy Technol 64:64–74
93. Domagala J, Sady M, Grega T, Pustkowiak H, Florkiewicz A, 2010. The influence of cheese type and fat extraction method on the content of conjugated linoleic acid. J Food Compos Anal 23:238–243
94. Kim JH, Kim Y, Kim YJ, Park Y (2016). Conjugated linoleic acid-potential health benefits as a functional food ingredient. Ann Rev Food Sci Tech 7:221–244
95. Ferguson LR (2010) Meat and cancer. Meat Sci 84:308–313
96. Santarelli RL, Pierre F, Corpet DE (2008) Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence. Nutr Cancer 60:131–144
97. Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, Sheard PR, Enser M (2004) Effects of fatty acids on meat quality: a review. Meat Sci 66:21–32
98. Wyness L, Weichselbaum E, O'Connor A, Williams EB, Benelam B, Riley H, Stanner S (2011) Red meat in the diet: an update. Brit Nutr Found Nutr Bull 36:34–77
99. Hubbard NE, Lim D, Erickson KL (2003) Effect of separate conjugated linoleic acid isomers on murine mammary tumorigenesis. Cancer Lett 190:13–19
100. Aro A, Mannisto S, Salminen I, Ovaskainen ML, Kataja V, Uusitupa M (2000) Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. Nutr Cancer 38:151–157
101. Ha YL, Grimm NK, Pariza MW (1989) Newly recognized anticarcinogenic fatty acids: identification and quantification in natural and processed cheeses. J Agric Food Chem 37:75–81
102. Ip C, Chin SF, Scimeca JA, Pariza MW (1991) Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res 51:6118–6124
103. Ochoa JJ, Farquharson AJ, Grant I, Moffat LE, Heys SD, Wahle KWJ (2004) Conjugated linoleic acids (CLAs) decrease prostate cancer cell proliferation: different molecular mechanisms for cis -9, trans -11 and trans -10, cis -12 isomers. Carcinogesis 25:1185–1191
104. Ovesen L (2004) Cardiovascular and obesity health concern. In: Jensen WK, Devine C, Dikeman M (eds) Encyclopedia of meat sciences. Elsevier Academic Press, Oxford
105. Arbonés-Mainar JM, Navarro MA, Guzman M., Arnal C, Surra JC, Acin S, Carnicer R, Osada J, Roche HM (2006) Selective effect of conjugatedlinoleic acid isomers on atherosclerotic lesion development in apolipoprotein E knockout mice. Atherosclerosis 189:318–327

106. Khosla P, Fungwe TV (2001) Conjugated linoleic acid: effects on plasma lipids and cardiovascular function. Curr Opin Lipidol 12:31–34
107. Kritchevsky D (2000) Antimutagenic and some other effects of conjugated linoleic acid. Br J Nutr 83:459–465
108. Moloney F, Yeow TP, Mullen A, Nolan J., Roche HM (2004) Conjugated linoleic acid supplementation, insulin sensitivity, and lipoprotein metabolism in patients with type 2 diabetes mellitus. Am J Clin Nutr 80:887–895
109. Atkinson RL (1999) Conjugated Linoleic Acid for altering body composition and treating obesity. In: Yurawecz MP, Mossoba MM, Kramer JKG, Pariza MW, Nelson GJ (eds) Advances in conjugated linoleic acid research, vol 1. AOCS Press, Champaign
110. Silveira MB, Carraro R, Monereo S, Tébar J (2007) Conjugated linoleic acid (CLA) and obesity. Public Health Nutr 10(10A):1181–1186
111. Rainer L, Heiss C (2004) Conjugated Linoleic Acid: health implications and effects on body composition. J Amer Diet Assoc 2004; 104:936–938
112. Lee HY, Park JH, Seok SH, Baek MW, Kim DJ, Lee KE, Paek KS, Lee Y, Park JH (2006) Human originated bacteria, Lactobacillus rhamnosus PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. BBA-Mol Cell Biol L 1761:736–744
113. House RL, Cassady JP, Eisen EJ, McIntosh MK, Odle J.(2005) Conjugated linoleic acid evokes delipidation through the regulation of genes controlling lipid metabolism in adipose and liver tissue. Obesity Reviews 6:247–258
114. Muniyappa R, Sullivan SD, Tella SH, Abel BS, Harman SM, Blackman MR (2017) Effects of growth hormone administration on luteinizing hormone secretion in healthy older men and women. Physiol Reports 5:e13516
115. Csillik Z, Faigl V, Keresztes M, Galamb E, Hammon HM, Tröscher A, Fébel H, Kulcsár M, Husvéth F, Huszenicza G, Butler, WR (2017) Effect of pre- and postpartum supplementation with lipid-encapsulated conjugated linoleic acid on reproductive performance and the growth hormone–insulin-like growth factor-I axis in multiparous high-producing dairy cows. J Dairy Sci 100:5888–5898
116. Kreider R, Ferreira M, Greenwood M, Almada A (2002) Effects of conjugated linoleic acid (CLA) supplementation during resistance training on body composition, bone density, strength and selected hematological markers. J Strength Conditioning Res 16:325–334
117. Silveira MB, Carraro R, Monerero S, Tébar J (2007) Conjugated linoleic acid (CLA) and obesity. Public Health Nutr 10(10A):1181–1186
118. Moloney F, Toomey S, Noone E, Nugent A, Allan B, Loscher C., Roche, HM (2007) Antidiabetic effects of cis-9, trans-11-conjugated linoleic acid maybe mediated via anti-inflammatory effects in white adipose tissue. Diabetes 56:574–582
119. Marcolla CS, Holanda DM, Ferreira SV, Rocha GC, Serão NVL, Duarte MS, Abreu MLT, Saraiva A (2017) Chromium, CLA, and ractopamine for finishing pigs. J Anim Sci https://doi.org/10.2527/jas.2017.1753
120. Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim H-J, Tange T, Okuyama H, Kasai M, Ikemoto S, Ezaki O (2000) Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes 49:1534–1542
121. Medina EA, Horn WF, Keim NL, Havel PJ, Benito P, Kelley DS, Nelson GJ, Erickson KL (2000) Conjugated linoleic acid supplementation in humans: effects on circulating leptin concentrations and appetite. Lipids 35:783–788

Part IV

Food Carbohydrates and Derived Compounds

Total Dietary Fiber Intake, Whole Grain Consumption, and Their Biological Effects

24

Semih Otles and Emine Nakilcioglu-Tas

Contents

Abstract

Unlike refined grains, whole grains, which consist of entire grain, contain high micronutrients and dietary fiber in their bran and seed. In the literature many studies showed that high-fiber diets may reduce the incidence of chronic diseases such as diverticulitis, diabetes, obesity, heart diseases, and some cancer types. Once upon a time, whole grains were neglected by researchers. Following the determination that dietary fibers are present in the whole grains together with micronutrients and phytochemicals, the focus of the studies has shifted towards observational studies related to whole grains intake. Cereal fibers have proven to have stronger health effects as a result of synergistic effects with phytochemicals and micronutrients in whole grains. This chapter describes the dietary fiber-related health effects of whole grain consumption after mentioning characteristics of dietary fiber and whole grains.

S. Otles (✉) · E. Nakilcioglu-Tas
Department of Food Engineering, Faculty of Engineering, Ege University, Bornova, Izmir, Turkey
e-mail: semih.otles@ege.edu.tr; semih.otles@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_7

Keywords
Cardiovascular diseases · Cereal fibers · Diabetes · Dietary fiber · Whole grains

Abbreviations

AACCI	American Association of Cereal Chemists International
BMI	Body mass index
CAD	Coronary artery disease
CCNFSDU	Codex Committee on Nutrition and Foods for Special Dietary Uses
CVD	Cardiovascular disease
DRI	Dietary Reference Intakes
FAO/WHO	Food and Agriculture Organization/World Health Organization
FDA	Food and Drug Administration
FOS	Fructooligosaccharide
GM	Galactomannan
HDL-C	High-density lipoprotein cholesterol
IDF	Insoluble dietary fiber
LDL-C	Low-density lipoprotein cholesterol
MCAD	Minimum coronary artery diameter
NTD	Neural tube defects
OD	Odds ratio
RDA	Recommended Dietary Allowance
RR	The effect size across studies
SDF	Soluble dietary fiber
US	United States

1 Introduction

Nutrition makes a significant contribution to the etiology, prevention, and progression of disease [1–4]. Diets such as the Mediterranean-type dietary patterns, which have high in fruits, vegetables, whole grains, and proteins from plant sources and lower in meat and meat products have been shown to have long-term beneficial effects on health [4–7]. It is not just happenstance that longevity is also characterized by similar dietary patterns, even though the influence of other lifestyle factors cannot be ignored [4, 8]. High levels of dietary fiber, unsaturated fatty acids, antioxidants, and foods that have low energy density are some of the protective components of the diet [4, 9]. The all bioactive components of the diet work together for promoting health.

One of the most important constituents in foods that have beneficial physiological effects in humans is dietary fiber. The consumption of dietary fiber has decreased considerably as a result of the fact that the foods have begun to be processed and refined more in the last two centuries [10, 11]. It has been determined that increased consumption of low-fiber foods in the diet as a result of the transition from high-fiber diets to low-fiber diets in human nutrition may lead to an increase in the prevalence

of chronic diseases such as diverticulitis, diabetes, obesity, heart diseases, and some cancer types [11, 12]. After this hypothesis, there has been an increase in interest and researches related with dietary fiber and foods which are thought to be important sources of dietary fiber [11].

Whole grains are rich sources of vitamins, minerals, and phytochemicals as well as fiber. The phytochemicals of whole grains contain phenolics, carotenoids, vitamin E, lignans, β-glucan, inulin, resistant starch, sterols, phytates, etc. Plant-based foods like fruits, vegetables, and whole grains, which include significant amounts of bioactive compounds, can also meet desirable health benefits beyond basic nutrition to decrease the risk of some diseases [13–15]. Epidemiological studies ensure evidence that whole foods, including whole grains, are protective against a broad range of diseases, and this protectiveness is usually greater than that seen with any individual ingredient [16, 17]. Dietary guidance suggests consumption of whole grains to decrease the risk of chronic diseases such as cancer and cardiovascular disease (CVD) [18]. Epidemiologic studies declare the opinions that whole grains are protective against diabetes, obesity, CVD, and cancers, especially gastrointestinal cancers including gastric and colonic [16, 18]. According to the results of research among a large group of women aged 55 to 69 years, eating at least one serving a day of whole grain foods significantly decreased the risk of mortality from all causes compared with women who ate almost no whole grain products [19, 20].

The protective effect of dietary fiber taken from whole grain foods on diseases is much greater than the individual effect of dietary fiber. It is thought that this is caused by the synergistic effect of phytochemicals with dietary fiber in whole grain foods. Therefore, it is more beneficial that dietary fiber intake by consuming complex food sources such as whole grains for human health.

In this chapter, we describe the definitions and intakes of dietary fiber and whole grains and health effects of dietary fiber intake by consuming whole grains.

2 Dietary Fiber

2.1 Definitions of Dietary Fiber

The definition of dietary fiber was used in 1953 by Hipsley to show the nondigestible constituents which consist of the plant cell wall [21–23]. Since then, the term has evolved and the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) established an internationally has approved the legal definition of dietary fiber in 2009 [23–25]. According to CCNFSDU, dietary fiber is defined as carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following three categories:

(1) Edible carbohydrate polymers that are naturally present in the food as it is consumed.

(2) Carbohydrate polymers that have been obtained physically, enzymatically, or chemically from food raw material and that have been shown to have beneficial physiological effects on health as proved by scientific evidence are accepted by competent authorities.
(3) Synthetic carbohydrate polymers that have a physiological effect on the health benefit as indicated by generally accepted scientific evidence by the competent authorities [24].

While the expression "dietary fiber" describes the nondigestible carbohydrates and lignin that are intrinsic and intact in plants, the term "functional fiber" expresses the isolated nondigestible carbohydrates that have beneficial physiological effects in human body [26]. When inulin is formed naturally in consumable plants such as onions and chicory, it is defined as a dietary fiber; if it is synthetically produced and added to yogurt, it is categorized as a functional fiber [21–23]. Total fiber is also the sum of dietary fiber and functional fiber, and dietary fiber on food labels includes both dietary and functional fiber [23]. The expression "nondigestible" means both not digested and not absorbed in the human small intestine [26].

2.2 Classification, Sources, and Physiological Effects of Dietary Fiber

According to the solubility of dietary fiber, it is classified into two categories: insoluble dietary fiber (IDF) such as cellulose, part of hemicelluloses, resistant starch, and lignin; and soluble dietary fiber (SDF) such as oligosaccharides, including fructooligosaccharide (FOS), pectins, β-glucans, galactomannan (GM) gums, alginate, and psyllium [23, 24, 27, 28].

2.2.1 Soluble Dietary Fiber

SDF is present in the form of a sticky or viscous, rather than a hard tissue in the food [11, 29]. Although SDF are found in fruits and vegetables, the best sources of SDF are oats and dried beans [30]. FOS, also known as oligofructose and inulin, are collectively entitled as fructans [24, 27]. They are found in plants such as agave, artichokes, asparagus, leeks, garlic, onions, yacon, jicama, and wheat.

Pectin is found in most primary cell walls and is particularly rich in the nonwoody parts of terrestrial plants. It is a linear polysaccharide mainly consisted of about 300 to 1000 D – galacturonic acid monosaccharide units [24, 31]. Although fruits are the major source, pectins represent 15–20% of the fiber in vegetables, legumes, and nuts [24, 32].

The β-glucans are polysaccharides of d-glucose monomers linked by β-glycosidic bonds, which occur most commonly as cellulose in plants, the bran of cereal grains, the cell wall of baker's yeast, certain fungi, mushrooms, and bacteria [24, 31].

GMs are also polysaccharides comprising of a mannose backbone with galactose side groups. They are commonly used in foods as stabilizers owing to their high water binding capacity and their emulsification and viscosity increasing properties.

GM gums, such as fenugreek gum (mannose:galactose; 1:1), guar gum (mannose: galactose; 2:1), tara gum (mannose:galactose; 3:1), and locust bean gum (mannose: galactose; 4:1), change according to their ratios of mannose and galactose [24, 33].

Alginates are unbranched polysaccharides consisting of 1–4 linked β-d-mannuronic acid and α-guluronic acid. Alginate is distributed widely in the cell walls of algae. It is also an exopolysaccharide of bacteria including *Pseudomonas aeruginosa* and commercially available alginates now come only from algae. It is binding with water and it forms which use as thickening agent. These viscous hydrogels are also useful for numerous biomedical applications [24, 34].

Psyllium is the common name used for several members of the plant genus Plantago. The seeds of this plant are used commercially for the production of mucilage. The phrase of psyllium is used for the seed husk, the seed, and the entire plant. The seed husk of Psyllium is a rich source of SDF that is known as psyllium hydrophilic mucilloid, psyllium hydrocolloid, and psyllium seed gum [24, 31].

Some SDF components including oat bran, pectin, and guar gum stimulate fecal excretion of bile acids. On the other hand, wheat bran has no such effect; it promotes a different composition of bile acids than does pectin [35–38]. Some SDFs also reduce serum cholesterol level [38, 39]. Although short-term studies (a single meal or a few days) show that SDFs enhanced glucose tolerance and increased insulin sensitivity in healthy subjects, the results of long-term studies do not support them [38, 40]. Dietary fiber influences colonic function and activities of the microflora. High fiber intakes increase bacterial mass but do not change the microflora composition [38, 41–43]. Colonic bacteria attack fermentable fiber components and then at least a portion of them degrades to short-chain fatty acids and gases [38]. These short-chain fatty acids decrease the colon cancer risks by suppressing toxigenic bacterial reactions [44, 45]. Some studies also express that SDFs prevent type II diabetes owing to the viscosity of the fibers [24, 46].

2.2.2 Insoluble Dietary Fiber

IDFs include cell wall components like cellulose, lignin, and hemicellulose that exist mainly in wheat, most grain products, and vegetables [30]. Cellulose is the best known, most widely distributed, and only truly fibrous component of the plant cell wall. It forms about 25% of the fiber in grains and fruit and about a third of the fiber in vegetables and nuts [24, 32]. It is a polymer that consists of glucose and the β-glucoside linkages. The β-linkages in cellulose are not hydrolyzed by the human intestinal enzymes, so cellulose is accepted as a dietary fiber. Cellulose also has the property of absorbing water (0.4 g water per gram of cellulose), and this explains its ability to increase fecal weight when added to the diet [47].

Hemicelluloses are polysaccharides containing sugars other than glucose, which are associated with cellulose in cell walls. They present in both water soluble and insoluble forms in cell walls. Hemicellulose constitutes about a third of the fiber in vegetables, fruits, legumes, and nuts. The primary dietary sources of hemicellulose are cereal grains [24, 32].

Lignin is a lipophilic phenolic polymer that can absorb bile acids [48]. It is composed of phenylpropane units which are thought to be chemically bound to

the carbohydrates in woody structure [49]. It presents only in small amounts in the diet [26]. Good sources of lignin are foods with a woody component such as celery and the outer layers of cereal grains [24].

Resistant starch (the sum of starch and starch-degradation products that do not digest in the small intestine) performs a function as dietary fiber, after it reaches the large intestine [26, 50]. Legumes are main sources of resistant starch and 35% of legumes starch is escaping digestion [26, 51]. Small amounts of resistant starch are formed by processing and baking of cereal and grain products. Actually, many new functional fibers are resistant starches which are added to processed foods [26].

The function of IDFs is to shorten bowel transit time, to increase fecal bulk, and to render feces softer [30]. The reasons for these are their high water-holding capacities and having insoluble characteristics [48]. Addition of some IDF sources in the diet may also cause a decrease in mineral retention [52].

Although about 75% of the dietary fiber in foods is found as the insoluble fraction, products known as soluble or insoluble fiber sources are often sources in both of these fiber types [30]. The energy value of dietary fiber has detected as 0 kcal g-1 if it is insoluble and 4 kcal g-1 if it is soluble [53].

2.3 Recommendations for Dietary Fiber Intake

Consuming foods with dietary fiber is very important for enhancing bowel function and reducing symptoms of chronic constipation, diverticular disease, and hemorrhoids. There are increasing proofs that a diet rich in these foods can reduce not only high blood cholesterol risk but also hypertension, stroke, and cancer risks [54]. On the other hand, people who consume low dietary fiber and complex carbohydrates and high fat, especially saturated fat, in diets incline to have more heart disease, obesity, and some cancers [30].

General recommendations for dietary fiber intake range between 25 and 35 g per day (g d-1) for adults. The data found in guidelines for dietary fiber consumption of children are the extrapolated data from studies in adults. According to the Dietary Reference Intakes (DRI), the average intake of people of all aged should be 14 g per 1000 kcal; for children 1 to 3 years of age, average intake should be 19 g d-1; and for children 4 to 8 years of age, average intake should be 25 g d-1. The American Academy of Pediatrics suggests dietary fiber intake of 0.5 g per kg body weight for children older than 2 years. In 1995, American Health Foundation developed the rule of "age plus five" which indicates that intake of dietary fiber should be greater than or equal to the age plus 5 g d-1 for children over 2 years [22, 23, 55]. Despite the recommendations for dietary fiber intake, the average dietary fiber intake among people is lower than it should be. It changes from 9.4 ± 4.0 g d-1 to 10.7 ± 3.6 g d-1 in females, and 10.7 ± 4.2 g d-1 to 12.0 ± 4.5 g d-1 in males [23].

Even though many benefits of high fiber diets are also naturally found in the nondietary fiber components such as natural antioxidants, it is best to take dietary fiber from foods in high amounts. But dietary fiber consumption with food is almost

half the recommended level, as can be seen. For this reason, it is thought that the consumption of fiber supplements may be beneficial when used moderately [30].

3 Whole Grains

3.1 Definition and Intake of Whole Grains

According to the guidelines of Food and Drug Administration (FDA), "whole grain" contains "cereal grains that consist of the intact and unrefined, ground, cracked or flaked fruit of the grains whose principal components – the starchy endosperm, germ and bran – present in the same relative proportions as they exist in the intact grain" [56]. The more common whole grain cereals are whole wheat, whole oats/oatmeal, whole grain cornmeal, popcorn, brown rice, whole rye, whole-grain barley, wild rice, triticale, millet, and sorghum, while emmer, farro, spelt, and wheat berries are less common whole grain cereals consumed worldwide [57].

Although American Association of Cereal Chemists International (AACCI) had identified "whole grains" in a similar way with FDA, in 2006 the AACCI expanded this definition to include pseudocereals. Pseudocereals such as amaranth (*Amarantus caudatus*), quinoa (*Chenopodium quinoa* Willd.), and buckwheat (*Fagopyrum* spp.) were included in the definition of "whole grains" because of their macronutrient composition and consumption trends similar to that of cereals. Also, some traditionally and minimally processed forms of whole grains including lightly pearled barley or wheat, bulgur (cracked wheat), and nixtamalized corn are considered whole grains [58, 59].

In April 2013, the AACCI further described a whole-grain product as being one which must include 8 g or more of whole grain per 30 g of product [4, 59]. The FDA acknowledged the whole grain definition created by AACCI but stated that at least 51% of the total weight of the product must be whole grains in order to qualify for whole grain health claims [4, 60]. Also, whole grain food must contain 1–7 g dietary fiber [61].

Whole grains can be consumed in whole, cracked, split, flaked, or ground. They are often milled into flour that is used to make breads, cereals, pasta, crackers, and other grain-based foods. Regardless of how the whole grain is processed, the proportions of bran, germ, and endosperm in a whole grain product must be approximately the same as an original grain [57]. A whole grain can be a complete food such as brown rice or can be also a food ingredient such as whole wheat flour in bread or cereal.

The 2005 Dietary Guidelines for Americans and Healthy People 2010 advises the consumption of at least three servings of whole grain (each equivalent to three ounces) per day [15]. Additionally, dietary guidelines expresses that the recommended grains should come from enriched or whole grain products. Generally, at least half the consumed grains should come from whole grains. Moreover, their consumption is low in the United States (US), mostly owing to taste, cost, and poor eating habits [56]. It is noteworthy that grains intake in Europe and North America is

even lower than in less developed countries, although grains ensure approximately two thirds of the calorie and protein intake in the world [62, 63]. Ninety percent of Americans do not meet the recommendation related with whole grain consumption, and the mean intake of whole grains in the United States is less than one serving per day [15, 64]. Even in Denmark, which has one of the highest whole grain intake in the world, only about 6% of people consume seven or more servings of whole grains [65].

3.2 Biochemical Constituents of Whole Grains and Their Roles in a Healthful Diet

Whole grains are composed of three main parts as starchy endosperm, germ, and bran. In order for a grain to be able to be characterized as a whole grain, it must contain all three parts mentioned above. The bran and germ contain the dietary fiber and most of the biologically active components, whereas endosperm is mainly structure of starch and protein [66]. About 50–75% of the endosperm is starch which is the major energy supply for the embryo during germination of the kernel. About 8–18% of the endosperm is storage proteins along with cell-wall polymers. Relatively few vitamins, minerals, fiber, or phytochemicals are found in the endosperm fraction [61]. The bran contains the aleurone that protects the grain from bacteria, molds, insects, and severe weather [66]. The germ contributes quite small to the dry weight of most grains, for example, 4–5% in wheat and barley. The germ of maize provides a much higher contributor to the total grain structure than that of wheat, barley, or oats [61].

Whole grains, except for rice, are high in dietary fiber and amino acids (arginine, lysine, etc.), low in fat, concentrated sources of starch, high in vitamins, particularly vitamins B (thiamin, niacin, riboflavin, pantothenic acid, etc.), and good sources of minerals (Ca, Mg, K, P, Na, Fe, etc.) [18, 61, 67]. Other components in whole grains associated with improved health status include tocotrienols, lignans, phytoestrogens, phenolic compounds (predominantly ferulic acid and diferulates), phytic acid, and some unique compounds to grain products (avenanthramides, avenalumic acid) [15, 18, 61, 67–69]. They are responsible for the high antioxidant activity of whole grains [61, 67]. The types and varieties of grains effect the concentration of phytochemicals in whole grains [15, 70]. Table 1 shows a comprehensive list of biochemical constituents in some whole grains products.

During the milling process, the bran and germ are separated from the starchy endosperm and then the starchy endosperm is ground to flour. Refined flours have lower nutrients than whole grains because of the higher concentrations of nutrients in the outer part of the grains [62, 63]. Milling process may open up the food matrix, thereby let the release of firmly bound phytochemicals from the grain structure [61]. Milling of grains results in significant nutrient loss [18, 63].

Grains are usually subjected to some types of processing such as cooking and parboiling before consumption in developed countries. Commercial cereals are generally extruded, puffed, flaked to make a desirable product. Most researchers

Table 1 Energy, macronutrient, fiber, some vitamin and mineral contents of common whole grain products [71]

Whole grain products	Serving size	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)	Fiber (g)	Vitamin E (mg)	Folate (μg)
Bread, whole wheat	1 slice	81	13.67	3.98	1.12	1.9	0.850	13
English muffin, whole wheat	1/2 muffin	67	13.33	2.90	0.69	2.2	0.140	16
Bread pita, whole wheat	1,4 in. Diameter	74	15.40	2.74	0.73	2.1	0.170	10
Crackers, whole wheat	6 crackers	118	19.20	2.92	3.90	2.8	0.390	8
Oats, regular or quick, cooked with water	1/2 cup	83	14.04	2.97	1.78	2.0	0.090	7
Ready-to-eat cereal, all bran	1/2 cup	81	23.01	4.07	1.52	9.1	0.380	406
Rice, brown, medium grain, cooked	1/2 cup	109	22.92	2.26	0.81	1.8	–	4
Spaghetti, whole wheat, cooked	1/2 cup	87	18.58	3.73	0.38	3.2	0.210	4
Popcorn, air popped	3.5 cups	108	21.78	3.62	1.29	4.1	0.080	9
		Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Pantothenic acid (mg)	Pyridoxine (mg)	Ca (mg)	Fe (mg)
Bread, whole wheat	1 slice	0.126	0.053	1.420	0.207	0.069	52	0.79
English muffin, whole wheat	1/2 muffin	0.099	0.046	1.125	0.229	0.054	87	0.81
Bread pita, whole wheat	1,4 in. Diameter	0.095	0.022	0.795	0.233	0.074	15	0.86
Crackers, whole wheat	6 crackers	0.050	0.006	1.278	0.230	0.051	10	0.92
Oats, regular or quick, cooked with water	1/2 cup	0.089	0.019	0.262	0.363	0.006	11	1.05
Ready-to-eat cereal, all bran	1/2 cup	0.704	0.840	4.588	0.329	3.720	121	5.46
Rice, brown, medium grain, cooked	1/2 cup	0.099	0.012	1.297	0.382	0.145	10	0.52

(*continued*)

Table 1 (continued)

Spaghetti, whole wheat, cooked	1/2 cup	0.076	0.032	0.495	0.293	0.055	10	0.74
Popcorn, air popped	3.5 cups	0.029	0.023	0.646	0.143	0.044	2	0.89
		Mg (mg)	P (mg)	K (mg)	Na (mg)	Zn (mg)	Cu (mg)	Mn (mg)
Bread, whole wheat	1 slice	24	68	81	146	0.57	0.073	0.696
English muffin, whole wheat	1/2 muffin	23	93	69	120	0.53	0.070	0.591
Bread pita, whole wheat	1,4 in. Diameter	19	50	48	124	0.43	0.081	0.487
Crackers, whole wheat	6 crackers	30	91	95	194	0.73	0.016	0.594
Oats, regular or quick, cooked with water	1/2 cup	32	90	82	5	1.17	0.087	0.679
Ready-to-eat cereal, all bran	1/2 cup	112	356	316	80	3.84	0.322	2.297
Rice, brown, medium grain, cooked	1/2 cup	43	75	77	1	0.6	0.079	1.070
Spaghetti, whole wheat, cooked	1/2 cup	21	62	31	2	0.57	0.117	0.965
Popcorn, air popped	3.5 cups	40	100	92	2	0.86	0.073	0.312

It is adopted from "USDA National nutrient database for Standard Reference 26. Nutrient Data Laboratory, https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/nutrient-data-laboratory/"

determined that biologically important compounds, like antioxidants, in the processed whole grains are not removed [61, 72]. According to the results of study with rye, many of the bioactive compounds are stable during food processing, and there can be an increase in their ratios with appropriate processing [61, 73].

Whole grains include significant amounts of bioactive phytochemicals that can provide decreasing the risk of chronic diseases [14, 15, 64, 74]. The phytochemicals in whole grains can be protective or can act synergistically to exhibit these protective effects [19]. The recent evidence shows that the complex mixture of bioactives in whole foods can be more healthful than individual isolated components [15, 74]. The beneficial effects related with whole grain consumption originate due to the presence of the unique phytochemicals in whole grains [15].

According to 2005 Dietary Guidelines, refined and whole grains are important sources of carbohydrates in particular. Carbohydrates provide energy to the body in the form of glucose. Glucose is the only energy source for red blood cells and the preferential energy source for the brain and central nervous system, and preferred energy source for the placenta and fetus during pregnancy. Carbohydrates in grains are mainly found in the form of starches and some fibers. Carbohydrates in grains are mainly found in the form of starches and some fibers. The US Recommended Dietary Allowance (RDA)'s recommendation for daily carbohydrate consumption is 130 g per day for adults and children. If glucose is not present in the diet or glycogen is depleted, which is the body's storage form of glucose, the body will transform protein to glucose for supplying the essential fuel to the brain and protect blood glucose levels [57]. Protein destruction will imperil the life and vital activities.

Compared to refined grains, whole grains are more abundant in nutrients. This is very important in the intake of some components such as folic acid [57]. Refined grain products can be fortified with folic acid and the consumption of folic acid is associated with decreased risk of birth defects, including neural tube defects (NTD), and heart disease [57, 75, 76]. Most whole grain foods, the exception of both hot and cold breakfast cereals, do not need to be fortified with folic acid or other vitamins and minerals. Consumptions of whole grains are more beneficial due to these and similar situations [57].

4 Biological Effects of Dietary Fiber Intake with Consumption of Whole Grains

Epidemiological evidences show that the consumption of whole grains is correlated with reduced risk of chronic diseases such as CVD, diabetes, the metabolic syndrome, and certain cancers. Also, whole grain consumption can play a positive role in weight management [4]. Some examples of similar studies are given below.

Relevant evidence was assessed by Montonen et al. (2003) [77], which high fiber intake with whole grain foods reduced the risk of type 2 diabetes. A cohort consisted of 4316 Finnish men and women aged 40–69 years were followed for 10 years and an inverse association between the intake of whole grain foods and the risk of type 2 diabetes was found. A similar relation was not found for the intake of fiber from

vegetables and fruit. The risk reduction of type 2 diabetes as a result of whole grain intake was approximately 35%.

Newby et al. (2007) [78] examined associations between the consumptions of whole grains, refined grains, and cereal fiber with weight management. It had been observed that compared with subjects in the lowest quintile of whole grain intake, subjects in the highest quintile had lower body mass index and weight and smaller waist circumference. It was determined that whole grains and grain fiber had similar and collective effects on weight, body mass index (BMI), waist circumference changes. It was thought that the cereal fiber and its constituents could in part mediate these relations. In the same study, the associations between the consumptions of whole grains, refined grains, and cereal fiber with both cholesterol and blood glucose level changes were also evaluated. According to the results of study, the consumption of whole grains was inversely related with total cholesterol and LDL cholesterol levels and 2-h glucose amount. Although the effects of cereal fiber on cholesterol and glucose levels were similar with whole grains, the refined grains were increased fasting insulin among women but not men.

Intakes of whole grains and cereal fiber were inversely associated with the risk of chronic diseases. But their relation with total and disease-specific mortality remains unclear. In the study of Huang et al. (2015) [79], it was aimed to evaluate the association of whole cereals and cereal fiber intake with all causes and cause-specific mortality. The study included 367,442 participants that followed from 1995 to 2009. Participants with cancer, heart disease, stroke, diabetes, and self-reported end-stage renal disease at baseline were not accepted to the study. A total of 46,067 deaths were documented over an average of 14 years of follow-up. At the end of the study, it was observed that consumption of whole grains were inversely related with risk of all-cause mortality and death from CVD, cancer, diabetes, infections, respiratory disease, and other causes. When individuals were compared according to their whole grain and cereal fiber intakes, the lowest all-cause mortality risk was found to be 17% for those who had the highest intake of whole grains and 19% for those who had the highest intake of cereal fiber. The lowest disease-specific mortality risk was also determined to be 11–48% for those who had the highest intake of cereal fiber and 15–34% for those who had the highest intake of whole grains. The associations between whole grains consumption with the deaths from CVD, respiratory disease, and infections were not found significant, but the associations between all-cause mortality with deaths from cancer and diabetes were significant ($p < 0.029$). In the result of study, it was determined that consumption of whole grains and cereal fiber could inversely associate with reduced total and cause-specific mortality and cereal fiber was one potentially protective component in whole grains.

The effect of fermented whole grain cereal kernels with a high content of amylose (40%) and/or beta-glucan (4.6%) on postprandial glucose and insulin responses in healthy adults was evaluated by Alminger and Eklund-Jonsson (2008) [80]. For this purpose, a glucose solution or tempe fermented whole-grain barley or tempe fermented whole grain containing 25 g carbohydrates randomly was given to 13 healthy volunteers between the ages of 20 and 75. Blood samples were collected immediately before meal and at 15, 30, 45, 60, 90, and 120 minutes after meal

started. For each individual according to Food and Agriculture Organization/World Health Organization (FAO/WHO) standards, the glycemic index and insulin index of the meals were calculated. It was detected that the lowest peak of insulin response occurred after the high β-glucan oat tempe meal, while the peak of glucose was lowest after the high-amylose/high β-glucan barley tempe meal. During the first 60 minutes after meal, the average blood glucose and insulin responses for both barley and oatmeal dishes were significantly lower than for the reference glucose load ($p < 0.05$). The glycemic indexes calculated for the barley and oat tempe were 30 and 63, respectively. The obtained data suggested that whole grain products that reduce postprandial glucose and insulin responses could be got by fermentation and by using grains with an increased amylose and/or β-glucan content.

Systematic review and meta-analysis of prospective observational studies were performed by Aune et al. (2011) [81] for determining the association between intake of dietary fiber and whole grains with risk of colorectal cancer. For these reasons, PubMed and several other databases up to December 2010 and the reference lists of studies included in the analysis as well as those listed in published meta-analyses were used as data sources. Among them, prospective cohort and nested case-control studies for dietary fiber or whole grain intake and incidence of colorectal cancer were examined. According to the investigated 25 prospective studies, the relative risk of developing colorectal cancer for 10 g daily consumption of total dietary fiber (16 studies) was 0.90 ($p < 0.05$), for fruit fiber (9 studies) was 0.93, for vegetable fiber (9 studies) was 0.98, for legume fiber (4 studies) was 0.62, and for cereal fiber (8 studies) was 0.90. The relative risk of increase in colorectal cancer in the case of three servings of daily whole grains consumption (six studies) was 0.83. In the study, a high intake of dietary fiber, especially cereal fiber and whole grains, resulted in a reduced risk of colorectal cancer.

Chen et al. (2016) [82] who believed that the association between whole and refined grain consumptions and stroke risk remained unclear conducted a study using the MEDLINE and EMBASE databases until February 29, 2016. Seven prospective studies with a total of 446,451 individuals and 5892 stroke cases were examined. Compared to low consumption, the relative risk of stroke in the high consumption was 0.95 for total grains, 0.92 for whole grains, and 0.99 for refined grains ($p < 0.05$). Diets rich in whole grains were inversely associated with ischemic stroke risk whose relative risk was determined as 0.75 ($p < 0.05$). Meta-analysis expressed that the consumptions of whole and refined grain were not associated with total stroke risk, but whole grain consumption was associated with decreased ischemic stroke risk. It was clear that this was caused by the high dietary fiber contents of whole grains.

Although Hollænder et al. (2015) [83] acknowledged the potential role of whole grains in the prevention of CVD, they believed that the results of randomized controlled studies on blood lipids were inconsistent due to the compositional differences of some foods containing dietary fiber. They evaluated the influence of whole grains on changes in levels of total cholesterol (TC), LDL cholesterol, HDL cholesterol, and triglycerides compared with non–whole grain foods by using a meta-analytic approach. In the study which contained systematic literature review of

selected databases, randomized controlled comparisons were performed between whole-grain foods and a non–whole grain control in adults. A total of 6069 articles were screened and data were extracted from most suitable 24 studies. Generally, whole-grain intake reduced LDL cholesterol (weighted mean difference: −0.09 mmol L-1) and TC levels (weighted mean difference: −0.12 mmol L-1) compared to the control ($p < 0.05$). The greatest effect on TC (weighted mean difference: −0.17 mmol L-1) was observed in the whole grain oat consumption. Whole grain foods tended to lower triglycerides compared with the control (weighted mean difference: −0.04), whereas no effect of whole grain foods on HDL cholesterol was observed. Also, no relation was found between whole-grain dose or baseline TC concentration and any of the outcomes, while study duration was positively related with changes of TC and LDL cholesterol levels. Researchers proved that the consumption of whole grain diets lowered LDL cholesterol and TC but did not lower HDL cholesterol or triglycerides. They also indicated that whole grain oats were the most effective grain to lower cholesterol.

Chen et al. (2016) [84] assessed the associations between whole-grain intake and risk of dying from any cause such as CVD, and cancer with a meta-analytic approach. Relevant works on subjects were determined by researching PubMed and EMBASE databases and bibliographies of full publications. In the study, 13 studies about total mortality (104,061 deaths), 12 studies about CVD mortality (26,352 deaths), and 8 studies about cancer mortality (34,797 deaths) were used. Three of these studies were related to whole-grain intake, while the remaining studies were related to whole-grain products intake. Summary RRs were also calculated using the random effect model at the 95% confidence interval and were used as the effect size across studies. In the dose-response analysis which converted the intake of whole-grain products to the amount of whole grain, the summary RRs for an increment in whole-grain intake of 50 g d-1 were 0.78 for total mortality, 0.70 for CVD mortality, and 0.82 for cancer mortality. A similar decrease in the mortality from ischemic heart disease (RR: 0.68) was also observed, but the same case was not observed for stroke (RR: 0.93). Nonlinear relationships between whole grain intake and total ($p < 0.001$) and CVD mortalities ($p < 0.001$) was proven. The relation about cancer mortality ($p = 0.12$) was not nonlinear because these relationships appeared slightly more steep at lower ranges (<35 g d-1) of the intake compared to higher ranges. The findings supporting the significant inverse relations between whole grain intake and mortality related to any cause, CVD or cancer, suggested that whole grain consumption could be increased to improve public health.

Whole grains had attracted attention due to their potential role in weight regulation. A high intake of whole grains was related with smaller weight gain according to prospective cohort studies, while the results from randomized controlled studies were less consistent. Using a meta-analytic approach, Pol et al. (2013) [85] evaluated the influences of whole grains on changes in body weight, percentage of body fat, and waist circumference compared with non–whole grain foods. A systematic literature search was conducted in selected databases and 26 studies comparing whole grains and non–whole grain control consumptions in adults were defined. According to data from 2060 participants, it was found that whole grain intake had

no effect on body weight (weighted mean difference: 0.06 kg) but had a little effect on body fat percentage (weighted mean difference: −0.48%) compared to a control ($p < 0.05$). Whole grain consumption did not reduce body weight compared to control consumption, but it could create a small beneficial effect on body fat. It was thought that the significant differences in body weight and fat were not determined because of the relatively short duration of intervention studies (16 weeks).

Behall et al. (2006) [86] studied the effect of a whole grain diet containing brown rice/whole wheat and/or barley on blood pressure. For this purpose after a 2-week controlled diet, 16 participants consumed a diet which had been replaced by 20% of its energy with whole wheat/brown rice, barley, or half whole wheat-brown rice/half barley during 5 weeks each. Participants were selected from those with systolic blood pressure <140 mmHg, diastolic blood pressure <90 mmHg, and cholesterol levels between 200 and 240 mg dL-1. While the measurements of blood pressure were performed weekly, their weights were determined daily before breakfast. As a result of the study, it was seen that systolic blood pressure decreased by 2.2 mmHg during the control diet and an extra 1.4 to 6.7 mmHg in the whole grain diets. Diastolic blood pressure also decreased by 2 mmHg in the control diet and an extra 2.9 to 3.7 mmHg in the whole grain diets. The replacement of white rice with brown rice, white bread with whole grain bread, and low fiber cereals with barley or whole wheat cereals resulted in reduced systolic and diastolic blood pressure in mildly hypercholesterolemic men and women, and whole grain diets helped to lower blood pressure.

Pancreatic cancer was known as the most fatal cancer type in the United States. The intake of grains and cereals within a large population-based case-control study of pancreatic cancer was evaluated by Chan et al. (2007) [87]. A 131-item semi-quantitative food frequency questionnaire was applied to 532 cases and 1701 controls. Participants were asked to inform their frequency of individual food items intake in the 1 year prior to cancer diagnosis (for cases) or 1 year before interview (for controls). In this study, consumption of whole grain, refined grains, mixed grains, and sweetened refined grains as well as individual grain items were studied. Odds ratio (OR) and 95% confidence intervals were calculated as predictions of relative risk. It was observed that participants who consumed > or =2 servings of whole grains daily had a lower risk of pancreatic cancer as compared to participants who consumed <1 serving per day (OR = 0.60, $p < 0.05$). Similar results were obtained for brown rice (OR = 0.72, $p < 0.05$) and tortillas (OR = 0.56, $p < 0.05$). If the consumption of doughnuts and cooked breakfast cereals were > or =2 servings per week as compared with <1 serving per month, the risk of cancer increased (OR = 1.8 for doughnuts, $p < 0.05$; OR = 1.3 for oatmeal/oat bran, $p < 0.05$; OR = 2.1 for other cooked breakfast cereals, $p < 0.05$). Dietary fiber was inversely related with cancer risk (for highest quartile vs. lowest, OR = 0.65, $p < 0.05$). The data obtained from the study supported the hypothesis that higher consumption of whole-grain or high fiber foods could decrease pancreatic cancer risk. Refined grains consumption was not also associated with risk.

One of the recommended mechanisms for the protective effects of whole grains against chronic disease risk was their gut microbiota effect. Fermentation of soluble

fibers in whole grains could possess a prebiotic effect by altering the composition and/or activity in the gastrointestinal microbiota. To prove this, Costabile et al. (2008) [88] compared the effectiveness of whole grain wheat and wheat bran to modulate the gastrointestinal microbiota by using double blind crossover studies. Thirty-one healthy individuals were chosen randomly to either consume a whole grain wheat breakfast cereal (48 g day-1) or wheat bran breakfast cereal (48 g day-1) daily during 3 weeks. After a period of 2 weeks, the participants consumed the other cereal type for another 3 weeks. It was found that the numbers of fecal bifidobacteria and lactobacilli, which are beneficial for human health, were significantly higher after consuming the whole grain cereal as compared to the wheat bran cereal (9.3 ± 0.4 vs. 8.8 ± 0.4 $\log_{10}$cells g-1 feces, $p < 0.001$ and 8.7 ± 0.2 vs. 8.4 ± 0.2 $\log_{10}$cells g-1 feces, $p < 0.05$, respectively). The 2.5-fold increase in the concentration of ferulic acid (an antioxidant in plant foods) in the blood compared to the baseline was also observed as a result of consuming both cereals.

Erkkilä et al. (2005) [89] examined the relations between dietary fiber and whole grain intakes with progression of coronary atherosclerosis in women due to the fact that increased dietary fiber and whole grain intake was associated with lower risks of coronary artery disease morbidity and mortality. Regular dietary intake was evaluated at baseline with a validated semi-quantitative food frequency questionnaire. Angiographic evaluations were made in a cohort of postmenopausal women with coronary artery disease (CAD) in the Estrogen Replacement and Atherosclerosis Trial (n = 229) at baseline and after 3 years. The relations between baseline and total, cereal, fruit, and vegetable fiber intake and changes in coronary artery diameter and appearance of new lesions were determined. Changes in minimum coronary artery diameter (MCAD) and mean percent stenosis of women who consumed >6 servings of whole grains per week as compared to women who consumed <6 servings per week tended to be smaller after multivariate adjustment ($p = 0.04$ and $p = 0.07$, respectively). Higher cereal fiber consumption than fruit and vegetable-derived fiber was associated with a smaller change in MCAD compared to lower cereal fiber consumption ($p = 0.05$). These findings showed that CAD risk was decreased with a high intake of cereal fiber but not with fruit and vegetable fiber. The results supported dietary recommendations regarding the consumption of foods containing rich fiber, especially whole grain products, among postmenopausal women.

Karmally et al. (2005) [90], who believed that Hispanic Americans had higher risk scores for coronary heart disease than non-Hispanic whites, conducted a study about the efficacy of fiber-rich foods in management of hypercholesterolemia in Hispanics. A total of 152 Hispanic Americans aged 30–70 with baseline low-density lipoprotein cholesterol (LDL-C) levels between 120 and 190 mg dL-1 and triglycerides <400 mg dL-1 joined the study. Participants were randomly chosen to consume 90 g of corn (no brand specified) or oat (Cheerios) cereal per day for the next 6 weeks after consuming a National Cholesterol Education Program Step 1 diet during 5 weeks. The compliance rate among the participants was 100% in the corn cereal group and 99% in the oat cereal group and their body weight was stable during the study. While adding the oat cereal in the diet resulted in significant reductions in total cholesterol (−10.9 mg dL-1; −4.5%) and LDL-C (−9.4 mg dL-1; −5.3%),

there were no significant changes in total cholesterol (1.2 mg dL-1) and LDL-C (1.2 mg dL-1) in the corn cereal group. Also, it was found that every cereal did not have a significant effect on high-density lipoprotein cholesterol (HDL-C), triglycerides, or apo A-1 plasma levels. According to obtained data, the ready-to-eat oat cereal was effective in lowering cholesterol levels of men and women with mild to moderate hypercholesterolemia. An oat cereal can be evaluated as a therapeutic option together with a cholesterol-lowering diet for cure of mild to moderate hypercholesterolemia.

It was thought that whole grains could help in preventing overweight and obesity by increasing satiety and by slowing down starch digestion and absorption. For the purpose of reaching more exact opinions in this topic, van de Vijver et al. (2007) [91] assessed the association between consumption of whole grain foods and dietary fiber with BMI, overweight, and obesity among participants in the Netherlands Cohort Study in a cross-sectional (n = 4237) and in a prospective (n = 1257) setting. A validated semi-quantitative food frequency questionnaire was used for assessing the dietary intake of participants. As the result of the study, the intake of whole grain was found to be inversely associated with BMI in both men and women after multivariate adjustment. Compared to normal weight, the risk of obesity in women and men is lower by 10% (2–16%) and 4% (1–7%), respectively, for each additional gram of dry whole grain consumption ($p < 0.05$). It was estimated that a decrease of 1 unit BMI was associated with a 33 g day-1 increase in dry whole grain intake, and there was a slight inverse relationship between baseline fiber intake and weight gain over 20 years. The results of the study with healthy middle-aged participants showed that men and women with a high whole grain intake had lower BMI, overweight, and obesity risks as compared to men and women with a low whole grain intake.

5 Conclusion

Cereals are a major source of energy in many countries, and surprisingly less attention has been paid to the quality of cereals such as “refined” or “whole” in the different dietary recommendations of different countries. Whereas whole grains have beneficial effects on glucose-insulin homeostasis, certain types of cancers, blood lipids, and gastrointestinal health, especially due to their high dietary fiber contents. When the literature is searched, it is seen that a large mass of evidence on the health outcomes of whole grains has accumulated in the last 10 or 15 years. The associations between whole grain intake and a lower risk of major diseases and death can be explained by several mechanisms. Meta-analysis, which combines the results of many scientific studies, has some weaknesses due to the fact that there are few studies for some endpoints, such as mortality from diabetes and infectious diseases, and it has poor information on the assessment of whole grain intake. Future studies should improve the evaluation of whole grain intake, which is similarly reported, using biomarkers to track compliance in randomized trials, and using validated assessment methods in observational studies. There is a need to further studies related to the biological mechanisms of health effects of whole grains and the

contribution to health of different whole grain types. For example, the numbers of studies suggesting that whole grain oats may be more beneficial in CDVs as comparing to whole grain wheat should be increased, and further research should be conducted in similar fields. For healthy life, whole grain consumption on a daily diet should not be forgotten.

References

1. McNaughton SA, Bates CJ, Mishra GD (2012) Diet quality is associated with all-cause mortality in adults aged 65 years and older. J Nutr 142(2):320–325
2. Samieri C, Sun Q, Townsend MK, Chiuve SE, Okereke OI, Willett WC et al (2013) The association between dietary patterns at midlife and health in aging. Ann Intern Med 159(9):584–591
3. Authors/Task Force Members, Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F et al (2013) ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J 34(39):3035–3087
4. Rebello CJ, Greenway FL, Finley JW (2014) Whole grains and pulses: a comparison of the nutritional and health benefits. J Agric Food Chem 62:7029–7049
5. Sofi F, Abbate R, Gensini GF, Casini A (2010) Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr 92(5):1189–1196
6. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D (2003) Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 348(26):2599–2608
7. Lopez-Garcia E, Rodriguez-Artalejo F, Li TY, Fung TT, Li S, Willett WC et al (2014) The Mediterranean-style dietary pattern and mortality among men and women with cardiovascular disease. Am J Clin Nutr 99(1):172–180
8. Boccardi V, Herbig U (2012) Telomerase gene therapy: a novel approach to combat aging. EMBO Mol Med (8):685–687
9. Schroder H (2007) Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. J Nutr Biochem 1(3):149–160
10. Kendall CWC, Esfahani A, Jenkins DJA (2010) The link between dietary fibre and human health. Food Hydrocoll 24(1):42–48
11. Bellikci Koyu E (2016) Dietary fiber and cancer. In: Otles S, Akcicek E (eds) Food and nutrition on protection against cancer, 1st edn. Sidas, Izmir, pp 147–168
12. Trowell H (1976) Definition of dietary fiber and hypotheses that it is a protective factor in certain diseases. Am J Clin Nutr 29(4):417–427
13. Liu RH (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 78(3 Suppl):517S–520S
14. Slavin JL (2000) Mechanisms for the impact of whole grain foods on cancer risk. J Am Coll Nutr 19(3 Suppl):300S–307S
15. Liu RH (2007) Whole grain phytochemicals and health. J Cereal Sci 46(3):207–219
16. Slavin J (2003) Why whole grains are protective: biological mechanisms. Proc Nutr Soc 62(1):129–134
17. Pereira MA, Pins JJ, Jacobs DR, Marquart L, Keenan JM (2001) Whole grains, cereal fiber and chronic diseases: epidemiologic evidence. In: Spiller G (ed) CRC handbook of dietary fiber in human nutrition. CRC Press, Boca Raton, pp 461–479
18. Slavin J, Jacobs D, Marquart L (1997) Whole-grain consumption and chronic disease: protective mechanisms. Nutr Cancer 27(1):14–21
19. Slavin J, Jacobs D, Marquart L, Wiemer K (2001) The role of whole grains in disease prevention. J Am Diet Assoc 101(7):780–785

20. Jacobs DR, Meyer KA, Kushi LH, Folsom AR, Folsom AR (1999) Is whole grain intake associated with reduced total and cause-specific death rates in older women? The Iowa Women's Health Study. Am J Public Health 89(3):322–329
21. Jones JR, Lineback DM, Levine MJ (2006) Dietary reference intakes: implications for fiber labeling and consumption: a summary of the International Life Sciences Institute North America Fiber Workshop, June 1–2, 2004, Washington, DC. Nutr Rev 64(1):31–38
22. Kranz S, Mitchell DC, Siega-Riz AM, Smiciklas-Wright H (2005) Dietary fiber intake by American preschoolers is associated with more nutrient-dense diets. J Am Diet Assoc 105(2):221–225
23. Maćkowiak K, Torlińska-Walkowiak N, Torlińska B (2016) Dietary fibre as an important constituent of the diet. Postępy Hig i Med Doświadczalnej [Internet] 70:104–109. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26943307
24. Perry J, Ying W (2016) A review of physiological effects of soluble and insoluble dietary fibers. J Nutr Food Sci 6(2):476–482
25. Codex Alimentarius Commission (2009) Guidelines on nutritional labeling. Report of the 31st session of the codex committee on nutrition and foods for special dietary uses, Dusseldorf [Internet]. [cited 2017 Jul 18] Available from: https://ec.europa.eu/food/sites/food/files/safety/docs/codex_ccnfsdu_31_ag_item_4.pdf
26. Slavin J (2008) Position of the American Dietetic Association: health implications of dietary fiber. J Am Diet Assoc 108(10):1716–1731
27. Maziarz M (2013) Role of fructans and resistant starch in diabetes care. Diabetes Spectr 26(1):35–39
28. NCEP Expert Panel (2002) On detection, evaluation and T of HBC in A. Third report of the national cholesterol education program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation 106(25):3143–3421
29. Eroglu Samur G, Mercanligil SM (2012). Dietary fiber and nutrition. Reklam Kurdu Ajansı, Ankara, 20 (in Turkish)
30. Cho SS, Dreher ML (2001) Handbook of dietary fiber. CRC Press, Inc., New York, 894 p
31. Theuwissen E, Mensink R (2008) Water-soluble dietary fibers and cardiovascular disease. Physiol Behav 94(2):285–292
32. Fuentes-Zaragoza E, Riquelme-Navarrete M, Sánchez-Zapata E, Pérez-Álvarez J (2010) Resistant starch as functional ingredient: a review. Food Res Int 43(4):931–942
33. Wu Y, Li W, Cui W, Eskin NAM, Goff HD (2012) A molecular modeling approach to understand conformation–functionality relationships of galactomannans with different mannose/galactose ratios. Food Hydrocoll 26(2):359–364
34. Pawar SN, Edgar KJ (2012) Alginate derivatization: a review of chemistry, properties and applications. Biomaterials 33(11):3279–3305
35. Hillman LC, Peters SG, Fisher CA, Pomare EW (1986) Effects of the fibre components pectin, cellulose, and lignin on bile salt metabolism and biliary lipid composition in man. Gut 27(1):29–36
36. Pomare EW, Heaton KW (1973) Alteration of bile salt metabolism by dietary fibre (bran). Br Med J 4(5887):262–264
37. Pomare EW, Heaton KW, Low-Beer TS, Espiner HJ (1976) The effect of wheat bran upon bile salt metabolism and upon the lipid composition of bile in gallstone patients. Am J Dig Dis 21(7):521–526
38. National Research Council Committee on Diet and Health (US) (1989) Dietary fiber. In: Diet and health: implications for reducing chronic disease risk. National Academy Press, Washington, DC, pp 291–310
39. Judd PA, Truswell AS (1985) Dietary fibre and blood lipids in man. In: Leeds AR, Avenell A (eds) Dietary fibre perspectives: reviews and bibliography. John Libbey and Company Ltd., London, pp 23–39

40. Pilch SM (1987) Physiological effects and health consequences of dietary fiber. Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, 236 p
41. Baird IM, Walters RL, Davies PS, Hill MJ, Drasar BS, Southgate DA (1977) The effects of two dietary fiber supplements on gastrointestinal transit, stool weight and frequency, and bacterial flora, and fecal bile acids in normal subjects. Metabolism 26(2):117–128
42. Drasar BS, Jenkins DJA, Cummings JH (1976) The influence of a diet rich in wheat fibre on the human faecal flora. J Med Microbiol 9(4):423–431
43. Finegold SM, Sutter VL (1978) Fecal flora in different populations, with special reference to diet. Am J Clin Nutr 31(10 Suppl):S116–S122
44. Reddy BS (1998) Prevention of colon cancer by pre- and probiotics: evidence from laboratory studies. Br J Nutr 80(4):S219–S223
45. Ozyurt VH, Otles S (2014) Prebiotics: an important food components for metabolism. Acad Food J 12(1):115–123
46. Mackie A, Bajka B, Rigby N (2016) Roles for dietary fibre in the upper GI tract: the importance of viscosity. Food Res Int 88:234–238
47. Spiller GA (2001) Dietary fiber in human nutrition, 3rd edn. CRC Press, Inc, Boca Raton, 709 p
48. Manthey FA, Hareland GA, Huseby DJ (1999) Soluble and insoluble dietary fiber content and composition in oat. Cereal Chem J 76(3):417–420
49. Furda I. (1983) Unconventional sources of dietary fiber-physiological and in vitro functional properties, vol 214. American Chemical Society, Washington, DC, 315 p
50. Asp NG (1994) Nutritional classification and analysis of food carbohydrates. Am J Clin Nutr 59(3 Suppl):679S–681S
51. Marlett JA, Longacre MJ (1996) Comparison of in vitro and in vivo measures of resistant starch in selected grain products. Cereal Chem 73(1):63–68
52. Furda I, Brine CJ (1990). New developments in dietary fiber – physiological, physicochemical, and analytical aspects. 1. Springer, New York, 338 p
53. Intakes. I of MP on the D of DF and the SC on the SE of DR (2001) Dietary reference intakes proposed definition of dietary fiber. National Academy Press, Washington, DC, 64 p
54. Coulston AM, Rock CL Monsen ER (2001) Nutrition in the prevention and treatment of disease. Academic Press, London, 801 p
55. Ruottinen S, Lagstrom HK, Niinikoski H, Ronnemaa T, Saarinen M, Pahkala KA et al (2010) Dietary fiber does not displace energy but is associated with decreased serum cholesterol concentrations in healthy children. Am J Clin Nutr 91(3):651–661
56. Rajasree Pai R, Raghesh V (2006) Health benefits of whole grains: a literature review. Internet J Nutr Wellness 4(2):1–15
57. IFIC Foundation. Whole grains fact sheet
58. Borneo R, León AE (2012) Whole grain cereals: functional components and health benefits. Food Funct 3(2):110–119
59. AACC International. Whole grain [Internet]. [cited 18 Jul 2017] Available from: http://www.aaccnet.org/initiatives/definitions/Pages/WholeGrain.aspx
60. Fardet A (2010 Jun) New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev 23(1):65–134
61. Slavin J (2004) Whole grains and human health. Nutr Res Rev 17:99–110
62. Slavin JL (1994) Epidemiological evidence for the impact of whole grains on health. Crit Rev Food Sci Nutr 34(5–6):427–434
63. Pedersen B, Knudsen KE, Eggum BO (1989) Nutritive value of cereal products with emphasis on the effect of milling. World Rev Nutr Diet 60:1–91
64. Liu S, Willett WC, Manson JE, FB H, Rosner B, Colditz G (2003) Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. Am J Clin Nutr 78(5):920–927
65. Kyrø C, Tjønneland A (2016) Whole grains and public health. Br Med J 353:i3046–i3047
66. Marquart L, Jacobs DR, McIntosh GH, Poutanen K, Reicks M (2007) Whole grains and health, 1st edn. Blackwell Science, Iowa, 335 p

67. Miller G, Prakash A, Decker E (2002) Whole-grain micronutrients. In: Marquart L, Slavin JL, Fulcher RG (eds) Whole-grain foods in health and disease. American Association of Cereal Chemists, St Paul, pp 243–258
68. Bunzel M, Ralph J, Marita JM, Hatfield RD, Steinhart H (2001) Diferulates as structural components in soluble and insoluble cereal dietary fibre. J Sci Food Agric 81(7): 653–660
69. Shahidi F, Naczk M (1995) Food phenolics: sources, chemistry, effects, applications. Technomic Publishing Company, Basel/Lancaste, 331 p
70. Adom KK, Rui HL (2002) Antioxidant activity of grains. J Agric Food Chem 50(21):6182–6187
71. USDA (2017) National nutrient database for standard reference 26. Nutrient data laboratory. https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/sr26-home-page/. Accessed 16 July 2017
72. Slavin JL, Jacobs D, Marquart L (2000) Grain processing and nutrition. Crit Rev Food Sci Nutr 40(4):309–326
73. Liukkonen KH, Katina K, Wilhelmsson A, Myllymaki O, Lampi AM, Kariluoto S et al (2003) Process-induced changes on bioactive compounds in whole grain rye. Proc Nutr Soc 62(1):117–122
74. Liu RH (2004) Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr 134(12 Suppl):3479S–3485S
75. Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY (2001) Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. JAMA 285(23):2981–2986
76. Malinow MR, Bostom AG, Krauss RM Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. Circulation 99(1):178–182
77. Montonen J, Knekt P, Järvinen R, Aromaa A, Reunanen A (2003) Whole-grain and fiber intake and the incidence of type 2 diabetes. Am J Clin Nutr 77(3):622–629
78. Newby PK, Maras J, Bakun P, Muller D, Ferrucci L, Tucker KL (2007) Intake of whole grains, refined grains, and cereal fiber measured with 7-d diet records and associations with risk factors for chronic disease. Am J Clin Nutr 86(6):1745–1753
79. Huang T, Xu M, Lee A, Cho S, Qi L (2015) Consumption of whole grains and cereal fiber and total and cause-specific mortality: prospective analysis of 367,442 individuals. BMC Med 13(1):59–67
80. Alminger M, Eklund-Jonsson C (2008) Whole-grain cereal products based on a high-fiber barley or oat genotype lower post-prandial glucose and insulin responses in healthy humans. Eur J Nutr 47(6):294–300
81. Aune D, Chan DSM, Lau R, Vieira R, Greenwood DC, Kampman E et al (2011) Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. BMJ 343:d6617–d6636
82. Chen J, Huang Q, Shi W, Yang L, Chen J, Lan Q (2016) Meta-analysis of the association between whole and refined grain consumption and stroke risk based on prospective cohort studies. Asia Pac J Public Health 28(7):563–575
83. Holloender PLB, Ross AB, Kristensen M (2015) Whole-grain and blood lipid changes in apparently healthy adults: a systematic review and meta-analysis of randomized controlled. Am J Clin Nutr 102(1):556–572
84. Chen GC, Tong X, JY X, Han SF, Wan ZX, Qin JB et al (2016) Whole-grain intake and total, cardiovascular, and cancer mortality: a systematic review and meta-analysis of prospective studies. Am J Clin Nutr 104:164–172
85. Pol K, Christensen R, Bartels EM, Raben A, Tetens I, Kristensen M (2013) Whole grain and body weight changes in apparently healthy adults: a systematic review and meta-analysis of randomized controlled the European Union Sixth Framework Program Integrated Project. Am J Clin Nutr 98(4):872–884

86. Behall KM, Scholfield DJ, Hallfrisch J (2006) Whole-grain diets reduce blood pressure in mildly hypercholesterolemic men and women. J Am Diet Assoc 106(9):1445–1449
87. Chan JM, Wang F, Holly EA (2007) Whole grains and risk of pancreatic cancer in a large population-based case-control study in the San Francisco Bay Area, California. Am J Epidemiol 166(10):1174–1185
88. Costabile A, Klinder A, Fava F, Napolitano A, Fogliano V, Leonard C et al (2008) Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. Br J Nutr 99(1):110–120
89. Erkkilä AT, Herrington DM, Mozaffarian D, Lichtenstein AH (2005) Cereal fiber and whole-grain intake are associated with reduced progression of coronary-artery atherosclerosis in postmenopausal women with coronary artery disease. Am Heart J 150(1):94–101
90. Karmally W, Montez MG, Palmas W, Martinez W, Branstetter A, Ramakrishnan R et al (2005) Cholesterol-lowering benefits of oat-containing cereal in Hispanic americans. J Am Diet Assoc 105(6):967–970
91. van de Vijver LPL, van den Bosch LMC, van den Brandt PA, Goldbohm RA (2009) Whole-grain consumption, dietary fibre intake and body mass index in the Netherlands cohort study. Eur J Clin Nutr 63(1):31–38

Inulin-Type Fructans Application in Gluten-Free Products: Functionality and Health Benefits

25

Natalia Drabińska, Cristina M. Rosell, and Urszula Krupa-Kozak

Contents

N. Drabińska (✉) · U. Krupa-Kozak
Department of Chemistry and Biodynamics of Food, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland
e-mail: n.drabinska@pan.olsztyn.pl; u.krupa-kozak@pan.olsztyn.pl

C. M. Rosell
Food Science Department, Institute of Agrochemistry and Food Technology (IATA-CSIC), Paterna, Valencia, Spain
e-mail: crosell@iata.csic.es

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_2

Abstract

The increasing demand on a high-quality gluten-free (GF) products and an increasing prevalence of GF consumers favors the development of research aimed to improve the overall quality of GF products. To obtain a functional GF product providing the additional health benefits, the fortification of GF food is applied. Recently, inulin-type fructans (ITFs) were proposed as multi-task ingredients of GF products, improving their nutritional and health-related properties. In this chapter, the most recent studies on GF products in which ITFs were applied as valuable ingredients affecting the rheological and technological parameters of GF products are presented. The literature data with the successful applications of ITFs in the GF products and with their health beneficial properties, as presented in this chapter, points to a great potential of ITFs in the GF technology. The promising evidences of beneficial impact of ITFs on characteristic of GF goods may contribute to further development and intensified research on new GF products of superior quality that will be dedicated to people suffering from gluten-related disorders.

Keywords

Inulin-type fructans · Inulin · Fructooligosaccharides · Gluten-free diet · Gluten-free products · Prebiotic

List of Abbreviations

AGA	Anti-gliadin
ASD	Autistic spectrum disorders
ATI	Amylase and trypsin inhibitor
BMI	Body mass index
CD	Celiac disease
DGP	Anti-deaminated gliadin
DP	Degree of polymerization
EMA	Anti-endomysium
FODMAPs	Fermentable oligo-, di-, and mono-saccharides and polyols
FOS	Fructooligosaccharides
GF	Gluten-free
GFD	Gluten-free diet
HLA	Human leucocyte antigen
IBD	Irritable bowel disease
IBS	Irritable bowel syndrome
IgG	Immunoglobulin G
ISAPP	The International Scientific Association of Probitics and Prebiotics
ITFs	Inulin-type fructans
JAR	Just-about-right
mRNA	Messenger RNA
MW	Molecular weight
NCGS	Non-celiac gluten sensitivity
QDA	Quantitative descriptive analysis

RDA	Recommended daily allowances
SCFA	Short-chain fatty acids
TLR	Toll-like receptor
tTG	Anti-tissue transglutaminase
WA	Wheat allergy
WDEIA	Wheat-dependent, exercise-induced anaphylaxis

1 Introduction

The term "functional food" is defined as a group of products, which may improve the general condition of the body and decrease the risk of specific diseases [1]. To obtain functional food products that will provide the additional health benefits above those arising from its basic composition, a fortification involving the addition of one or more biologically active compounds or nutrients is applied. Fortification is described as one of the most effective strategies available to improve micronutrient status [2]. Recently, due to the westernization of diet, increasing prevalence of food intolerances, including gluten intolerance, are noted [3]. In gluten-related disorders, nutritional deficiencies of vitamins, minerals, proteins, and fiber are frequently observed [4, 5]. The nutritional deficiencies are mainly associated with a lower nutritional value of the gluten-free (GF) products as compared to the gluten-containing counterparts and because they are rarely fortified [4, 6]. To meet the growing demands of GF food consumers, the enrichment of GF products aimed to improve their palatability and nutritional value seems to be important and reasonable [7, 8]. Recently, inulin-type fructans (ITFs) were proposed as multi-task ingredients of GF products, improving their nutritional and health-related properties [9].

2 Structures, Sources, and Functionality of Inulin-Type Fructans

Generally, fructans are water-soluble mixtures of oligo- or polysaccharides containing at least two adjoining fructose units. The molecule of glucose can be linked by α-(1–2) linkages, similar as in sucrose molecule, at the beginning of chain but it is not obligatory. Fructans can vary based on the degree of polymerization (DP) and chemical structure. In higher plants, five types of fructans can be distinguished based of their structure: inulin-type fructans, inulin neoseries-type fructans, levan-type fructans, levan neoseries-type fructans and mixed levan-type fructans [9] (Fig. 1). ITFs and levan-type fructans are ketose-based fructans with mostly or exclusively β-(2–1) and β-(2–6) fructosyl-fructose linkages, respectively. Therefore, both groups of fructans are mostly linear; however, a small amount of branched units with β-(2–6) and β-(2–1) configuration for ITFs and levan-type fructans, respectively, can occur [10]. Neoketose is a base for both inulin neoseries-type fructans and levan neoseries-type fructans, which are characterized by the presence of glycosidic

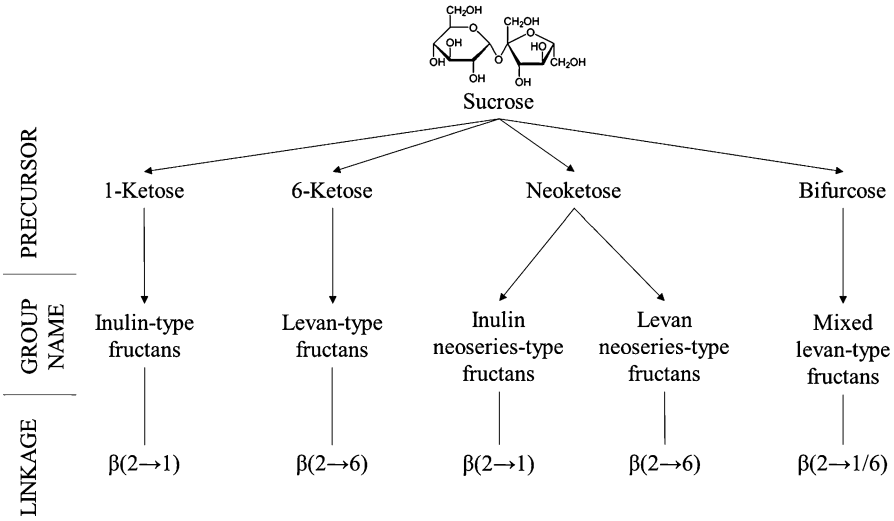


Fig. 1 Representation of fructans' types with their precursors and type of fructofuranosyl linkages

bonds like inulin- and levan-type fructans, respectively, and glucose on both ends of the molecule [10]. Elongation of inulin neoseries-type fructans starts from the C1 of the fructose group and/or from the C6 of the glucose unit of the initial sucrose, while elongation of levan neoseries-type fructans comprises molecules for which the elongation starts from the C6 of the glucose moiety. Mixed levan-type fructans include both types of fructofuranosyl linkages: β-(2–1) and β-(2–6) leading to formation of branched molecules [10].

In plants, ITFs composed of β-D-fructosyl units linked by (2–1) glycosidic bonds consist from 2 to 200 monomers, while ITFs synthesized by bacteria can be much longer reaching even 100,000 units [11]. Based on the length of the chain (DP), ITFs can be divided into long-chain inulin (DP $>$ 10) and short-chain oligofructose (DP $<$ 10) [9, 12]. DP determines the physicochemical properties of ITFs. Among commercially available ITFs, Synergy 1 (Raftilose®, Orafti, Tienen, Belgium) that is a mixture of long- and short-chain fructans have the features of both types of polymers.

The prevalence of fructans in the plant kingdom is very common, and they can be found in approximately 36,000 species [11]. The main taxonomic orders containing the largest amounts of ITFs are *Asteraceae* represented by chicory (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*) – the main sources of ITFs; as well as *Liliales*, including leek, onion, asparagus, and garlic [13]. Tubers and roots of *Asteraceae* are the richest source of ITFs. Plant genotype, cultivar conditions tissue, the developmental stage as well as the conditions of post-harvest storage are factors influencing the chemical structure and yield of ITFs [14, 15]. The majority of ITFs used by food industry is obtained from chicory, containing even 15–20% of inulin. Non-fractioned inulin extracted from chicory roots consists of oligomers and polymers ranged from 3 to 70 DP. The contents of ITFs in different

Table 1 Content (% of fresh weight) and chain length of inulin in various plant

Source	Inulin content	Chain length
Chicory (root)	15–20	DP[a] $< 40 = 83\%$ DP $\geq 40 = 17\%$
Jerusalem artichoke (tuber)	14–20.5	DP $< 40 = 94\%$ DP $\geq 40 = 6\%$
Globe artichoke	2–7	DP $\geq 40 = 87\%$ DP $\geq 5 = 95\%$
Dandelion (leaves)	12–15	
Garlic (bulb)	9–16	DP $\geq 5 = 75\%$
Leek (bulb)	3–10	DP = 12 – majority
Onion (bulb)	1.1–7.1	DP 2–12
Asparagus (modified stem)	2–3	
Wheat (bran)	1–4	DP $\leq 5 = 50\%$
Barley (cereal)	0.5–1.5	
Rye (cereal)	0.5–1	
Banana (fruit)	0.3–1	DP $< 5 = 100\%$

[a]*DP* degree of polymerization

plant sources are summarized in Table 1. Inulin can also be produced enzymatically from sucrose following the transglycosilation and hydrolysis catalyzed by fructosyl-transferase [16]. Synthetic inulin is characterized by a lower polydispersity as compared to plant-derived one [17].

Main role of fructans in plants is related with storing the energy, but they also take part in the osmoregulation and in the response for abiotic-stress [18, 19]. Inulin obtained from chicory is present in a form of white fine powder with a slightly sweet taste due to the presence of both long- and short-chain fractions. The unique chemical structure of ITFs having the furanose conformation and the backbone with the polyethylene oxide determines its higher molecule flexibility as compared to pyranose configuration [17]. The properties of individual fraction depend on DP, determining the versatility of ITFs applications. The short-chain oligofructose is characterized by sweet taste and its sweetness constitutes approximately 35% of sucrose. The technological properties of oligofructose are similar to features of glucose syrup, therefore it can be used to improve the mouth-feel of light-type food products with lower caloric value without compromising sweet taste [20]. Long-chain inulin has neutral taste and it is not sweet but is able to form the gel network when it is mixed at high concentration with water. The aqueous liquid emulsion can be used to replace fat in food production, what is commonly applied in the production of low-fat dairy products with smooth, creamy texture [21]. Fat resembling features are also used in the meat and bakery industries to formulate low caloric products [22–25]. ITFs can be applied not only in the food industry but also for pharma. They can be used as targeted colon deliverer, to stabilize the protein-based pharmaceuticals, to enhance the dissolution rate, and to diagnose kidney failure by urine secretion rate [17, 26, 27].

3 Health-Related Properties of Inulin-Type Fructans

3.1 Prebiotic Effect

B-configuration of ITFs is responsible for their unique properties. ITFs are not hydrolyzed by the enzymes of the upper intestinal digestive tract as they only have the ability to hydrolyze linkages in α-configuration, therefore ITFs reach the colon unchanged [28]. This specific property of ITFs is one of the crucial features which allow considering ITFs as prebiotics. Nevertheless, the definition of prebiotic has evolved many times since 1995, when Gibson and Robertfroid [29] explained prebiotic as "a nondigestible food ingredient that selectively stimulates growth and/or activity of one or a limited number of bacteria in the colon, thereby improving host health" to the final, the most recent definition developed in 2017 by The International Scientific Association of Probiotics and Prebiotics (ISAPP) that simplified it to "a substrate that is selectively utilized by host microorganism conferring a health benefit" [30]. To specify this definition, three criteria should be accepted: prebiotic must be selectively utilized by the commensal microorganisms; have adequate evidence of health benefit for the target host; and must not be degraded by the target host enzymes. ITFs meet all the abovementioned criteria [30]. The fermentation of prebiotics causes changes in the gut microbiota composition. ITFs are preferably degraded by β-fructanoidase synthesized by bifidobacteria [30]. The presence of carbohydrates stimulates the fermentation process leading to an increase in bacterial quantity and fecal mass. The final products of ITFs metabolism are hydrogen, methane, carbon dioxide, lactate, and short-chain fatty acids (SCFA) [31]. SCFA are considered to have beneficial direct and indirect effects on human health. Higher amount of SCFA decrease pH in the intestines inhibiting growth of pathogenic bacteria. Butyrate is the energy substrate for colonic epithelial cells, and it is a key factor in the colonocytes proliferation and development. Water-soluble SCFA can easily pass the intestinal barrier getting into bloodstream, and then spread in the body. Acetate is used by the brain, muscles, and tissue, while propionate is metabolized by liver, reducing the hepatic production of cholesterol [31].

Inulin has been found to stimulate the development of *Lactobacillus* and *Bifidobacterium*, with simultaneous inhibiting of amount of harmful bacteria like *Escherichia coli* and *Clostridium* [32]. The products of fermentation of *Bifidobacterium* are mainly lactate and acetate. The in vitro chemostat study conducted by Langlands and co-workers [33], aimed to evaluate the effect of the prebiotic carbohydrates oligofructose and inulin on the mucosal microorganisms, showed a significant increase ($P < 0.05$) in the number of *Bifidobacterium* and *Lactobacillus* in biopsies taken from the caecum, transverse and descending colon, and rectum. Authors reported a significant increase of eubacteria in subjects consuming fructooligosaccharides (FOS) and inulin but no changes in the total anaerobes clostridia, bacteroides, or coliforms, neither in their proliferation indices [33].

However, the in vitro models do not allow analyzing the complexity of the intestinal ecosystem; therefore to confirm the probiotic effect of ITFs, the in vivo studies and human trial were necessary. Recent in vivo study on inulin

administration in rats' diet of reduced amount of calcium showed a stimulation of bifidobacteria, particularly *Bifidobacterium animalis*, and an increase in SCFA in the groups fed with ITFs [34]. The stimulation of microbiota was dependent on calcium intake, and the positive changes were observed only in animals fed with a recommended amount of calcium. The DP-dependent influence of ITFs on gut microbiota was determined in the study on C57BL/6 J mice [35]. Authors reported that ITFs with lower DP stimulated the intestinal microbiota more efficiently than long-chain inulin. However, in both groups, the growth stimulation of the butyric-producing bacteria was observed. In the group receiving FOS, the dominant bacteria in *Verrucomicrobia* phylum was *Akkermansia muciniphila*, belonging to mucin-degrading species [35].

Numerous human studies, varying the applied dose of prebiotic, the DP, and the duration of experiment confirmed the prebiotic effect of ITFs [33, 36–39]. Randomized, double-blind placebo-controlled study on obese women receiving 16 g of oligofructose-enriched inulin or placebo (maltodextrin) was designed by Salazar and co-authors [40]. After 3 months supplementation, the number of *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum,* and *Bifidobacterium adolescentis* increased in the ITFs-consuming group. In general, in the obese and overweight human, the SCFA concentration is elevated [41]. In the study by Salazar and co-workers [40], the normalization of the SCFA concentration, with the significant decrease in acetate, propionate and total SCFA content, was noted in the prebiotic group. The increase in *Bifidobacterium* counts was determined in the randomized trial with 4-week intake of 8 g of FOS by 12 healthy elderly volunteers [42]. Prebiotic effect of inulin was also reported in the study of Tuohy and co-workers [39]. Authors reported the significant increase in *Bifidobacterium* in pooled fecal samples collected from ten healthy volunteer after 14-days intake of 8 g of inulin. Small but significant increase was observed also in clostridium group. Inulin intake had no effect on the total bacteria number, *Bacteroides* spp., *Lactobacillus* sp., and *Enterococcus* sp. The authors noticed that the more prominent increase in *Bifidobacterium* was observed in volunteers with a lower initial number of these bacteria [39]. Fecal microbiota composition was weekly monitored in the study with Jerusalem artichoke and chicory inulin-supplemented snacks introduced to 45 volunteers [43]. The increase in the count of *Bifidobacterium* with simultaneous reduction in *Bacteroides – Prevotella* ratio and *Clostridium histolyticum/Clostridium lituseburense* was observed in both experimental groups fed with ITFs [43].

3.2 Dietary Fiber

According to the definition of Institute of Medicine of The National Academies, dietary fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants [44]. Dietary fibers should be resistant to the enzymes of early sections of intestinal track and should be metabolized in the colon by intestinal microbiota [45]. The interest in dietary fibers is related with a wide spectrum of health beneficial properties, including reducing the risk of type 2 diabetes,

hypertension, cardiovascular disease and helping in the body mass management [46]. The consumption of fibers should consist of 25–38 g per day [47]. ITFs are characterized by the low caloric value (1–2 kcal/g) as well as by β-configuration, allowing to reach large intestine in the intact form in almost 90% determines that ITFs belong to the dietary fiber group, thus they are considered as dietary fiber [20, 48, 49]. Precisely, based on their physiochemical characteristic, ITFs belong to the group of soluble dietary fibers, being better fermentable and viscous [31]. Therefore, ITFs-containing products are labeled as a source of dietary fiber.

3.3 Intestinal Morphology and Gut Function

In general, it is well known that intestinal microbiota has a crucial role in the whole body homeostasis and maintenance of the gut health. Prebiotics, including ITFs, stimulating the development of the beneficial strains of bacteria are considered to improve the gut health in terms of morphological changes, maintenance of intestinal integrity, and alleviating the symptoms of intestinal diseases.

ITFs were applied as a supplement in a study aimed to compare the morphometry of germ-free rats and rats with human fecal microbiota [50]. The height of villi and depth of goblet cells was improved in groups receiving ITFs. Moreover, ITFs had beneficial effect of epithelial mucus layer, especially in a group with fecal microbiota transplantation [50]. The fragility and spontaneous bleeding are considered as a symptom of low-fiber diet due to the reduced amount of SCFA [51]. In other study, 10% of inulin, FOS, and cellulose were applied in the diet of obese mice for 4 weeks to evaluate the effect of fibers on gastroenterology physiology [52]. The authors noticed that in groups fed ITFs, the cecal crypt depth was improved as compared to cellulose-fed and control groups. Additionally, inulin-fed mice had better cecal transmutal resistance. Moreover, a lower expression of colonic mRNA of genes encoding occludin, zonulin 1, AMP kinase, and monocarboxylic transporter 1 was observed in inulin-fed mice, pointing the negative changes in the gut barrier functioning. However, as authors underlined, because the deteriorating in the transmutal resistance and morphological failures were not observed in inulin-fed mice, this mechanism may be more complex and require further studies [52]. Unfavorable effect of ITFs on gut barrier was reported in other studies with animal model [53–55]. However, in the human studies no effect of ITFs on gut barrier was observed [56–58]. On the contrary, Russo and co-workers [59] reported improvement in the circulating level of intestinal permeability markers, such as zonulin and glucagon-like peptide 2 after administration of an inulin-enriched pasta, suggesting the potential role of inulin in the prevention of intestinal disorders [59].

The randomized, controlled trial evaluated the effect of oligofructose on the prevalence of diarrhea in 282 infants aged 6–12 month [60]. Diet supplemented with prebiotics did not affect the prevalence of diarrhea; however, the mean number of days with diarrhea was smaller in the oligofructose group than in the control group. Nonetheless, authors concluded that a prebiotic supplementation cannot be substitutive of breast-feeding [60]. Klessen co-workers [61] analyzed the influence

of inulin on the constipation in ten elderly women and found that in most cases, prebiotic increased the stool frequency from 1–2 to 8–9 per week and modified a stool consistency. In the initial part of this study, the daily dose of 20 g of inulin was applied and after increased gradually up to 40 g per day. In most of women, the inulin dose has no impact on the stool frequency, except of one [61]. Similar findings were reported in the studies conducted on 56 healthy children (age 16–46 weeks) [62]. The authors reported that FOS in a dose of 0.74 g per day taken for 28 days resulted in improved stool frequency and consistency as compared to placebo-fed children. Contrary results were obtained by Bouhnik and co-workers [42] who reported no influence after 4 weeks' supplementation with 8 g FOS on the stool weight, water percentage and stool frequency in six elderly persons.

3.4 Body Mass Management

The increasing evidence of obesity and overweight became a significant problem in the well-developed countries. It is speculated that the increased intake of dietary fibers can help in the body weight management. Thus, a growing number of studies have attempted to develop products with higher content of dietary fibers as well as to answer the question if dietary fibers are actually able to reduce obesity.

Single-blinded, crossover, placebo-controlled pilot study in ten healthy persons taking 8 g of oligofructose or placebo twice a day for 2 weeks was conducted by Cani and co-workers [63]. Morning consumption of ITF significantly improved satiety ($P = 0.04$), while after evening intake, significantly improved satiety and reduced hunger. The energy intake at breakfast and lunch and total daily energy intake were reduced in the group receiving oligofructose as compared to placebo group. Therefore, oligofructose was proposed as dietary supplement in obese people [63]. Similar findings were reported by Genta and co-authors [64], who stated that FOS can help in the obesity management. In their study, 0.29 g or 0.14 g FOS per kg per day in the form of yacon syrup, being a rich source of FOS, were taken for 120 days by 35 obese and slightly dyslipidemic premenopausal women divided into subgroups. The intake of FOS, resulted in reduced body weight, waist circumference, and body mass index (BMI). Moreover, women receiving yacon syrup had lower fasting serum insulin and serum LDL-cholesterol levels and reduced Homeostasis Model Assessment index [64]. Parnell and Reimer [65] examined the effect of oligofructose supplementation on body weight and the concentration of satiety hormones in overweight and obese adults. Forty-eight obese subjects, without any other health problems, ingested 21 g oligofructose or a placebo (maltodextrin) per day for 12 weeks. Supplementation of diet with oligofructose significantly reduced ($P = 0.01$) the body weight, while in the placebo group, the body weight increased up to 0.5 kg. The level of glucose and insulin decreased in the ITFs group and increased in placebo group. The profile of satiety hormone secretion changed after prebiotic intake with increased level of peptide YY and reduced ghrelin, suggesting the contribution in the reduction in energy intake. On the other hand, oligofructose intake did not affect the secretion of glucagon-like peptide 1 [65]. A randomized

double-blind, cross-over intervention with oligofructose (10 g/d or 16 g/d) or placebo for 13 days conducted in healthy adults with average BMI of 24.8 kg/m^3 have evidenced a 11% reduction in the energy intake in the 13th day as compared to the baseline in a group receiving oligofructose [66]. The difference in the energy intake was also noticed between both groups receiving different doses of oligofructose with 2801 kJ and 3177 kJ for 10 g and 16 g of oligofructose, respectively. The reduction of energy intake may be explained by higher secretions of peptide YY and glucagon-like peptide 1 [66]. The most recent randomized double-blind, placebo-controlled trial in obese and overweight children where Synergy 1 (8 g/d) was administered for 16 weeks, showed a significant increase in the feeling of fullness and lower prospective food consumption during breakfast in ITFs group at the end of experiment as compared to the baseline [67]. The energy intake was dependent on age, and 11–12 years old children receiving prebiotic had significantly reduced ($P = 0.04$) energy intake as compared to placebo group, while in younger children (7–10 years old) the difference was not detected. Moreover, the concentration of adiponectin and ghrelin was higher in the group receiving ITFs as compared to placebo [67].

Contrary to that, few studies reported no effect of ITFs supplementation of appetite control, and body weight management. Archer and co-authors [68] substituted fat in sausage patty with inulin and lupin-kernel fiber and evaluated their effect on palatability, perceptions of satiety, and food intake in 33 men. Inulin-enriched patty had no impact on satiety, while lupin-kernel patty was more satiating. However, the total fat intake was 18 g lower in group consuming inulin-enriched patty [68]. Another study with two doses of FOS per day (5 g at breakfast and 8 g 2 h before dinner) where applied to measure satiety [69]. Low morning dose did not affect satiety or food intake during a lunch, while a higher dose influenced satiety in the gender-dependent manner: in women the food intake decreased, however in men increased [69]. Another study also reported that bars enriched with 10 g of different fibers, including inulin and FOS, did not change satiety and food intake in women [70]. These divergent results could be explained by the differences in the DP and/or doses of applied ITFs. Therefore, this issue requires further studies.

3.5 Lipid Profile

Inadequate diet, rich in fat and sugar, with insufficient amount of dietary fiber is suggested to be a factor of cardiovascular diseases, in particular of hypertension, stroke, and heart failure [71]. Therefore, several studies were conducted to assess if changing of dietary habits or dietary supplements could help in reducing the risk of cardiologic conditions. So far, the studies directly evaluating the effect of ITFs on cardiovascular diseases have not been performed; however, their influences on the serum lipid profile have been examined widely.

The study of Letexier and co-workers [72] showed that 10 g of inulin per day significantly reduced the level of serum triglyceride and liver liponeogenesis, but at the same time, it did not affect total cholesterol, HDL- and LDL-cholesterol

concentrations [72]. Other results were obtained by Balcazar-Munoz and co-workers [73] in a 4-weeks randomized placebo-controlled trial in 12 obese individuals who received 7 g of inulin per day. Authors showed a significant decrease in total cholesterol, LDL-cholesterol, VLDL-cholesterol, and triglyceride levels as compared to placebo [73]. The study on healthy men (22 subjects) fed for 5 weeks with inulin-enriched pasta were performed by Russo and co-workers [74] and indicated an increase in HDL-cholesterol by 36% in the group fed with inulin-pasta. At the same time, total cholesterol to HDL cholesterol ratio, concentrations of triglycerides and lipoprotein (a) were reduced by 22%, 23%, and 17%, respectively. In other clinical study, 10% decrease in LDL-cholesterol was reported in type-2 diabetes patients with elevated lipid level after 14 days intake of 8 g/d of FOS [75].

On the other hand, no effect of ITFs on lipid profile was determined in many studies in healthy people [76–78]. The meta-analysis of randomized controlled trial showed that ITFs are able to reduce LDL-cholesterol across all study population, while HDL-cholesterol improvement and reduced fasting glucose trend was noticed only in the type-2 diabetes mellitus subgroup [79]. Therefore, further study on the effect of ITFs on lipid profile, especially studies explaining their dose- and DP-dependent influence are necessary for a definitive conclusion.

3.6 Mineral Absorption

Several in vitro and in vivo studies as well as in human trials have attempted to determine the effect of the intake of ITFs on mineral absorption. Recent in vitro study conducted by Krupa-Kozak and co-authors [80] evaluated the effect of inulin and FOS on calcium uptake and absorption from the calcium-enriched GF bread. The results showed that short-chain FOS improved the cellular calcium uptake from calcium-fortified GF bread. The authors concluded that the absorption of calcium is dependent of DP [80].

Rat model was used to assess the effect of ITFs with different chain length and their combinations on the calcium and magnesium absorption in a 50 male Wistar rats [81]. A significant improvement of magnesium absorption was reported for all ITFs formulations. In case of calcium, only the mixture of short-chain and long-chain ITFs increased absorption significantly; however, the increasing trend was observed also in single formulations [81]. Recent 6-week nutritional experiment on rat model was conduct by Krupa-Kozak and co-workers [34], who aimed to evaluate the effect of inulin of calcium absorption depending on the amount of this mineral in diet. The authors reported that inulin stimulated the absorption of calcium especially in rats fed a diet of reduced calcium level [34]. The results of this study are somehow in agreement with the results of a human trial on adolescent girls consuming a diet supplemented with oligofructose-enriched inulin [82, 83]. Synergy 1 intake in a dose of 8 g per day resulted in the improvement of calcium absorption in girls with normalized calcium intake and in girls with calcium deficiency; however, the beneficial effect was more prominent in the second group [82, 83].

Although the majority of in vivo studies are focused on the evaluation of the impact of ITFs on calcium and magnesium absorption, in one study the effect of 5 week supplementation of mixture of ITFs with different DP (Synergy 1) on iron utilization in piglets fed corn and soybean meal diet was assessed [84]. Final blood hemoglobin concentrations and the overall hemoglobin repletion efficiency of pigs increased significantly ($P < 0.01$) by 28% and 15%, respectively, in pigs supplemented with 4% of ITFs. Changes in the iron utilization were positively ($r = 0.55$ and 0.69, $P < 0.01$) correlated with dietary ITFs concentrations. Also iron concentration in the digesta of proximal, middle, and distal colon were significantly ($P < 0.05$) higher in pig supplemented with 4% ITFs [84].

The effect of ITFs on mineral absorption was evaluated in numerous human trials. Yap and co-workers [85] reported that absorption of iron, magnesium, and zinc were significantly improved in infants fed with inulin-enriched formulations. On the other hand, the retention of calcium and copper were not affected by inulin intake [85].

However, early studies showed that intake of 15 g of oligofructose per day improve the calcium absorption in adolescent girls [86]; numerous studies indicated that the most prominent effect on mineral absorption is observed with oligofructose-enriched inulin as compared to individual ITFs [81, 87, 88]. Supplementation for 6 week with 10 g of Synergy 1 per day resulted in the increase of fractional absorption of calcium and magnesium in 15 postmenopausal women as compared to control [89]. Moreover, the beneficial effect of ITFs was observed also in terms of bone turnover biomarkers, including serum osteocalcin and urinary deoxypyridinoline cross-link [89]. One year interventional randomized, placebo-controlled study showed that 8 g of Synergy 1 significantly improved calcium absorption in a group of 100 adolescents (9–13 years old) [87]. Authors emphasized that the significant increase in true calcium absorption was observed just after 8 weeks of trial, and it was maintained till the end of study in Synergy 1 group. At the end of the experiment, the bone condition parameters were evaluated and showed an improvement in bone mineral content and bone mineral density in ITFs group as compared to control [87].

4 Gluten and the Application of Gluten-Free Diet

4.1 Gluten

Gluten is a mixture of storage proteins found in the endosperm of the mature grain of wheat and some other cereals within the grasses family, including rye and barley. It consists mainly of protein (75–85%) and lipids (5–10%), while most of the remainder is starch and nonstarch carbohydrates, being the residue occurring after washed out the starch granules and other soluble substances from the wheat flour [90]. Gluten proteins are the main source of nitrogen for a growing seedling [91]. Mixture of gluten proteins is composed by monomeric gliadins and polymeric glutenins [92]. Gliadins and glutenins differ in their alcohol solubility. Gliadins are soluble in ethanol-water mixtures, while glutenins are insoluble. Gliadins belong to prolamins

of wheat. The prolamins present in rye, barley, and oats are secalins, hordeins, and avenins, respectively. Gliadins can be subdivided into α/β, γ, and ω-gliadins based on their electrophoretic mobility. α/β and γ-gliadins are low molecular weight proteins (MW 28–35 kDa) with six and eight cysteine residues respectively, whereas ω-gliadins (MW 40–75 kDa) are sulfur poor [90]. The structure of gliadins consists of approximately 300 amino acids with the high percentage of proline and glutamine and low amount of lysine and methionine [90]. The amino acid characteristic is responsible for the harmful effect of gliadins with 13- and 33-mer oligopeptides involved in the alleviated immunological response in celiac patients [93]. The glutenin fraction comprises aggregated proteins linked by inter-chain disulfide bonds; they have varying size ranging from about 500,000 to more than 10 million [94]. Amino acid characteristic of glutenins is similar to gliadins with high content of glutamine and proline [95]. The amount of gluten in wheat grain has changed in time. Genetic modification aimed to improve yielding of crops, result in the arriving of new varieties with multiplied genes also coding the gluten proteins. These changes in the wheat sensitivity are widely reviewed [91, 96].

4.2 Gluten-Related Disorders

A growing popularity of a gluten-free diet (GFD) is related with increasing incidence of gluten-related disorders [97]. In certain group of individuals, the intake of gluten can lead to the development of diseases, which can be divided into two main groups based on the pathogenesis: allergic and autoimmunological. Allergic diseases include wheat allergy (WA), while celiac disease (CD), *Dermatitis herpetiformis,* and ataxia belong to the second group. Nowadays, many cases of patients suffer from hard to diagnose disease, non-fitting to none of defined gluten-related disorders but for who treatment with GFD helps and are enclosed in the so-called non-celiac gluten sensitivity (NCGS).

4.2.1 Celiac Disease

Celiac disease is autoimmunological disease, observed in genetically predisposed individuals, resulting in the villous atrophy as a consequence of presence of gluten in diet. The diagnosed CD patients constitute approx. 1% of general population [98]; however, in many cases CD remain unrecognized or incorrectly diagnosed [99]. CD is observed mainly in highly developed countries due to the westernization of a diet [100]. CD can be diagnosed at every age, not as erroneously thought, only in children. The prevalence of CD is twice more frequent in women as compared to men [101].

Pathogenesis of CD is complex and includes genetic, environmental, immunological, metabolic, and infectious factors [102]. The genetic predisposition is inherited dominantly. Most of person diagnosed with CD contain genes of human leukocyte antigen (HLA): HLA DQ2 and HLA DQ8, placed on a chromosome 6, encoding proteins presenting gluten to lymphocyte CD4+ [103]. Approximately 90–95% of individuals with CD inherit alleles coding HLA-DQ2, while in majority

of the rest, HLA-DQ8 is detected [104]. It is suspected that also other genes may be involved in the pathogenesis of CD. The expression of HLA-DQ is necessary but not sufficient for the development of CD. Breast feeding, time of gluten introduction into the diet of the child, a history of infectious, certain medicals and smoking can be environmental factors contributing to the CD development [105–108]. Groups with higher risk of the incidence of CD include: relatives of CD patients and people suffering from type-1 diabetis mellitus, Hashimoto's thyroiditis, IgA deficiency, and genetic disorders such as Down's syndrome and Turner's syndrome [92].

The immune response to ingested gluten depends on the presentation of gluten peptides for lymphocytes T. In colon, the gluten protein undergoes deamidation by enzyme, tissue transglutaminase, with formation of strongly immunogenic peptides. Intraepithelial lymphocyte infiltrates and immune system stimulation manifested by increased production of interleukin 15 is observed in genetically predisposed individuals [109]. Activation of lymphocytes T, following the gluten peptide recognition, results in damage to the intestinal mucosa by direct action of inflammatory mediators and extracellular matrix metalloproteinases secreted by stimulated fibroblasts, leading to the development of inflammation of the endothelium and lamina propria of small intestine, cryptic hypertrophy, and the atrophy of intestinal villi [103, 110]. One of the consequences of morphological intestinal changes in CD is a limitation of the absorption process. Malabsorption is related to deficiencies of important alimentary nutrients, leading to growth deficit, anemia, vitamin deficiencies, thyroid diseases, mental disorders, fatigue, and infertility.

A wide range of manifestations of CD complicates its diagnosis. Based on the clinical picture, three main types can be distinguished: classic, atypical, and silent [111]. Some of researchers suggest also the addition of a term latent CD and a genetic susceptibility for CD to this classification. Classic CD is manifested by gastrointestinal symptoms such as diarrhea, fatty stools with bad odor, constipations, abdominal pain, loss of appetite, and weight loss. This CD type is diagnosed mainly in children. As compared to classic form, the atypical CD is more common, and it is diagnosed in older children, teenagers, and adults. Except of typical gastrointestinal symptoms, atypical CD can be manifested with verity of extraintestinal symptoms, including anemia, mental disorders, and bone alterations [112–114]. Silent CD is diagnosed accidently in relatives of CD patients or during screening studies [115].

The diagnosis of CD is confirmed by clinical picture, duodenal biopsy, genetic tests, and serological tests. Serological tests include measurements of several antibodies: anti-tissue transglutaminase (tTG), anti-endomisium (EMA), anti-gliadin (AGA) and anti-deaminated gliadin (DGP) [92].

4.2.2 Wheat Allergy

Wheat allergy is a classic form of food allergy being a defense response to a contact with wheat proteins exclusively. Proteins present in other cereals do not cause an immunological response in WA patients. The incidence of WA is estimated at 0.4% of worldwide population, wherein the most frequently is diagnosed in children [91, 116]. The frequency of the WA is age-dependent, in fact in 2 years old children WA incidence was estimated in 2%, while in older children it increased up to 9% [117].

Main signs of WA are skin lesions, typical for food allergies, and gastrointestinal symptoms such as diarrhea, abdominal pain, and constipation [118]. It is possible to distinguish few types of WA depending on the way of exposure to the allergen and the mechanism of immune response. First type is classic WA manifested by the gastrointestinal, respiratory, and skin symptoms; second is wheat-dependent, exercise-induced anaphylaxis (WDEIA), which appears after physical activity; and the last type is the baker's asthma, commonly appearing in the workers of bakeries and mills because of the exposition of the wheat particles irritating the respiratory track. The incidence of WA is the smallest among gluten-related disorders but the consequences of WA can be the most serious ones because of the possible anaphylactic shock, which can be fatal.

Gluten proteins are not the only triggers responsible for allergic reactions in WA. Another wheat proteins belonging to albumin and globulin group have similar ability. Gliadin ω-5 is the main allergen in WDEIA and skin allergies [119], while gliadin α- and γ- as well as protein ATI CM3 (amylase trypsin inhibitors), belonging to albumin group, are responsible for atopic dermatitis [120]. Baker's asthma is a response to the presence of low mass glutenins (α-, ω-gliadins), but the main role in the pathogenesis of Baker's asthma is played by several albumins and globulins, including ATI, lipid transporting proteins, and serpins [121].

Diagnosis of WA is based on the analysis of serum IgE concentration and skin tests. Because of the similarities of immunological epitopes in phylogenetically close varieties, the presence of cross-allergies with allergens of grass and weeds is possible. So far, the most reliable method of food allergies diagnosis is the challenge with allergy-causing food, and then attempt of its elimination from a diet [122]. Therefore, in the diagnosis of WA, gluten-challenge is applied.

4.2.3 Non-Celiac Gluten Sensitivity

Non-celiac gluten sensitivity belongs to gluten-related disorders; however, its characteristic and course does not fit CD or WA [97]. It is suspected that the main cause of NCGS is an imbalanced diet containing foods of high gluten content [123]. NCGS is diagnosed mainly in adults, particularly in female [124]. Individuals with irritable bowel syndrome (IBS) and allergy are particularly vulnerable to incidence of NCGS [125, 126]. High rate of NCGS has been reported in relatives of CD patients, amounting 13% [127]. NCGS patients suffer from symptoms similar to classic CD, including abdominal pain, diarrhea, as well as extraintestinal symptoms such as depression, fatigue, and musculoskeletal pain. In NCGS, symptoms appear in short time after gluten intake. Contrary to CD, NCGS patients do not have elevated concentration of antibodies characteristic for CD (anti-tTG, EMA). There are also no changes in the allergic tests including the measurement of immunoglobulins levels and skin tests. Therefore, the diagnosis of NCGS is based only on the elimination of CD and WA.

Pathogenesis of NCGS is not fully known; however, in its development an important role of the activation of innate immune response, changes in the intestinal barrier functioning and intake of food containing inhibitors of amylases was indicated [100, 127, 128]. It is also suspected that NCGS can be related with a high

intake of fermentable oligo-, di-, and monosaccharides and polyols (FODMAPs) [124]. Persons suffering from NCGS show markedly increased expression of toll-like receptors (TLR) involved in the innate gastrointestinal immune response and lower expression of FOXP3 Treg markers associating with the development of autoimmune diseases such as CD [100].

4.3 Gluten-Free Diet as a Treatment of Other Diseases

Nowadays, a GFD is considered as a treatment alleviating several symptoms and diseases. The attempts have been made to check the possibility of application of GFD in other diseases, in particular a GFD was proposed as a potential treatment for the selected symptoms of irritable bowel disease (IBD). A controlled trial of application of a GFD in patients with IBS-diarrhea indicated that a GFD can reduce the frequency and change the consistency of stool, improve the intestinal barrier functions, and alter rectosigmoid messenger RNA expression of tight junction proteins [129]. Gluten changed the level of various cytokines in peripheral blood mononuclear cells in IBD, and it was concluded that the extent of these alterations were associated with HLA-DQ2 or HLA-DQ8 genotype [129]. To identify the subgroup of IBD patients who potentially respond to a GFD, another approach proposed the evaluation of serum IgG antibodies against gliadin or tissue-transglutaminase in combination with HLA-DQ2 expression [130]. Moreover, an internet-based cross-sectional study utilizing a GFD questionnaire in 1647 patients with IBD reported that among the analyzed group 0.6% and 4.9% of participants were diagnosed with CD and NCGS, respectively [131]. Amongst the respondents, 19.1% had previously tried a GFD, while only 8.2% admitted currently following of GFD. What was interesting, 65.6% of participants reported an improvement of gastrointestinal symptoms after the elimination of gluten from the diet, and 38.3% described symptoms of disease as less frequent and severe [131]. Studies conducted on patients with fibromyalgia both with IBS or CD indicated that a GFD can slightly alleviate the symptoms of those diseases [132, 133].

Emerging evidence suggested the importance of insufficient enzymatic activity, increased gastrointestinal permeability, and the absorption of toxic byproducts of incompletely digested proteins from dairy (casein) and cereals (gluten) in the development of autistic spectrum disorders (ASD) [134]. Therefore, gluten-free and casein-free diets are proposed as a treatment of ASD. The results of the randomized single-blind trials indicated the improvement in the communication, social isolation, repetitive and challenging behavior, and better development in children on a GFD, casein-free diet as compared to those on control diet [135, 136]. On the other hand, another studies reported no significant ($P < 0.05$) differences in verbal and nonverbal communication and behavioral indexes in children following a GFD as compared to controls [137, 138]. The efficiency of a GFD in ASD was widely reviewed [134, 139].

4.4 Gluten-Free Diet as a Dietary Trend

The total occurrence of gluten-related disorders is observed in low percent of the whole population, meanwhile the increasing trend in the GF products consumption and thus production is noticed, projecting that the GF food market will reach 4.89 billion dollars in 2021 [140]. Surprisingly, an emerging number of healthy people begin a GFD, believing that gluten can have harmful health effects [141]. The prevalence of a GFD consumers in US society, who were not diagnosed in any of gluten-related disorders consists at least 0.5% [142]. Another study evaluating this trend showed that the prevalence of CD remained constant (0.58–0.77% of general population), while the amount of a GFD followers increase from 0.52% in 2009–2010 up to 1.69% in 2013–2014 [141]. Authors explained this rising GFD popularity phenomena by the popular opinion that gluten carries negative health effects, therefore a GFD is healthier than a conventional gluten-containing diet, easier access to GF products, and the increasing prevalence of self-diagnosed gluten-related disorders and following of diet without the consultation with physician [141]. Recent prospective cohort study performed in 64,714 women and 45,303 men responding for food frequency questionnaires for 26 years showed no relation between presence of gluten in food and the development of coronary heart disease [143]. On the contrary, authors suggest that a non-necessary GFD application is related with the avoidance of whole grains having beneficial effects on heart conditions, more likely may result in the coronary heart disease development. The authors underline that the promotion of a GFD among people without diagnosed gluten-related disorders should not be recommended [143].

4.5 Controversy Concerning a Gluten-Free Diet

GFD is based on the elimination of gluten-containing foodstuff from a daily regime. The commercially available GF products have higher content of fat and sugar as compared to corresponding gluten-containing counterparts [6]. Studies on children indicated significantly higher energy intakes in CD patients as compared to controls [144]. Additionally, GF products are poor in several important nutrients, especially proteins and mineral components, as well as non-nutritional but physiologically important components, like dietary fiber [6]. For that reason, the efforts have been made to improve the nutritional value of GF products by addition of non-gluten natural additives. Highly nutritional grains, like quinoa, teff, buckwheat, and amaranth, dietary ingredients, and mineral supplements were applied in the development of new gluten-free bakery goods [145–148]. These procedures resulted in improving the nutritional quality as well as augmented the technological properties of GF products.

The difficulty in the proper nutrients balance in the GFD is not the only problem. Recent studies indicated that a GFD can increase the exposure to certain toxins as compared to conventional diet. Fourfold increase in the serum levels of mercury in

CD patients following a GFD as compared to the healthy controls following regular diet was reported by Elli and co-workers [149]. The source of mercury in a GFD is not known; however, these results are worrisome because of the harmful effect caused by mercury, especially in the nervous system [150]. Recently, emerging number of evidence underlie the higher content of arsenic in rice [151]. Rice, rice flour, and other rice products are common replacement of wheat in a GFD; therefore they might elevate the arsenic levels in CD patients, but this must be studied.

Because of all abovementioned controversy of GFD, the proper dietary balance should be established with an assistance of a dietician to keep diversity of the diet [152] and the development of new, high-nutrition, and safe GF products should be explored.

5 Technological Importance of Gluten

Gluten plays an important role in the technology of the cereal-based food products. Due to its unique properties, gluten contributes to batter emulsification and visco-elasticity, provides cohesiveness to dough during processing, retains leavening gases, sets the crumb structure, and imparts elasticity to the bread texture [90, 153]. Gluten properties result of the ability of gliadins and glutenins to aggregate and form a network, the structure of which result from the presence of noncovalent (ionic, hydrogen, and hydrophobic) bonds [90]. The unique physiochemical properties of gluten derive from the presence of both protein fractions, equally affecting the quality of dough. Dough strength and elasticity is related with insoluble glutenin, being more elastic and cohesive. Properties of glutenins are correlated with the presence of disulfide bonds. When these bonds are reduced, glutenins become soluble in aqueous solutions of alcohols, such as gliadins, and they lose the strength features [90]. The soluble gliadin is mainly responsible for the viscosity and extensibility of the dough, because they are inflexible and less cohesive than glutenins. The viscosity is a consequence of high content of proline, allowing the dough to flow and rice [91]. Under conditions providing sufficient dough hydration and when the dough is optimally developed by the mixing process, the disruption of the initially spherical protein particles takes place, together with the stretching and alignment of proteins, which leads to the formation of a three-dimensional structure. By holding gases produced during dough proofing or fermentation, a gluten network allows bread to rise. Finally, during the baking, gluten changes its characteristics from elastic to semi rigid, contributing to the change from dough to crumb. The crumb structure is then formed. These functions of the gluten network give bread its chewy and elastic texture.

Many studies on gluten-free baking products have focused on the design of a GF matrix to overcome the negative impacts of the absence of the gluten network. The replacement of gluten is yet a technological challenge, as it is an essential structure-building protein which is necessary for formulating high-quality cereal-based goods, thus the production of such products is difficult.

A technology of GF baked products is different from the traditional processing of gluten-containing bakery foods. GF dough itself varies considerably from wheat dough, being closer to batter [154, 155]. The consistency of GF dough is greatly dependent on the amount of water or hydration, showing very low consistency during mixing when water adsorption is higher than 90% [156]. For that reason, GF dough does not require kneading, like conventional bread dough, and instead it is blended in a mixer [154]. Therefore, this dough is more like a batter of cake than bread dough [155]. Despite the intensive development of the GF production, many baked GF breads and confectionary products currently on sale are still of inferior quality. The lack of gluten makes it very difficult to obtain an acceptable texture and sufficient volume of baked goods because of the absence of a proper protein network necessary to hold the carbon dioxide produced during proofing [7]. The obtained GF products have a crumbling texture and pale crust [157, 158]. Another problem with GF-baked goods is the reduced volume of obtained loaves due to low carbon dioxide-binding activity during rising. Furthermore, baked GF products, mainly composed of different starches, stale very quickly and are characterized by reduced sensory qualities compared with their gluten-containing counterparts. Gluten is also responsible for the structure of dry pasta and its texture during cooking. A gluten network coagulates during cooking and creates strengthened network, preventing the starch granules to leach [159]. Lack of gluten network results in sticky pasta with poor consistency [159].

The imitation of viscoelastic properties of gluten is a key technological task, and numerous studies have therefore been conducted to overcome this problem. Hydrocolloids and gums, due to their water-binding capacity and ability to form a gel network, were proposed as substitutes for a gluten network to improve of the rheological properties, structure, mouth-feel, acceptability, and shelf life of GF-baked products [160, 161]. Enzymes were applied to improve the quality and extend the shelf-life of GF products [156, 162]. Positive effects were also obtained by using sourdough fermentation as an effective way to improve the texture, flavor, shelf-life, and nutritional value of bread [163].

Recently, Padalino and others [164] studied a range of vegetable flours in the formulation of GF spaghetti and indicated that yellow pepper pasta was the most desirable vegetable flour due to its orange color, homogeneity, and pleasant taste; additionally, yellow pepper pasta decreased the hardness of the pasta, compared to the control. Susanna and Prabhasankar [165] analyzed the inclusion of high protein flours (soya, channa, and sorghum flours) for the production of GF pasta and showed that formulations containing the highest level of soya flour and channa flour, along with hydrocolloids and whey protein concentrate, produced the best cooked pasta characteristics, having the lowest cooking loss, a soft texture, and a pasta with higher protein content compared to the control of *Triticum durum* flour.

Poor structure and texture are not the only problems of GF products. They are also characterized by inferior nutritional qualities. Therefore, to increase their palatability and dietary quality, aside from the typically used corn and rice, a wide range of naturally GF grains (oat, sorghum, and millet) and pseudocereals (buckwheat, amaranth, quinoa, and teff), rich in valuable proteins, minerals, dietary fibers,

and bioactive compounds, were proposed as GF substitutes [8, 145, 146, 166, 167]. Legumes, nut and carob germ, and chia containing important nutrients have been described as valuable components of GF baked products [168, 169]. Another important approach of the GF production is the addition of animal (dairy and egg proteins) and non-gluten plant proteins (zein), as those substances have both a nutritional and a technological role [156, 170–173]. Nowadays, a number of research have been conducted to evaluate the effect of ITFs on the quality and nutritional properties of GF products.

6 State of Art of Application of Inulin-Type Fructans in Gluten-Free Products

6.1 Inulin

The application of inulin and Jerusalem artichoke in the traditional bread of improved technological properties without compromised sensory acceptance [174, 175] has attracted scientists to explore widely this topic. This interest resulted in a number of researches aimed to utilize inulin in traditional bread making [176–179], as well as in other bakery products such as quick breads (scones) [180], pasta [181, 182], different types of cakes [183–185], muffins [24], biscuits [186], and extruded snacks [187].

Although the application of inulin in baked products resulted in decrease technological parameters (lower volume, darkening of the crust, increased crumb hardness, higher rate of staling), but it improved the palatability and flavor, therefore 5% inulin addition was assumed as a compromise between the improvement in nutritional and sensory quality and decreased technological features [177, 188]. However, a recent study indicated that it is possible to optimize the formulation with even 30% incorporation of fibers without compromising the quality of bread [179]. As a sweetener and fat replacement, ITFs were proposed as additive to gluten-containing confectionery products. Muffins with up to 50% fat substitution with inulin were characterized by increased fiber content, moisture, as well as crumb density and springiness [24]. In cakes, even 70% substitution of fat by inulin was possible, resulting in a product with acceptable sensory properties and reduced caloric value [189]. Incorporation of inulin in wheat pasta allowed to obtain pasta with improved nutritional value, without compromising the sensory and cooking quality [182, 190]. On the other hand, Bustos and co-workers [181] showed that inulin is completely lost during cooking and its incorporation into pasta affects negatively its technological properties.

The successful experience of inulin utilization in the traditional cereal products technology contributed to the development of studies on GF foodstuff. As bread is a staple food, consumed daily by the majority of population, thus the majority of research on GF product refers to it. The supplementation of GF bread with different percent of inulin showed that the amount of incorporated inulin plays the main role in changing the properties of GF bread [191]. Volume of loaves increased with the

increasing share of inulin, reaching even 9% increase in bread with 8% inulin addition. This phenomenon can be explained by high water binding capacity of inulin. Unfortunately, bread fortified with higher amount of inulin (8%) was characterized by reduced crumb cohesiveness and springiness and wrinkled crust classifying inulin-rich GF bread as second-grade quality product [191]. The enrichment of GF bread with inulin significantly ($P < 0.05$) reduced the crumb hardening rate as compared to the control non-fortified GF bread. The lower hardening can be attributed to the slower migration of water from crumb to crust. The results of this study showed that the GF bread preparation enriched with 5% of inulin allows to obtain the product with the highest quality.

Hager and co-workers [192] demonstrated that native inulin from chicory determined the rheological properties of fortified GF batter and consequently the quality of GF bread. Moisture content was 5% higher in the GF formulation fortified with inulin as compared to unfortified controls, suggesting that inulin microcrystals led to formation of a gel structure enclosing large amounts of water. During the formation of gel, water is immobilized in inulin macromolecular zones, enclosing large amounts of water, leading to less elastic and more viscous dough [192]. Rheological and viscoelastic properties of GF bread enriched with inulin were a focus of research of Juszczak and co-authors [193]. In their study, the increasing amount of inulin resulted in lower consistency and paste viscosity of the GF batter. The increase of the viscoelastic compliance values and gelatinization temperatures were also reported. Changes in the rheological properties can be associated with the reduced volume of starch granules and limited friction. Water binding capacity of inulin may reduce the swelling of starch granules affecting their size [193].

Chicory-fortified GF bread was characterized by darker crust being a consequence of Maillard reaction, attributed to a larger number of reducing ends formed due to degradation of inulin [192]. On the other hand, as compared to control, the obtained bread had an increased rate of staling and harder crumb, what can play critical role in the consumer acceptance [192]. Contrary results were obtained by Juszczak and co-workers [193], who reported reduction in crumb hardness in parallel with the increasing amount of the inulin addition, suggesting that it can be a method of extension of shelf-life of GF bread.

The addition of inulin to GF bread fortified with bovine plasma proteins were analyzed by Rodriguez Furlán, and co-workers [194]. The enrichment of GF bread with inulin resulted in decreased moisture loss what was attributed to greater number of hydrophilic groups in the inulin molecule and consequently higher water retention [195]. Reduced moisture loss contributed to delay the staling rate of GF bread. Additionally, the incorporation of inulin improved the quality of GF bread crumb by reducing its hardness [194]. It can be explained by uniformity and stabilization of the air bubbles diameter and decreasing the thickness of the walls surrounding the small air cells. The cracked surface of the crust of GF bread disappeared after inulin addition, making it smoother and the volume of loaves increased [194].

Recent studies of Scarini and co-authors [196] aimed to compare the effect of soluble and insoluble fibers addition on the technological and nutritional properties of GF bread, indicated that inulin used at 5% and 10% substitution level decreased

the dough firmness. Addition of inulin at 10% level resulted in GF bread with less compact crumb and the highest cell area of crumb as compared to control GF and GF breads supplemented with other fibers. The texture of loaves was also influenced by inulin addition with lower firmness and increased specific volume of bread in both inulin-enriched formulation. The proteins digestibility increased in GF bread with 5% substitution and then decreased in bread with 10% inulin level [196].

The popularity of confectionary products and their high amount of sugar motivated scientist to develop modified sweets with higher nutritional quality, including dietary fiber. In GF products, as gluten is replaced with fat and sugar to maintain the texture, the problem of unhealthy sweets is especially important. ITFs as fat and sugar substitute were proposed as additives to GF confectionary products. Maghaydah and co-workers [197] demonstrated that different levels of inulin increased moisture of GF cookies in parallel with increasing share of inulin. Moreover, inulin increased the dietary fiber content in fortified GF cookies. Cookies are considered as a good deliverer of nutritional components, especially for children, because of sweet taste, long shelf-life, and low cost. Additionally, the results showed no impact of inulin addition on sensory quality, including flavor, texture, aroma, and softness as well as the technological properties, especially width, thickness, and spread factor [197].

The effectiveness of application of Jerusalem artichoke powder, containing even 50–60% of inulin in dry mass, as a replacement of both fat and sugar in GF biscuits formulation have been reported by Sharoba and co-workers [198]. Jerusalem artichoke powder was used as 25%, 50%, 75%, and 100% substitution of sugar and corn oil in the GF formulation separately, indicating that inulin enriched product can be successfully replaced control biscuits. The inulin-enriched biscuits were characterized by 29% increase in protein and even fourfold increase in fiber contents than control unfortified GF biscuits. Additionally, the caloric value reduced with the increasing amount of inulin. The mineral content, including calcium, magnesium, and iron was 26, 54% and even twofold higher in inulin-rich biscuits as compared to control. The substitution of oil or sugar lower than 75% did not cause significant changes in both physical and sensory properties of biscuits but allowed them to deliver the recommended daily allowances (RDA) of protein, fiber, and minerals [198].

Gularte and co-workers [199] examined the effect of incorporation of both soluble or insoluble fibers and their mixes into a GF layer cake. The addition of blended soluble (inulin) and insoluble (oat fiber) fibers improved the technological properties, particularly specific volume of the fortified cake. Addition of inulin alone resulted in decreased properties of dough, including density and springiness, and reduced the amount of resistant starch [199]. It is well known that the amount of resistant starch is a parameter of concern in carbohydrate-based products [200].

Recent studies aimed to analyze the rheological parameters of chickpea flour-based GF muffins substituted with different concentrations of different biopolymers, including inulin [201]. Authors reported that an inulin-enriched batter was similar to batter supplemented with wheat protein in terms of heating pattern. The value of loss tangent during non-isothermal heating increased, resulting in more clear decreases in

elastic modulus as compared to dissipative modulus at early stage of heating. Higher values of loss tangent are associated with reduced viscoelasticity of batter [201].

Pasta is a staple food based on starches, being a good source of carbohydrates in human diet. Simplicity of cooking, storing, and handling supports the high popularity of this product. Majority of GF pasta is produced of corn and rice starch. Like other GF products, technological properties and nutritional value of GF pasta are poorer as compared to semolina-based product [9]. Therefore, many researchers have attempted to improve the properties of pasta by incorporation of additives having beneficial effect on texture with simultaneous revised impact of human health.

First, the use of inulin was proposed as a supplement of semolina-based pasta. The nutritional quality of inulin-enriched pasta improved by inhibiting the release of sugar during in vitro starch digestion is what reduced the predicted glycemic index by even 15% in formulation with 10% inulin concentration [202]. Inulin influenced the swelling index but did not affect the cooking loss of pasta, adhesiveness, and elasticity. Moreover, the incorporation of inulin reduced firmness of pasta, owing to the ability of inulin to compete with starch for available water, limiting the swelling and gelatinization of starch granules [202].

Contrary results were obtained in term of GF pasta. Mastromatteo and co-workers [203] prepared GF pasta based on maize starch with addition of inulin up to 20%, and they found that enrichment with 5% and 7.5% of inulin resulted in pasta with the highest values of elongation and shear viscosity. Authors stated that the incorporation of inulin over 12.5% increase the firmness of the pasta dough, and finally, too high share of inulin, amounting 15% and 20%, resulted in pasta with such high firmness that made impossible to conduct rheological analysis. However, the dough preparation might affect the rheological properties. The authors applied a pregelatinization process [203]. Consecutive heating and cooling of starch in water condition results in the breaking of amylose and amylopectin chains, and consequently their rearrangement into a crystalline form. It contributes to the retrogradation of starch and starts physical changes in the dough including higher viscosity, improved gel formation and increased degree of crystallinity and exudation of water [204]. The main difference in the starch retrogradation between GF and gluten-containing pasta can be related with the properties of proteins. Gluten proteins have been shown to have no impact on amylopectin; however, glutenin fraction was found to inhibit the starch retrogradation [205]. In contrast, another fractions of wheat protein, including gliadin, albumin and globulin have the ability to improve the retrogradation process [205]. The sensory analysis of dry and cooked gluten-free pasta enriched with inulin showed that these products had an improved quality, wherein the pasta fortified with 5% inulin was characterized by the best sensorial properties, being the most similar to control pasta [203]; however, the higher amount of inulin in spaghetti was associated with a decrease in sensory score, but these products were still acceptable.

Traditional white sauce is based on the wheat flour; however, people suffering from gluten-related disorders can replace it with non-gluten counterparts, such as rice and corn starches [206, 207]. Authors reported that inulin added to the gluten-

and lactose-free, fat-reduced white sauce based on rice starch improved gelatinization of the starch and reduced the viscosity [206]. Additionally, the high water-binding capacity of inulin inhibited starch degradation and hindered the leaching of amylose. Inulin added to sauce limited the formation of bonds between proteins and starch, and consequently, the sauce was more homogeneous. Another study of the same group of authors showed that also a corn starch-based white sauce can be successfully supplemented with inulin [207]. Inulin-enriched white starch-based sauces were characterized by acceptable sensory quality and stability during refrigerating, therefore they were proposed as a suitable substitute for people suffering from CD and lactose intolerance [206].

Tárrega and co-authors [20] studied the effect of fat substitution with different DP inulin on the quality of custard – creamy dessert based on milk or cream and egg yolk which can include also starch or gelatin. The rheological properties of analyzed custard based on hydroxypropyl-distarch phosphate from Tapioca starch and fortified with inulin significantly differed from whole-fat dessert. However, the inulin-enriched custard was characterized by higher thickness, sweetness, and vanilla flavor perception. The formulation with higher amount of inulin as compared to short-chain FOS (ratio 75:25) gave a product with the best texture and pseudoplasticity [20]. Another studies conducted on custard confirmed that inulin can positively influence texture of dessert, due to the water binding capacity of inulin allowing to maintain the thermally stable complexes [207, 208]. The inulin-enriched desserts were characterized by increase of creaminess and sensation of roughness, the improvement of consistency, and simultaneously the decrement of smoothness [20, 208].

In general, dairy products are naturally GF products consumed all over the world. The beneficial effects of dairy fat on human health as well as on the physiochemical and technological properties of dairy products are commonly known [210]. Recently, consumers are looking for low-fat and low-caloric products without compromising the texture and sensory properties of products. Long-chain inulin, having the ability to form crystals, is used as a texture-creator in low-fat dairy products. This ability was used in the studies conducted on acid casein-processed cheese analogs resulting in improved meltability, density, cohesiveness, and viscosity with simultaneous reduction in hardness and adhesiveness [211]. Another study confirmed that substitution of 10% of fat in cream cheese with inulin allow to obtain the product with chemical features similar to control high-fat cheese but with reduced caloric value [212]. Additionally, inulin incorporated into cheese reduced the rate of syneresis in spreads and fresh cheese [212]. Dave and co-workers [213] reported that the inulin percent of substitution of fat can affect the rheological properties of cheese spreads. They showed that 6% fat replacement result in nonacceptable cheese with a mushy texture. However, increasing concentration of inulin (7% and 8% of fat substitution) caused improvement of the spreadability, reaching the parameters close to a control full-fat cheese [213]. Moreover, inulin was applied to improve the nutritional properties of petit-suisse cheese due to the prebiotic effect [214]. Authors reported that inulin added simultaneously with probiotics resulted in the most promising formulation having the potential health benefit.

The stability of inulin in moderate pH condition was used in studies on fermented milk resulting in the increase in the total solid contents leading to increase of the acidity of fermented product. The modified fermented milk was characterized by higher viscosity as compared to control unfortified milk. Additionally, thixotropy of inulin-rich fermented milk increased in parallel with increasing temperature, while hysteresis decreased [215].

6.2 FOS

Short-chain ITFs are characterized by completely different properties as compared to long-chain inulin; therefore, the effect of their incorporation into GF products is diverse. The influence of the addition of ITFs with different DP into GF bread on the physiochemical properties and staling rate was studied by Ziobro and co-workers [216]. The application of FOS significantly ($P < 0.05$) increased GF bread volume in parallel with the increasing amount of the ITF addition. Authors explained it by the competition for unbound water between short-chain ITFs, consisting of mono- and oligosaccharides, and starch, resulting in the delay of starch gelatinization, and consequently the increase of loaves volume. FOS improved porosity and uniformity of bread crumb and reduced staling rate during storage [216]. The authors concluded that FOS can be successfully used to improve the technological quality of GF bread, while the inulin with high DP can be used in bakery technology only to improve the nutritional value, when the improvement in the structure and mechanical properties in not necessary.

Short-chain FOS is characterized by a higher sweetness which can influence the sensory perception of GF products with their application. The study conducted by Morais and co-workers [217] evaluated the effect of different ITFs on the sensory properties of GF bread, using a quantitative descriptive analysis (QDA) performed by a trained sensory panel or consumer tests of CD patients. FOS-enriched GF bread was characterized by better taste and aroma as well as improved appearance parameters such as porosity, texture, and crust color [217].

Cassava starch is used for making special cheese GF bread, popular in the countries of South America [218]. FOS was proposed as a supplement of this special type of GF bread influencing water absorption index and starch solubility. The pasting viscosity decreased with increasing concentration of FOS in GF bread formulation due to higher solubility of FOS as compared to starch. Authors reported changes in GF cheese bread with 9% FOS addition; however, still this amount was not sufficient to consider FOS-enriched bread as a good source of dietary fiber [218].

Addition of 6% of short-chain FOS into custard improved consistency, viscosity, and elasticity of dessert [20, 208, 209]. However, authors stated that these effects were strongly dependent on the presence of carrageenan. High water-binding capacity of water-soluble FOS contributes to increased viscosity [20]. Sensory analysis showed that FOS-enriched custard is characterized by higher sweetness and intensive flavor [20, 208].

FOS can be also used as sugar substitute in low-caloric yoghurt [219]. The increasing concentration of FOS (up to 8%) affects the physicochemical and rheological properties of yoghurt, increasing thixotropy, viscosity, as well as storage and loss moduli. Texture of FOS-enriched yoghurt has been characterized as a weak gel. The authors evaluated also post-acidification of FOS-supplemented yoghurts showing no changes in acidity, what is desirable in modern yoghurts and concluded that FOS was not utilized by applied bacteria (*Streptococcus thermofilus* and *Lactobacillus bulgaricus*) as a carbon source. Survival analysis of consumer acceptance showed that increasing amount of FOS reduced the sensory quality of yoghurt. The addition of approx. 2.6% of FOS was reported as the most accurate for yoghurts [219].

6.3 Synergy Between Inulin and FOS

As short-chain FOS and long-chain inulin differ in their properties, studies were performed aiming to evaluate the effect of a mixture of both fractions to obtain product with improved properties. Commercially available oligofructose-enriched inulin (Synergy 1) was used in a study on GF bread [220]. In general, the technological properties of GF bread supplemented with Synergy 1 were improved. Irrespective of the amount of fructans added to a formulation, breads have higher specific volume which could be attributed to higher CO_2 retention capacity. Additionally, ITFs containing reducing sugars positively affected the crust color of gluten-free bread, what resulted from the formation of brown nitrogenous polymers and melanoidins as the ends products of Maillard reaction during baking [220]. Moisture of Synergy 1-enriched batter and bread was reduced, leading to faster staling. The authors also confirmed the improvement of the sensory qualities of gluten-free breads fortified with ITFs. Irrespective of the amount of fructans added, the enriched breads received higher scores in terms of appearance, color, texture, and taste. The nutritional quality of GF bread was improved after addition of Synergy 1 in term of higher concentration of dietary fiber and reduced glycemic index (from 71 to 48) and glycemic load (from 12 to 8) in formulation with 12% addition of ITFs [220].

Synergistic effect of FOS and inulin was used also to improve the quality of chocolate dairy dessert [221]. Sensory analysis showed that formulation with 7.5% of ITFs and 0.2% guar gum had the best scores in terms of appearance, aroma, taste, texture, and overall acceptability of chocolate dairy desserts. On the contrary, addition of 10% of ITFs decreased dramatically the sensory acceptance of that product. The study indicated that the moderate concentration of ITFs (7.5%) and guar gum (0.2%) was the most appropriate in term of creaminess of chocolate evaluated using a 9-point just-about-right (JAR) scale [221].

Table 2 Summarized effect of the addition of ITFs with diversified DP on the quality parameters of gluten-free products.

Product	Percent of additive	Effect	Reference
Inulin			
Bread	3–8	Increased loaves volume Reduced crumb cohesiveness and springiness Wrinkled crust Reduced hardening	[190]
Bread	9	Increased moisture Increased specific volume Darker crust Reduced hardness	[191]
Bread	4–12	Lower dough consistency and paste viscosity Increased the viscoelastic compliance values Increased gelatinization temperatures Reduced crumb hardness	[192]
Bread	0.5–3.5 PUFI[a]	Reduced moisture loss Reduced staling Increased loaves volume Improved crumb color and porosity	[193]
Bread	5, 10	Reduced dough firmness Improved porosity Increased specific volume Reduced firmness	[195]
Cookies	3–4.5	Increased moisture Increased content of dietary fiber No influence on sensorial quality	[196]
Biscuits	25–100% sugar and oil substitution	Improved protein and dietary fiber content Reduced caloric value Improved mineral content No influence of physical and sensorial quality	[197]
Layer cake	20 or mixed with oat fiber (1:3 ratio)	Decreased density and springiness (inulin alone) Reduced starch resistance (inulin) Improved specific volume (inulin + oat fiber)	[198]
Muffins	5–15	Heating pattern similar to wheat protein-containing dough Reduced viscoelasticity of batter	[200]
Pasta	2.5–10	Reduced predicted glycemic index Reduced firmness Reduced starch swelling and gelatinization No effect on cooking loss, adhesiveness and elasticity	[201]

(*continued*)

Table 2 (continued)

Product	Percent of additive	Effect	Reference
Pasta	5–20	5–7.5% – improved elongation and shear viscosity Over 12.5% – increased firmness Improved sensory quality	[202]
White sauce	2.5	Improved gelatinization Reduced viscosity Reduced starch degradation Improved homogeneity	[205, 206]
Custard	7.5	Increased thickness, sweetness and vanilla flavor perception Improved texture and pseudoplasticity Increased creaminess and smoothness	[20, 207, 208]
Cheese analogs	1–3	Improved meltability, density, cohesiveness, viscosity Reduced hardness and adhesiveness	[210]
Cream cheese	8–12	Cheese with reduced caloric value but with properties similar to full-fat cheese Reduced syneresis rate	[211]
Cheese	6–8	Mushy texture (6%) Improved spreadability (7–8%)	[212]
Petit-suisse cheese	10	Stimulation of growth of probiotic bacteria	[213]
Fermented milk	5	Improved viscosity Increasing thixotropy and decreasing hysteresis parallel with increasing temperature	[214]
FOS			
Bread	3–8	Increased loaves volume (5–8%) Increased hardness (5–8%) Decreased volume and hardness (3%)	[190]
Bread	4–12	Increased loaves volume Delayed starch gelatinization Improved porosity and uniformity Reduced staling	[215]
Bread	0.75	Improved sensory characteristic in terms of taste and aroma Improved porosity, texture and crust color	[216]
Cheese bread	9–29	Increased starch solubility and decreased water absorption index Decreased specific volume	[217]
Custard	7.5	Improved consistency, viscosity and elasticity Higher sweetness and intensified flavor	[20, 207]
Yoghurt	2–8	Increased thiotrophy and viscosity Reduced texture Reduced sensory perception	[218]

(*continued*)

Table 2 (continued)

Product	Percent of additive	Effect	Reference
Synergy 1			
Bread	4–12	Reduced moisture Increased volume and specific volume of loaves Darker crumb and crust color Reduced glycemic index and increased dietary fiber content Improved sensory perception	[219]
Chocolate dairy dessert	5–10	Acceptable sensory properties and creaminess	[220]

[a]A formulation of ultrafiltred and freeze-dried bovine plasma with the addition of inulin (63%)

7 Conclusions

The increasing demand on high-quality GF products and an increasing prevalence of GF consumers favors the development of research aimed to improve the overall quality of GF products. The literature data on the application of ITFs in the GF products and on their health beneficial properties, as presented in this chapter, points to a great potential of ITFs in the GF living. In this chapter, we have presented the most recent studies on GF products in which ITFs were applied as valuable ingredients affecting the rheological and technological parameters of GF products (Table 2). ITFs added to GF products interact with other ingredients and additives, but, in general, they improved the sensory perception of obtained GF products. The evidences of beneficial impact of ITFs on characteristic of GF goods presented in this chapter are promising and therefore, could contribute to further development and intensified research on new GF products of superior quality that will be dedicated to people suffering from gluten-related disorders.

References

1. Siró I, Kápolna E, Kápolna B, Lugasi A (2008) Functional food. Product development, marketing and consumer acceptance – a review. Appetite 51(3):456–467
2. Martorell R, Ascencio M, Tascan L, Alfaro T, Young MF, Addo OY, Dary O, Flores-Ayala R (2015) Effectiveness evaluation of the food fortification program of Costa Rica: impact on anemia prevalence and hemoglobin concentration in women and children. Am J Clin Nutr 101:201–217
3. Sicherer SH, Sampson HA (2014) Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. J Allergy Clin Immunol Pract 133:291–307
4. Saturni L, Ferretti G, Bacchetti T (2010) The gluten-free diet: safety and nutritional quality. Forum Nutr 2:16–34

5. Ilus T, Kaukinen K, Virta LJ, Pukkala E, Collin P (2014) Incidence of malignancies in diagnosed celiac patients: a population-based estimate. Am J Gastroenterol 109(9):1471–1477
6. Matos ME, Rosell CM (2011) Chemical composition and starch digestibility of different gluten free breads. Plant Foods Hum Nutr 66:224–230
7. Gallagher E, Gormley TR, Arendt EK (2004) Recent advances in the formulation of gluten-free cereal-based products. Trends Food Sci Technol 15:143–152
8. Giménez-Bastida JA, Piskuła MK, Zieliński H (2015) Recent advances in development of gluten-free buckwheat products. Trends Food Sci Technol 44:58–65
9. Drabińska N, Zieliński H, Krupa-Kozak U (2016) Technological benefits of inulin-type fructans application in gluten-free products – a review. Trends Food Sci Technol 56:149–157
10. Van Laere A, Van Den Ende W (2002) Inulin metabolism in dicots: chicory as a model system. Plant Cell Environ 25:803–813
11. Shoaib M, Shehzad A, Omar M, Rakha A, Raza H, Sharif HR, Shakeel A, Ansari A, Niazi S (2016) Inulin: properties, health benefits and food applications. Carbohydr Polym 147:444–454
12. Kelly G (2008) Inulin-type prebiotics: a review: part 1. Altern Med Rev 13(4):315–329
13. Bosscher D (2009) Fructan prebiotics derived from inulin. In: Charalampopoulos D, Rastall A (eds) Prebiotics and probiotics science and technology. Springer, New York
14. Barclay T, Ginic-Markovic M, Cooper P, Petrovsky N (2010) Inulin – a versatile polysaccharide with multiple pharmaceutical and food chemical uses. J Excipients Food Chem 1(3):27–50
15. Ronkart SN, Blecker CS, Fourmanoir H, Fougnies C, Deroanne C, Van Herck JC, Paquot M (2007) Isolation and identification of inulooligosaccharides resulting from inulin hydrolysis. Anal Chim Acta 604(1):81–87
16. Ozimek LK, Kralj S, Van der Maarel MJ, Dijkhuizen L (2006) The levansucrase and inulosucrase enzymes of lactobacillus reuteri 121 catalyse processive and non-processive transglycosylation reactions. Microbiology 152:1187–1196
17. Mensink MA, Frijlink HW, Maarschalk KV, Hinrichs WLJ (2015) Inulin, a flexible oligosaccharide I: review of its physicochemical characteristics. Carbohydr Polym 130:405–419
18. Apolinario AC, Damasceno BPGD, Beltrao NED, Pessoa A, Converti A, da Silva JA (2014) Inulin-type fructans: a review on different aspects of biochemical and pharmaceutical technology. Carbohydr Polym 101:368–378
19. Livingston DP, Hincha DK, Heyer AG (2009) Fructan and its relationship to abiotic stress tolerance in plants. Cell Mol Life Sci 66(13):2007–2023
20. Tarrega A, Rocafull A, Costell E (2010) Effect of blends of short and long-chain inulin on the rheological and sensory properties of prebiotic low-fat custards. LWT-Food Sci Technol 43(3):556–562
21. Lopez-Molina D, Navarro-Martinez MD, Melgarejo FR, Hiner ANP, Chazarra S, Rodriguez-Lopez JN (2005) Molecular properties and prebiotic effect of inulin obtained from artichoke (Cynara Scolymus L.) Phytochemistry 66(12):1476–1484
22. Garcia ML, Caceres E, Selgas MD (2006) Effect of inulin on the textural and sensory properties of mortadella, a Spanish cooked meat product. Int J Food Sci Technol 41(10):1207–1215
23. Capriles VD, Soares RAM, Silva MEMPE, Areas JAG (2009) Effect of fructans-based fat replacer on chemical composition, starch digestibility and sensory acceptability of corn snacks. Int J Food Sci Technol 44(10):1895–1901
24. Zahn S, Pepke F, Rohm H (2010) Effect of inulin as a fat replacer on texture and sensory properties of muffins. Int J Food Sci Technol 45(12):2531–2537
25. Beriain MJ, Gomez I, Petri E, Insausti K, Sarries MV (2011) The effects of olive oil emulsified alginate on the physico-chemical, sensory, microbial, and fatty acid profiles of low-salt, inulin-enriched sausages. Meat Sci 88(1):189–197
26. Hinrichs WLJ, Prinsen MG, Frijlink HW (2001) Inulin glasses for the stabilization of therapeutic proteins. Int J Pharm 215(1–2):163–174

27. Imran S, Gillis RB, Kok MS, Harding SE, Adams GG (2012) Application and use of inulin as a tool for therapeutic drug delivery. Biotechnol Genet Eng Rev 28:33–45
28. Roberfroid MB (2007) Inulin-type fructans: functional food ingredients. J Nutr 137(11):2493–2502
29. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125(6):1401–1412
30. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G (2017) Expert consensus document: the international scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 14(8):491–502
31. Slavin J (2013) Fiber and prebiotics: mechanisms and health benefits. Forum Nutr 5:1417–1435
32. Madrigal L, Sangronis E (2007) Inulin and derivates as key ingredients in functional foods. Arch Latinoam Nutr 57(4):387–396
33. Langlands SJ, Hopkins MJ, Coleman N, Cummings JH (2004) Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. Gut 53(11):1610–1616
34. Krupa-Kozak U, Markiewicz L, Lamparski G, Juśkiewicz J (2017) Administration of inulin-supplemented gluten-free diet modified calcium absorption and caecal microbiota in rats in a calcium-dependent manner. Forum Nutr 9(7):702
35. Zhu L, Qin S, Zhai S, Gao Y, Li L (2017) Inulin with different degrees of polymerization modulates composition of intestinal microbiota in mice. FEMS Microbiol Lett 364(10):fnx075
36. Gibson GR, Beatty ER, Wang X, Cummings JH (1995) Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 108(4):975–982
37. Buddington RK, Williams CH, Chen SC, Witherly SA (1996) Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. Am J Clin Nutr 63:709–716
38. Rao VA (2001) The prebiotic properties of oligofructose at low intake levels. Nutr Res 21:843–848
39. Tuohy KM, Finlay RK, Wynne AG, Gibson GR (2001) A human volunteer study on the prebiotic effects of HP-inulin – faecal bacteria enumerated using fluorescent in situ hybridization (FISH). Anaerobe 7:113–118
40. Salazar N, Dewulf EM, Neyrinck AM, Bindels LB, Cani PD, Mahillon J, de Vos WM, Thissen JP, Gueimonde M, de Los Reyes-Gavilán CG, Delzenne NM (2015) Inulin-type fructans modulate intestinal Bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. Clin Nutr 34(3):501–507
41. Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, Hardt PD (2010) Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring) 18(1):190–195
42. Bouhnik Y, Raskine L, Champion K, Andrieux C, Penven S, Jacobs H, Simoneau G (2007) Prolonged administration of low-dose inulin stimulates the growth of bifidobacteria in humans. Nutr Res 27:187–193
43. Kleessen B, Schwarz S, Boehm A, Fuhrmann H, Richter A, Henle T, Krueger M (2007) Jerusalem artichoke and chicory inulin in bakery products affect faecal microbiota of healthy volunteers. Br J Nutr 98:540–549
44. Institute of Medicine, Food and Nutrition Board (2002) Dietary reference intakes: energy, carbohydrates, fiber, fat, fatty acids, cholesterol, protein and amino acids. National Academies Press, Washington, DC
45. Turner ND, Lupton JR (2011) Dietary fiber. Adv Nutr 2(2):151–152
46. Anderson JW, Baird P, Davis RH, Ferreri S, Knudtson M, Koraym A, Waters V, Williams CL (2009) Health benefits of dietary fiber. Nutr Rev 67:188–205
47. Romo C, Mize K, Warfel K (2008) Addition of hi-maize, natural dietary fiber, to a commercial cake mix. J Am Diet Assoc 108:76–77
48. Roberfroid M, Slavin J (2000) Nondigestible oligosaccharides. Crit Rev Food Sci Nutr 40(6):461–480

49. Cherbut C (2002) Inulin and oligofructose in the dietary fibre concept. Br J Nutr 87(Suppl 2): S159–S162
50. Kleessen B, Hartmann L, Blaut M (2003) Fructans in the diet cause alterations of intestinal mucosal architecture, released mucins and mucosa-associated bifidobacteria in gnotobiotic rats. Br J Nutr 89(5):597–606
51. Strugala V, Allen A, Dettmar PW, Pearson JP (2003) Colonic mucin: methods of measuring mucus thickness. Proc Nutr Soc 62(1):237–243
52. Liu TW, Cephas KD, Holscher HD, Kerr KR, Mangian HF, Tappenden KA, Swanson KS (2016) Nondigestible fructans alter gastrointestinal barrier function, gene expression, histomorphology, and the microbiota profiles of diet-induced obese C57BL/6J mice. J Nutr 146(5):949–956
53. Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, Van der Meer R (2003) Dietary fructo-oligosaccharides dose-dependently increase translocation of salmonella in rats. J Nutr 133:2313–2318
54. Bovee-Oudenhoven IM, ten Bruggencate SJ, Lettink-Wissink ML, van der Meer R (2003) Dietary fructo-oligosaccharides and lactulose inhibit intestinal colonisation but stimulate translocation of salmonella in rats. Gut 52:1572–1578
55. Barrat E, Michel C, Poupeau G, David-Sochard A, Rival M, Pagniez A, Champ M, Darmaun D (2008) Supplementation with galactooligosaccharides and inulin increases bacterial translocation in artificially reared newborn rats. Pediatr Res 64(1):34–39
56. Jain PK, McNaught CE, Anderson AD, MacFie J, Mitchell CJ (2004) Influence of synbiotic containing Lactobacillus acidophilus La5, Bifidobacterium lactis Bb 12, Streptococcus thermophilus, Lactobacillus bulgaricus and oligofructose on gut barrier function and sepsis in critically ill patients: a randomised controlled trial. Clin Nutr 23:467–475
57. Olguin F, Araya M, Hirsch S, Brunser O, Ayala V, Rivera R, Gotteland M (2005) Prebiotic ingestion does not improve gastrointestinal barrier function in burn patients. Burns 31:482–488
58. Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, Katan MB, van der Meer R (2006) Dietary fructooligosaccharides affect intestinal barrier function in healthy men. J Nutr 136:70–74
59. Russo F, Linsalata M, Clemente C, Chiloiro M, Orlando A, Marconi E, Chimienti G, Riezzo G (2012) Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers. Nutr Res 32(12):940–946
60. Duggan C, Penny ME, Hibberd P, Gil A, Huapaya A, Cooper A, Coletta F, Emenhiser C, Kleinman RE (2003) Oligofructose-supplemented infant cereal: 2 randomized, blinded, community-based trials in Peruvian infants. Am J Clin Nutr 77:937–942
61. Kleessen B, Sykura B, Zunft HJ, Blaut M (1997) Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. Am J Clin Nutr 65:1397–1402
62. Moore N, Chao C, Yang L, Storm H, Oliva-Hemker M, Saavedra JM (2003) Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. Br J Nutr 90:581–587
63. Cani PD, Joly E, Horsmans Y, Delzenne NM (2006) Oligofructose promotes satiety in healthy human: a pilot study. Eur J Clin Nutr 60(5):567–572
64. Genta S, Cabrera W, Habib N, Pons J, Carillo IM, Grau A, Sánchez S (2009) Yacon syrup: beneficial effects on obesity and insulin resistance in humans. Clin Nutr 28(2):182–187
65. Parnell JA, Reimer RA (2009) Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. Am J Clin Nutr 89(6):1751–1759
66. Verhoef SP, Meyer D, Westerterp KR (2011) Effects of oligofructose on appetite profile, glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. Br J Nutr 106(11):1757–1762

67. Hume MP, Nicolucci AC, Reimer RA (2017) Prebiotic supplementation improves appetite control in children with overweight and obesity: a randomized controlled trial. Am J Clin Nutr 105(4):790–799. https://doi.org/10.3945/ajcn.116.140947
68. Archer BJ, Johnson SK, Devereux HM, Baxter AL (2004) Effect of fat replacement by inulin or lupin-kernel fibre on sausage patty acceptability, post-meal perceptions of satiety and food intake in men. Br J Nutr 91(4):591–599
69. Hess JR, Birkett AM, Thomas W, Slavin JL (2011) Effects of short-chain fructooligosaccharides on satiety responses in healthy men and women. Appetite 56(1):128–134
70. Karalus M, Clark M, Greaves KA, Thomas W, Vickers Z, Kuyama M, Slavin J (2012) Fermentable fibers do not affect satiety or food intake by women who do not practice restrained eating. J Acad Nutr Diet 112(9):1356–1362
71. Mozaffarian D, Ludwig DS (2015) Dietary cholesterol and blood cholesterol concentrations-reply. JAMA 314(19):2084–2085
72. Letexier D, Diraison F, Beylot M (2003) Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentrations in humans. Am J Clin Nutr 77:559–564
73. Balcazar-Munoz BR, Martinez-Abundis E, Gonzalez-Ortiz M (2003) Effect of oral inulin administration on lipid profile and insulin sensitivity in subjects with obesity and dyslipidemia. Rev Med Chil 131:597–604
74. Russo F, Chimienti G, Riezzo G, Pepe G, Petrosillo G, Chiloiro M, Marconi E (2008) Inulin-enriched pasta affects lipid profile and Lp(a) concentrations in Italian young healthy male volunteers. Eur J Nutr 47(8):453–459
75. Yamashita K, Kawai K, Itakura M (1984) Effects of fructooligosaccharides on blood glucose and serum lipids in diabetic subjects. Nutr Res 4:961–966
76. Luo J, Rizkalla SW, Alamowitch C, Boussairi A, Blayo A, Barry JL, Laffitte A, Guyon F, Bornet FR, Slama G (1996) Chronic consumption of short-chain fructooligosaccharides by healthy subjects decreased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism. Am J Clin Nutr 63:939–945
77. Pedersen A, Sandstrom B, van Amelsvoort JM (1997) The effect of ingestion of inulin on blood lipids and gastrointestinal symptoms in healthy females. Br J Nutr 78:215–222
78. van Dokkum W, Wezendonk B, Srikumar TS, van den Heuvel EG (1999) Effect of non-digestible oligosaccharides on large-bowel functions, blood lipid concentrations and glucose absorption in young healthy male subjects. Eur J Clin Nutr 53:1–7
79. Liu F, Prabhakar M, Ju J, Long H, Zhou HW (2017) Effect of inulin-type fructans on blood lipid profile and glucose level: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr 71(1):9–20
80. Krupa-Kozak U, Świątecka D, Bączek N, Brzóska MM (2016) Inulin and fructooligosaccharide affect in vitro calcium uptake and absorption from calcium-enriched gluten-free bread. Food Funct 7:1950–1958
81. Coudray C, Tressol JC, Gueux E, Rayssiguier Y (2003) Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats. Eur J Nutr 42:91–98
82. Griffin IJ, Davila PM, Abrams SA (2002) Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes. Br J Nutr 87(Suppl 2):S187–S191
83. Griffin IJ, Hicks PMD, Heaney RP, Abrams SA (2003) Enriched chicory inulin increases calcium absorption mainly in girls with lower calcium absorption. Nutr Res 23:901–909
84. Yasuda K, Roneker KR, Miller DD, Welch RM, Lei XG (2006) Supplemental dietary inulin affects the bioavailability of iron in corn and soybean meal to young pigs. J Nutr 136(12):3033–3038
85. Yap KW, Mohamed S, Yazid AM, Maznah I, Meyer DM (2005) Dose response effects of inulin on fecal short-chain fatty acids content and mineral absorption of formula fed infants. Nutr Food Sci 35:208–219

86. van den Heuvel EG, Muys T, van Dokkum W, Schaafsma G (1999) Oligofructose stimulates calcium absorption in adolescents. Am J Clin Nutr 69:544–548
87. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, Ellis KJ (2005) A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am J Clin Nutr 82:471–476
88. Legette LL, Lee WH, Martin BR, Story JA, Campbell JK, Weaver CM (2012) Prebiotics enhance magnesium absorption and inulin-based fibres exert chronic effects on calcium utilization in a postmenopausal rodent model. J Food Sci 77(4):H88–H94
89. Holloway L, Moynihan S, Abrams SA, Kent K, Hsu AR, Friedlander AL (2007) Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in postmenopausal women. Br J Nutr 97:365–372
90. Wieser H (2007) Chemistry of gluten proteins. Food Microbiol 24(2):115–119
91. Kucek LK, Veenstra LD, Amnuaycheewa P, Sorrells ME (2015) A grounded guide to gluten: how modern genotypes and processing impact wheat sensitivity. Compr Rev Food Sci Food Saf 14:285–302
92. Elli L, Villalta D, Roncoroni L, Barisani D, Ferrero S, Pellegrini N, Bardella MT, Valiante F, Tomba C, Carroccio A, Bellini M, Soncini M, Cannizzaro R, Leandro G (2017) Nomenclature and diagnosis of gluten-related disorders: a position statement by the Italian Association of Hospital Gastroenterologists and Endoscopists (AIGO). Dig Liver Dis 49(2):138–146
93. Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Auricchio S, Picard J, Osman M, Quaratino S, Londei M (2003) Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. Lancet 362:30–37
94. Wieser H, Bushuk W, MacRitchie F (2006) The polymeric glutenins. In: Wrigley C, Bekes F, Bushuk W (eds) Gliadin and glutenin: the unique balance of wheat quality. American Association of Cereal Chemistry, St. Paul
95. Jansens KJA, Lagrain B, Rombouts I, Brijs K, Smet M, Delcour JA (2011) Effect of temperature, time and wheat gluten moisture content on wheat gluten network formation during thermomolding. J Cereal Sci 54(3):434–441
96. Malalagoda M, Simsek S (2017) Celiac disease and cereal proteins. Food Hydrocoll 68:108–113
97. Arranz E, Fernandez-Bañares F, Rosell CM, Rodrigo L, Peña AS (2015) Advances in the understanding of gluten related pathology and the evolution of gluten-free foods. OmniaScience, Barcelona. http://www.omniascience.com/monographs/index.php/monograficos/issue/view/24
98. Ludvigsson JF, Card TR, Kaukinen K, Bai J, Zingone F, Sanders DS, Murray JA (2015) Screening for celiac disease in the general population and in high-risk groups. United European Gastroenterol J 3(2):106–120
99. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA, Horvath K (2003) Prevalence of coeliac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med 163:286–292
100. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PHR, Hadjivassiliou M, Kaukinen K, Rostami K, Sanders DS, Schumann M, Ullrich R, Villalta D, Volta U, Catassi C, Fasano A (2012) Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med 10:13
101. Megiorni F, Mora B, Bonamico M, Barbato M, Montuori M, Viola F, Trabace S, Mazzilli MC (2008) HLA-DQ and susceptibility to celiac disease: evidence for gender differences and parent-of-origin effects. Am J Gastroenterol 103(4):997–1003
102. Grzymisławski M, Stankowiak-Kulpa H, Włochal M (2010) Celiakia – standardy diagnostyczne i terapeutyczne 2010 roku. Forum Zaburzeń Metabolicznych 1(1):12–21
103. Troncone R, Ivarsson A, Szajewska H, Mearin ML (2008) Review article: future research on coeliac disease – a position report from the European multistakeholder platform on coeliac disease (CDEUSSA). Aliment Pharmacol Ther 27(11):1030–1043

104. Johnson TC, Diamond B, Memeo L, Negulescu H, Hovhanissyan Z, Verkarre V, Rotterdam H, Fasano A, Caillat-Zucman S, Grosdidier E, Winchester R, Cellier C, Jabri B, Green PH (2004) Relationship of HLA-DQ8 and severity of celiac disease: comparison of New York and Parisian cohorts. Clin Gastroenterol Hepatol 2:888–894
105. Akobeng AK, Ramanan AV, Buchan I, Heller RF (2006) Effect of breast feeding on risk of coeliac disease: a systematic review and meta-analysis of observational studies. Arch Dis Child 91:39–43
106. Plot L, Amital H (2009) Infectious associations of coeliac disease. Autoimmun Rev 8:316–319
107. Cammarota G, Cuoco L, Cianci R, Pandolfi F, Gasbarrini G (2000) Onset of coeliac disease during treatment with interferon for chronic hepatitis C. Lancet 356:1494–1545
108. Vazquez H, Smecuol E, Flores D, Mazure R, Pedreira S, Niveloni S, Mauriño E, Bai JC (2001) Relation between cigarette smoking and coeliac disease: evidence from a case-control study. Am J Gastroenterol 96:798–802
109. Mention JJ, Ben Ahmed M, Bègue B, Barbe U, Verkarre V, Asnafi V, Colombel JF, Cugnenc PH, Ruemmele FM, McIntyre E, Brousse N, Cellier C, Cerf-Bensussan N (2003) Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. Gastroenterology 125(3):730–745
110. Di Sabatino A, Corraza GR (2009) Coeliac disease. Lancet 373:1480–1493
111. Bai JC, Fried M, Corazza GR, Schuppan D, Farthing M, Catassi C, Greco L, Cohen H, Ciacci C, Fasano A, González A, Krabshuis JH, LeMair A (2013) World Gastroenterology Organisation global guidelines on celiac disease. J Clin Gastroenterol 47:121–126
112. Rajalahti T, Repo M, Kivelä L, Huhtala H, Mäki M, Kaukinen K, Lindfors K, Kurppa K (2017) Anemia in pediatric celiac disease: association with clinical and histological features and response to gluten-free diet. J Pediatr Gastroenterol Nutr 64(1):e1–e6
113. Smith D, Gerdes L (2012) Meta-analysis on anxiety and depression in adult celiac disease. Acta Psychiatr Scand 125:183–193
114. Krupa-Kozak U (2014) Pathologic bone alterations in celiac disease: etiology, epidemiology, and treatment. Nutrition 30:16–24
115. Iwańczak F, Iwańczak B (2012) Nowe wytyczne dotyczące diagnostyki i leczenia choroby trzewnej u dzieci i młodzieży. Prz Gastroenterol 7(4):85–191
116. Zuidmeer L, Goldhahn K, Rona RJ, Gislason D, Madsen C, Summers C, Sodergren E, Dahlstrom J, Lindner T, Sigurdardottir ST, McBride D, Keil T (2008) The prevalence of plant food allergies: a systematic review. J Allergy Clin Immunol 121(5):1210–1218
117. Matricardi PM, Bockelbrink A, Beyer K, Keil T, Niggemann B, Grüber C, Wahn U, Lau S (2008) Primary versus secondary immunoglobulin E sensitization to soy and wheat in the multi-centre allergy study cohort. Clin Exp Allergy 38:493–500
118. Hischenhuber C, Crevel R, Jarry B, Mäki M, Moneret-Vautrin DA, Romano A, Troncone R, Ward R (2006) Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. Aliment Pharmacol Ther 23:559–575
119. Morita E, Matsuo H, Chinuki Y, Takahashi H, Dahlstrom J, Tanaka A (2009) Food-dependent exercise-induced anaphylaxis importance of omega-5 gliadin and HMW-glutenin as causative antigens for wheat-dependent exercise-induced anaphylaxis. Allergol Int 58:493–498
120. Tanabe S (2004) IgE-binding abilities of pentapeptides, QQPFP and PQQPF, in wheat gliadin. J Nutr Sci Vitaminol 50:367–370
121. Sandiford CP, Tatham AS, Fido R, Welch JA, Jones MG, Tee RD, Shewry PR, Newman Taylor AJ (1997) Identification of the major water/salt insoluble wheat proteins involved in cereal hypersensitivity. Clin Exp Allergy 27:1120–1129
122. Matuszewska E, Kaczmarski M (1999) Próby prowokacji pokarmowej w diagnostyce alergii/nietolerancji pokarmowej u dzieci. Alergia Astma Immunologia 4:245–249
123. Gibert A, Espadaler M, Angel Camela M, Sanches A, Vague C, Refecas M (2006) Consumption of gluten free products: should be the threshold value for traces amounts of gluten be at 20, 100 czy 200 p.p.m? Eur J Gastroenterol Hepatol 18:1187–1195

124. Stępień M, Bogdański P (2013) Nadwrażliwość na gluten – fakty i kontrowersje. Forum Zaburzeń Metabolicznych 4(4):183–191
125. Massari S, Liso M, De Santis L, Mazzei F, Carlone A, Mauro S, Musca F, Bozzetti MP, Minelli M (2011) Occurrence of nonceliac gluten sensitivity in patients with allergic disease. Int Arch Allergy Immunol 155:389–394
126. Mastrototaro L, Castellaneta S, Gentile A (2012) Gluten sensitivity in children: clinical, serological, genetic and histological description of the first pediatric series. Dig Liver Dis 44:254–255
127. Volta U, de Gorgio R (2012) New understanding of gluten sensitivity. Nat Rev Gastroenterol Hepatol 9:295–299
128. Junker Y, Zeissig S, Kim SJ, Barisani D, Wieser H, Leffler DA, Zevallos V, Libermann TA, Dillon S, Freitag TL, Kelly CP, Schuppan D (2012) Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor. J Exp Med 209(13):2395–2408
129. Vazquez-Roque MI, Camilleri M, Smyrk T, Murray JA, Marietta E, O'Neill J, Carlson P, Lamsam J, Janzow D, Eckert D, Burton D, Zinsmeister AR (2013) A controlled trial of gluten-free diet in patients with irritable bowel syndrome-diarrhea: effects on bowel frequency and intestinal function. Gastroenterology 144(5):903–911
130. Wahnschaffe U, Schulzke JD, Zeitz M, Ullrich R (2007) Predictors of clinical response to gluten-free diet in patients diagnosed with diarrhea-predominant irritable bowel syndrome. Clin Gastroenterol Hepatol 5(7):844–850
131. Herfarth HH, Martin CF, Sandler RS, Kappelman MD, Long MD (2014) Prevalence of a gluten free diet and improvement of clinical symptoms in patients with inflammatory bowel diseases. Inflamm Bowel Dis 20(7):1194–1197
132. Rodrigo L, Blanco I, Bobes J, de Serres FJ (2014) Effect of one year of a gluten-free diet on the clinical evolution of irritable bowel syndrome plus fibromyalgia in patients with associated lymphocytic enteritis: a case-control study. Arthritis Res Ther 16(4):421
133. Rodrigo L, Blanco I, Bobes J, de Serres FJ (2013) Clinical impact of a gluten-free diet on health-related quality of life in seven fibromyalgia syndrome patients with associated celiac disease. BMC Gastroenterol 13:157
134. Mulloy A, Lang R, O'Reilly M, Sigafoos J, Lancioni G, Rispoli M (2010) Gluten-free and casein-free diets in the treatment of autism spectrum disorders: a systematic review. Res Autism Spectr Disord 4(3):328–339
135. Knivsberg AM, Reichelt KL, Høien T, Nødland M (2002) A randomised, controlled study of dietary intervention in autistic syndromes. Nutr Neurosci 5(4):251–261
136. Knivsberg AM, Reichelt KL, Høien T, Nødland M (2003) Effect of a dietary intervention on autistic behavior. Focus Autism Other Dev Disabl 18(4):248–257
137. Elder JH, Shankar M, Shuster J, Theriaque D, Burns S, Sherrill L (2006) The gluten-free, casein-free diet in autism: results of a preliminary double blind clinical trial. J Autism Dev Disord 36(3):413–420
138. Seung H, Rogalski Y, Shankar M, Elder J (2007) The gluten- and casein-free diet and autism: communication outcomes from a preliminary double-blind clinical trial. J Med Speech-Lang Pathol 15(4):337–345
139. Millward C, Ferriter M, Calver SJ, Connell-Jones GG (2008) Gluten- and casein-free diets for autistic spectrum disorder. Cochrane Database Syst Rev 2:CD003498
140. Transparency Market Research (2015) Gluten free food market – global industry analysis, size, share, growth, trends, and forecast, 2015–2021. http://www.transparencymarketresearch.com/GF-products-market.html Published Date 21.10.2015. Accessed 22 July 2016
141. Kim HS, Patel KG, Orosz E, Kothari N, Demyen MF, Pyrsopoulos N, Ahlawat SK (2016) Time trends in the prevalence of celiac disease and gluten-free diet in the US population: results from the National Health and Nutrition Examination Surveys 2009–2014. JAMA Intern Med 176(11):1716–1717
142. Digiacomo DV, Tennyson CA, Green PH, Demmer RT (2013) Prevalence of gluten-free diet adherence among individuals without celiac disease in the USA: results from the continuous

National Health and Nutrition Examination Survey 2009–2010. Scand J Gastroenterol 48:921–925
143. Lebwohl B, Cao Y, Zong G, Hu FB, Green PHR, Neugut AI, Rimm EB, Sampson L, Dougherty LW, Giovannucci E, Willett WC, Sun Q, Chan AT (2017) Long term gluten consumption in adults without celiac disease and risk of coronary heart disease: prospective cohort study. BMJ 357:j1892
144. Zuccotti G, Fabiano V, Dilillo D, Picca M, Cravidi C, Brambilla P (2012) Intakes of nutrients in Italian children with celiac disease and the role of commercially available gluten-free products. J Hum Nutr Diet 26:436–444
145. Alvarez-Jubete L, Auty M, Arendt EK, Gallagher E (2010) Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. Eur Food Res Technol 230(3):437–445
146. Krupa-Kozak U, Wronkowska M, Soral-Śmietana M (2011) Effect of buckwheat flour on microelements and proteins contents in gluten-free bread. Czech J Food Sci 29(2):103–108
147. Krupa-Kozak U, Altamirano-Fortoul R, Wronkowska M, Rosell CM (2012) Breadmaking performance and technological characteristicof gluten-free bread with inulin supplemented with calcium salts. Eur Food Res Technol 235(3):545–554
148. Brito IL, de Souza EL, Felex SSS, Madruga MS, Yamashita F, Magnani M (2015) Nutritional and sensory characteristics of gluten-free quinoa (Chenopodium quinoa Willd)-based cookies development using an experimental mixture design. J Food Sci Technol 52(9):5866–5873
149. Elli L, Rossi V, Conte D, Ronchi A, Tomba C, Passoni M, Bardella MT, Roncoroni L, Guzzi G (2015) Increased mercury levels in patients with celiac disease following a gluten-free regimen. Gastroenterol Res Pract 2015:953042
150. Sanfeliu C, Sebastià J, Kim SU (2001) Methylmercury neurotoxicity in cultures of human neurons, astrocytes, neuroblastoma cells. Neurotoxicology 22(3):317–327
151. Lai PY, Cottingham KL, Steinmaus C, Karagas MR, Miller MD (2015) Arsenic and rice: translating research to address health care providers' needs. J Pediatr 167:797–803
152. Reilly NR (2016) The gluten-free diet: recognizing fact, fiction, and fad. J Pediatr 175:206–210
153. Delcour JA, Joye IJ, Pareyt B, Wilderjans E, Brijs K, Lagrain B (2011) Wheat gluten functionality as a quality determinant in cereal-based food products. Annu Rev Food Sci Technol 3:469–492
154. Moore MM, Schober TJ, Dockery P, Arendt EK (2004) Textural comparisons of gluten-free and wheat-based doughs, batters, and breads. Cereal Chem 81(5):567–575
155. Houben A, Hochstotter A, Becker T (2012) Possibilities to increase the quality in gluten-free bread production: an overview. Eur Food Res Technol 235(2):195–208
156. Marco C, Rosell CM (2008) Breadmaking performance of protein enriched, gluten-free breads. Eur Food Res Technol 227(4):1205–1213
157. Gallagher E, Gormley TR, Arendt EK (2003) Crust and crumb characteristics of gluten free breads. J Food Eng 56:153–161
158. Primo-Martin C, de Pijpekamp AV, van Vliet T, de Jongh HHJ, Plijter JJ, Hamer RJ (2006) The role of the gluten network in the crispness of bread crust. J Cereal Sci 43(3):342–352
159. Marti A, Pagani MA (2013) What can play the role of gluten in gluten free pasta? Trends Food Sci Technol 31(1):63–71
160. Lazaridou A, Duta D, Papageorgiou M, Belc N, Biliaderis CG (2007) Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. J Food Eng 79:1033–1047
161. Hager AS, Arendt EK (2013) Influence of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten-free breads based on rice, maize, teff and buckwheat. Food Hydrocoll 32(1):195–203
162. Renzetti S, Rosell CM (2016) Role of enzymes in improving the functionality of proteins in non-wheat dough systems. J Cereal Sci 67:35–45

163. Moroni AV, Dal Bello F, Arendt EK (2009) Sourdough in gluten-free bread-making: an ancient technology to solve a novel issue? Food Microbiol 26(7):676–684
164. Padalino L, Mastromatteo M, Lecce L, Cozzolino F, Del Nobile MA (2013) Manufacture and characterization of gluten-free spaghetti enriched with vegetable flour. J Cereal Sci 57(3):333–342
165. Susanna S, Prabhasankar P (2013) A study on development of gluten free pasta and its biochemical and immunological validation. LWT-Food Sci Technol 50(2):613–621
166. Schober TJ, Messerschmidt M, Bean SR, Park SH, Arendt EK (2005) Gluten-free bread from sorghum: quality differences among hybrids. Cereal Chem 82:394–404
167. Gawlik-Dziki U, Dziki D, Swieca M, Seczyk L, Rozylo R, Szymanowska U (2015) Bread enriched with Chenopodium quinoa leaves powder – the procedures for assessing the fortification efficiency. LWT-Food Sci Technol 62(2):1226–1234
168. Ouazib M, Garzón R, Zaidi F, Rosell CM (2016) Germinated, toasted and cooked chickpea as ingredients for breadmaking. J Food Sci Technol 53(6):2664–2672
169. Tsatsaragkou K, Kara T, Ritzoulis C, Mandala I, Rosell CM (2017) Improving carob flour performance for making gluten-free breads by particle size fractionation and jet milling. Food Bioprocess Technol 10:831–841
170. Gallagher E, Kenny S, Arendt EK (2005) Impact of dairy protein powders on biscuit quality. Eur Food Res Technol 221:237–243
171. Schober TJ, Bean SR, Boyle DL, Park SH (2008) Improved viscoelastic zein-starch doughs for leavened gluten-free breads: their rheology and microstructure. J Cereal Sci 48:755–767
172. Krupa-Kozak U, Bączek N, Rosell C (2013) Application of dairy proteins as technological and nutritional improvers of calcium-supplemented gluten-free bread. Forum Nutr 5(11):4503–4520
173. Ziobro R, Juszczak L, Witczak M, Korus J (2016) Non-gluten proteins as structure forming agent in gluten-free bread. J Food Sci Technol 53(1):571–580
174. Praznik W, Cieslik E, Filipiak-Florkiewicz A (2002) Soluble dietary fibres in Jerusalem artichoke powders: composition and application in bread. Nahrung/Food 46(3):151–157
175. Wang J, Rosell CM, de Barber CB (2002) Effect of the addition of different fibres on wheat dough performance and bread quality. Food Chem 79(2):221–226
176. Peressini D, Sensidoni A (2009) Effect of soluble dietary fibre addition on rheological and breadmaking properties of wheat doughs. J Cereal Sci 49:190–201
177. Skara N, Novotni D, Cukelj N, Smerdel B, Curi D (2013) Combined effects of inulin, pectin and guar gum on the quality and stability of partially baked frozen bread. Food Hydrocoll 30:428–436
178. Ronda F, Quilez J, Pando V, Roos YH (2014) Fermentation time and fiber effects on recrystallization of starch components and staling of bread from frozen part-baked bread. J Food Eng 131:116–123
179. Arufe S, Chiron H, Dore J, Savary-Auzeloux I, Saulnier L, Della Valle G (2017) Processing & rheological properties of wheat flour dough and bread containing high levels of soluble dietary fibres blends. Food Res Int 97:123–132
180. Rößle C, Ktenioudaki A, Gallagher E (2011) Inulin and oligofructose as fat and sugar substitutes in quick breads (scones): a mixture design approach. Eur Food Res Technol 233:167
181. Bustos MC, Pérez GT, León AE (2011) Effect of four types of dietary fiber on the technological quality of pasta. Food Sci Technol Int 17(3):213–221
182. Padalino L, Costa C, Conte A, Melilli MG, Sillitti C, Bognanni R, Raccuia SA, Del Nobile MA (2017) The quality of functional whole-meal durum wheat spaghetti as affected by inulin polymerization degree. Carbohydr Polym 173:84–90
183. Volpini-Rapina LF, Sokei FR, Conti-Silva AC (2012) Sensory profile and preference mapping of orange cakes with addition of prebiotics inulin and oligofructose. LWT-Food Sci Technol 48:37–42

184. Celik I, Isik F, Gursoy O, Yilmaz Y (2012) Use of Jerusalem artichoke (Helianthus tuberosus) tubers as a natural source of inulin in cakes. J Food Process Preserv 37:483–488
185. Zbikowska A, Marciniak-Lukasiak K, Kowalska M, Onacik-Gür S (2017) Multivariate study of inulin addition on the quality of sponge cakes. Pol J Food Nutr Sci 67(3):201–210
186. Serial MR, Blanco Canalis MS, Carpinella M, Valentinuzzi MC, León AE, Ribotta PD, Acosta RH (2016) Influence of the incorporation of fibers in biscuit dough on proton mobility characterized by time domain NMR. Food Chem 192:950–957
187. Peressini D, Foschia M, Tubaro F, Sensidoni A (2015) Impact of soluble dietary fibre on the characteristics of extruded snacks. Food Hydrocoll 43:73–81
188. Morris C, Morris GA (2012) The effect of inulin and fructo-oligosaccharide supplementation on the textural, rheological and sensory properties of bread and their role in weight management: a review. Food Chem 133(2):237–248
189. Rodríguez-García J, Puig A, Salvador A, Hernando I (2012) Optimization of a sponge cake formulation with inulin as fat replacer: structure, physicochemical, and sensory properties. J Food Sci 77(2):C189–C197
190. Aravind N, Sissons MJ, Fellows CM, Blazek J, Gilbert EP (2012) Effect of inulin soluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti. Food Chem 132(2):993–1002
191. Korus J, Grzelak K, Achremowicz K, Sabat R (2006) Influence of prebiotic additions on the quality of gluten-free bread and on the content of inulin and fructooligosaccharides. Food Sci Technol Int 12(6):489–495
192. Hager AS, Liam AM, Schwab C, Gänzle MG, O'Doherty AEK (2011) Influence of the soluble fibres inulin and oat b-glucan on quality of dough and bread. Eur Food Res Technol 232:405–413
193. Juszczak L, Witczak T, Ziobro R, Korus J, Cieslik E, Witczak M (2012) Effect of inulin on rheological and thermal properties of gluten-free dough. Carbohydr Polym 90(1):353–360
194. Rodriguez Furlan LT, Padilla AP, Campderrós ME (2015) Improvement of gluten-free bread properties by the incorporation of bovine plasma proteins and different saccharides into the matrix. Food Chem 170:257–264
195. Rosell CM, Rojas JA, de Barber CB (2001) Influence of hydrocolloids on dough rheology and bread quality. Food Hydrocoll 15:75–81
196. Sciarini LS, Bustos MC, Vignola MB, Paesani C, Salinas CN, Pérez GT (2017) A study on fibre addition to gluten free bread: its effects on bread quality and in vitro digestibility. J Food Sci Technol 54(1):244–252
197. Maghaydah S, Abdul-Hussain S, Ajo R, Obeidat B, Tawalbeh Y (2013) Enhancing the nutritional value of gluten-free cookies with inulin. Adv J Food Sci Technol 5(7):866–870
198. Sharoba AM, El-Salam AM, Hafez HH (2014) Production and evaluation of gluten free biscuits as functional foods for celiac disease patients. J Agroaliment Process Technol 20(3):203–214
199. Gularte MA, de la Hera E, Gomez M, Rosell CM (2012) Effect of different fibers on batter and gluten-free layer cake properties. LWT-Food Sci Technol 48(2):209–214
200. Fardet A, Leenhardt F, Lioger D, Scalbert A, Remesy C (2006) Parameters controlling the glycaemic response to breads. Nutr Res Rev 19(1):18–25
201. Alvarez MD, Cuesta FJ, Herranz B, Canet W (2017) Rheometric non-isothermal gelatinization kinetics of chickpea flour-based gluten-free muffin batters with added biopolymers. Foods 6(1):3
202. Brennan CS, Kuri V, Tudorica CM (2004) Inulin-enriched pasta: effects on textural properties and starch degradation. Food Chem 86(2):189–193
203. Mastromatteo M, Iannetti M, Civica V, Sepielli G, Del Nobile MA (2012) Effect of the inulin addition on the properties of gluten free pasta. Food Nutr Sci 3:22–27
204. Hoover R, Hughes T, Chung HJ, Liu Q (2010) Composition molecular structure, properties, and modification of pulse starches: a review. Food Res Int 43:399–413

205. Lian XJ, Guo JJ, Wang DL, Lin L, Zhu JR (2014) Effects of protein in wheat flour on retrogradation of wheat starch. J Food Sci 79:C1505–C1511
206. Guardeño LM, Puig A, Hernando I, Quiles A (2013) Effect of different corn starches on microstructural, physical and sensory properties of gluten-free white sauces formulated with soy protein and inulin. J Food Process Eng 36(4):535–543
207. Guardeño LM, Vazquez-Gutierrez JL, Hernando I, Quiles A (2013) Effect of different rice starches, inulin, and soy protein on microstructural, physical, and sensory properties of low-fat, gluten, and lactose free white sauces. Czech J Food Sci 31(6):575–580
208. Gonzalez-Tomas L, Bayarri S, Costell E (2009) Inulin-enriched dairy desserts: physicochemical and sensory aspects. J Dairy Sci 92(9):4188–4199
209. Gonzalez-Tomas L, Bayarri S, Coll-Marques J, Costell E (2009) Flow behaviour of inulin-enriched dairy desserts: influence of inulin average chain length. Int J Food Sci Tech 44(6):1214–1222
210. de Morais EC (2016) Prebiotic addition in dairy products: processing and health benefits. In: Watson RR, Preevdy VR (eds) Probiotics, prebiotics, and synbiotics: bioactive foods in health promotion, 1st edn. Elsevier Int, Amsrerdam
211. Solowiej B, Glibowski P, Muszynski S, Wydrych J, Gawron A, Jelinski T (2015) The effect of fat replacement by inulin on the physicochemical properties and microstructure of acid casein processed cheese analogues with added whey protein polymers. Food Hydrocoll 44:1–11
212. Fadaei V, Poursharif K, Daneshi M, Honarvar M (2012) Chemical characteristics of low-fat wheyless cream cheese containing inulin as fat replacer. Eur J Exp Biol 2:690–694
213. Dave P (2012) Rheological properties of low-fat processed cheese spread made with inulin as a fat replacer. University of Wisconsin-Stout
214. Cardarelli HR, Saad SMI, Gibson GR, Vulevic J (2007) Functional petit-suisse cheese: measure of the prebiotic effect. Anaerobe 13:200–207
215. Debon J, Prudencio ES, Petrus JCC (2010) Rheological and physico-chemical characterization of prebiotic microfiltered fermented milk. J Food Eng 99:128–135
216. Ziobro R, Korus J, Juszczak L, Witczak T (2013) Influence of inulin on physical characteristics and staling rate of gluten-free bread. J Food Eng 116(1):21–27
217. Morais EC, Cruz AG, Faria JAF, Bolini HMA (2014) Prebiotic gluten-free bread: sensory profiling and drivers of liking. LWT-Food Sci Technol 55(1):248–254
218. Rodriguez-Sandoval E, Franco CML, Manjarres-Pinzon K (2014) Effect of fructooligosaccharides on the physicochemical properties of sour cassava starch and baking quality of gluten-free cheese bread. Starch-Starke 66(7–8):678–684
219. Cruz AG, Cavalcanti RN, Guerreiro LMR, Sant'Ana AS, Nogueira LC, Oliveira CAF, Deliza R, Cunha RL, Faria JAF, Bolini HMA (2013) Developing a prebiotic yogurt: rheological, physico-chemical and microbiological aspects and adequacy of survival analysis methodology. J Food Eng 114:323–330
220. Capriles VD, Areas JAG (2013) Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance and glycemic response of gluten-free breads. Food Funct 4(1):104–110
221. Morais EC, Morais AR, Cruz AG, Bolini HMA (2014) Development of chocolate dairy dessert with addition of prebiotics and replacement of sucrose with different high-intensity sweeteners. J Dairy Sci 97:2600–2609

Development of Dietary Fiber-Rich Meat Products: Technological Advancements and Functional Significance

26

Nitin Mehta, Manish Kumar Chatli, Pavan Kumar, Om Prakash Malav, Akhilesh Kumar Verma, Yogesh Kumar, and Dinesh Kumar

Contents

N. Mehta (✉) · M. K. Chatli · P. Kumar · O. P. Malav · D. Kumar
Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India
e-mail: nmvets220@gmail.com; mkchatli@gadvasu.in; pavankumar@gadvasu.in; drommalav@gmail.com; dr.d.sharma26@gmail.com

A. K. Verma
Department of Livestock Products Technology, College of Veterinary Science, Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya evam Go Anusandhan Sansthan (DUVASU), Mathura, Uttar Pradesh, India
e-mail: vetakhilesh@gmail.com

Y. Kumar
ICAR – Central Institute of Post-Harvest Engineering and Technology (CIPHET), Ludhiana, Punjab, India
e-mail: ysomvanshi@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_9

Abstract

As a source of proteins having high biological value, essential nutrients like minerals, vitamins, and surfeit of bioactive compounds, meat is considered as an ideal food for masses. With the advancement of technology and change in socioeconomic status, the consumers have housed high preference for processed and value-added meat products. These products generally lack nutrients like complex carbohydrates that include dietary fiber. Fiber is considered as an important component in diet owing to its multifarious utilities as cardioprotective, weight reducer, management of diabetes, antioxidant, and stress reliever. Its inclusion in diet has been stressed in many studies and incorporation in products particularly high energy-dense products like meat is imperative. For adults, the recommended acceptable intakes of dietary fiber are 28–36 g/day and out of that, 70–80% must be insoluble fiber. Apart from acting as an integral fraction of diet, dietary fiber performs many functions in meat products, viz., improvement in yield, desirable processing attributes, fat reduction, texture modification, etc. Being a by-product of agriculture, these fiber sources are comparatively cheaper and its inclusion on meat products helps in reducing its overall cost of production. Thus, the inclusion of fiber in meat products helps in improving processing and technological functionality with proven health benefits for consumers.

Keywords

Dietary fiber · Meat · Technological · Processing · Cooking yield · Sensory

1 Introduction

An increasing awareness among people regarding their health and well-being has propelled the research in food science to a new level. The inclination to natural sources has been increased in recent times owing to many nutritional diseases of affluence that have posed a challenge to both processors as well as medical professionals [1]. However, rapid urbanization and globalization has introduced ready-to-eat, ready-to-cook, and packaged processed products on consumer's platter. These products are rich in fat, salt, and calories but are highly deficient in dietary fiber. Earlier, unrefined grains and vegetables which contain a significantly high amount of fiber were an essential dietary component but the industrial revolution brought a dynamic change in lifestyle resulting in removal of key components from diet, particularly fiber [2]. The extensive study of dietary fiber in recent times could be directly attributed to its multifunctional roles. An improvement in technological and processing characteristics of products along with beneficial physiological effects like lowering blood sugar, cholesterol, improving cardiac health, etc., led the researchers to search new sources of fiber which can be readily incorporated in food products [3].

Meat is considered as a nutrient-dense food item with a high amount of protein, fat, minerals, and vitamins that have a comparatively better bioavailability than other

food stuffs [4]. However, a damaging crusade against meat as a culprit for many health hazards has blemished its image to great extent. The deficiency of complex carbohydrates like dietary fiber in meat has been implicated with an increase in number of diseases like colon cancer and cardiovascular diseases [5]. A solution that has been proposed is enrichment of meat products with various fiber sources. It will not only lead in designing meat product with desired nutritional attribute but also a superior technological advantage attributed to characteristic features of fiber like water- and oil-binding capacity in addition to antioxidant function [6]. A number of studies have already been carried out by using fiber alone or in combinations for formulation of various categories of meat products [7, 8]. This chapter highlights about the functionality of dietary fiber, its physiological and technological significance with a major emphasis on its application in meat products.

2 Dietary Fiber: Definition and Classification

Since 1953 when Eben Hipsely first coined the term dietary fiber, its definition has been revised many a times and till date it is evolving. Initially referred to as unavailable carbohydrate content in foods that lowered the rates of pregnancy toxaemia [9], it included lignin, cellulose, and hemicellulose. Later, investigations regarding correlation of disease incidence and fiber intake led the researchers to recommend increased stool volume and softness through higher dietary fiber intake and hence a new definition was proposed [10]. The most unswerving definition was proposed by Trowell [11] that dietary fiber consists of plant components resistant to digestion by enzymes in alimentary canal of humans, and it includes cellulose, hemicellulose, lignin, oligosaccharides, pectin, gums, and waxes. So thereafter, a categorization of carbohydrates into two basic groups depending upon their digestibility in GIT was done. The first group included starch, fructans, and simple sugars that are easily hydrolyzed and absorbed in small intestine. They are sometimes referred to as nonstructural or nonfibrous polysaccharides. The second group included cellulose, hemicellulose, lignin, and pectin, that are resistant to hydrolysis in small intestine, and are referred to as nonstarch polysaccharides or structural carbohydrates. Contemporary definition of dietary fiber has been proposed by American Association of Cereal Chemists (AACC) and Codex Alimentarius Commission (CAC). According to AACC, 2001, "Dietary fiber refers to the edible part or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. According to this definition dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" [12]. The latest definition proposed by CAC states that dietary fiber are carbohydrate polymers with degree of polymerization not lesser than 3 and are resistant to digestion as well as absorption in small intestine. Further, it possesses properties like capability to increase stool bulk by decreasing intestinal transit time,

reduction of cholesterol, and postprandial glucose levels and fermentable by intestinal microbiota [13].

Dietary fiber can be classified in a number of ways such as on the basis of source from which they are derived (plant, animal, or synthetic), structure (linear, nonlinear, or branched), or solubility. The most acceptable classification is on basis of enzymatic fermentation behavior in a simulated system of that of human alimentary canal. It can be divided into two broad categories, viz., soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Insoluble fiber comprises cellulose, part of hemicelluloses, and lignin, whereas, soluble fiber constitutes pectins, gums, pentosans, and mucilage [16, 17].

Various components of dietary fiber are presented in Table 1 and classification of fiber on basis of solubility is presented in Table 2.

Cellulose: Cellulose is an unbranched chain of β(1→4) linked glucose monomers and is the most abundant polysaccharide found in nature which forms major constituent of cell walls in green plants and vegetables. The close packing of linear polymers renders it mechanically strong, water insoluble, and resistant to digestive enzymes in human gut. However, a partial microbial degradation in the large intestine may produce beneficial short chain fatty acids. It has an ability to bind water, thereby, increase fecal bulk, and relieve constipation. Two different components of cellulose had been studied by Aspinall [19] on basis of relative hydrolysis. The major portion of cellulose is crystalline component that has noncovalent hydrogen bonds responsible for its immense mechanical strength, resistance to degradation by gut microbes, and water insolubility. Whereas, the amorphous component constitutes a minor portion, i.e., 10–15% and is readily hydrolysable.

Hemicellulose: Similar to cellulose, these are also integral component of plant cell wall. It consists of chain of β(1→4) linked glucose monomers which are smaller in size than cellulose, usually branched and contain a number of sugar moieties like pentose (xylose and arabinose) and hexose units (mannose, galactose, rhamnose) [3, 20]. The main function in human system is binding of cholesterol and promotion of smooth bowel movements.

Lignin: It is a complex polymer that consist about 40 oxygenated phenylpropane units including coniferyl, sinapyl, and p-coumaryl alcohols that have undergone a complex dehydrogenative polymerization [21, 22]. Variable molecular weight and inertness owing to strong intamolecular bonding are characteristic features of this polymer.

Pectin: Composed of galacturonic acid chains connected with α(1→4) bonds, this linear polymer forms important structural component of plant cell wall and outer skin of fruits and vegetables. It is highly water soluble polysaccharide which is easily degraded by the microflora of the colon. A unique gel-forming capability of pectin makes it an ideal constituent of health foods as it decreases the rate of gastric emptying, improves cholesterol and lipid metabolism [23], and prevents and control diabetes in human beings [24]. Further, technologically it is applied in food as a gelling and thickening agent.

Hydrocolloids: It includes a wide range of polysaccharides that are viscous in nature. Majorly formed in secretory cells of plants, these are highly branched and

Table 1 Various components of dietary fiber [1, 14, 15]

Fiber components	Principal groupings	Description	Fiber sources
Nonstarch polysaccharides and oligosaccharides	Cellulose	Principal structural component of cell wall in plants; β-(1, 4) glucose polymer chain; soluble in concentrated acids	Cellulose plants (vegetable, sugarbeet, various brans)
	Hemicellulose	Polysaccharides in cell wall with β-(1, 4) glycosidic linkage	Arabinogalactans,β-glucans, arabinoxylans, glucuronoxylans, xyloglucans, galactomannans, pectic substances
	Polyfructoses	A polysaccharide of fructose that may contain some other sugars too, e.g., β-(2–1)-D-fructosyl-fructose	Inulin, oligofructans
	Gums and mucilages	Secreted by plants at site of injury; may contain rahmnose, arabinose, xylose, galactouronic acid, mannuronic acid, etc.	Seed extracts (galactomannans – guar and locust bean gum), tree exudates (gum acacia, gum karaya, gum tragacanth), algal polysaccharides (alginates, agar, carrageenan), psyllium
Carbohydrate analogues	Pectins	Cell wall components with D-galactouronic acid polymer chains; homogalactouronan, rhamnogalactouronan, xylogalactouronan, etc.	Fruits, vegetables, legumes, potato, sugarbeets
	Resistant starches and maltodextrins	Starch and starch degradation products; contain α-(1,4)-D-glucose polymer chain with α-(1,6)-D- glucose branching	Various plant such as maize, pea, potato
	Chemical synthesis	Synthetic in nature	Polydextrose, lactulose, cellulose derivatives
	Enzymatic synthesis	Produced by enzymatic hydrolysis; may possess prebiotic properties	Neo sugar or short chain, guar hydrolysate fructooligosaccharides, levan, xanthan gum, transgalactooligosaccharides, oligofructose, xylooligosaccharide, curdlan
Lignin	Lignin	Noncarbohydrate cell wall component that resists bacterial degradation; polyphenols like	Woody plants

(*continued*)

Table 1 (continued)

Fiber components	Principal groupings	Description	Fiber sources
		syringyl alcohol, guaiacyl alcohol as main chain	
Substances associated with nonstarch polysaccharides	Waxes, cutin, suberin	Protective in nature too	Plant fibers
Animal-origin fibers	Chitin, chitosan, collagen, chondroitin	Glucosamine linkages may be present	Fungi, yeasts, invertebrates

Table 2 Classification of dietary fiber on basis of water solubility [18]

Class	Examples
Insoluble	Cellulose
Soluble (only in hot water)	Agars, amylose, algins, kappa-type carrageenans (in the presence of K^+ or Ca^{2+}), gelan, konjac, mannan, locust bean gum, low-methoxyl pectins, granular starches, and starch derivatives
Soluble (in water at room temperature but insoluble in hot water)	Curdlan, hydroxylpropyl celluloses, hydroxylpropyl methylcelluloses, and methylcelluloses
Soluble (in water at room temperature and hot water)	Alginates, amylopectins, carboxymethyl celluloses, dextrins, iota-type carrageenan, guar gum, gum Arabic, high-methoxyl pectins, polydextrose, and xanthan gum

bind with water to form gels. This property is utilized in food systems as a gelling agent and thickener, e.g., gums, mucilage, and seaweed extracts (agar, alginate, carrageenan, etc.). The physiological effect of hydrocolloids is exerted by its enzymatic hydrolysis owing to higher soluble fraction of dietary fiber. It is widely used as cholesterol lowering and weight reducing component in diets.

Resistant starches (RS): Starches that escape the enzymatic degradation in small intestine are referred to as resistant starch. Divided into four categories, it has a wide application in food matrix. Type 1 (RS1) is made up of starch granules that are encapsulated by an indigestible plant matrix rendering it physically inaccessible and heat stable [25]. Type 2 (RS2) occurs in its native form such as in an uncooked potato and are resistant to enzymatic hydrolysis due to its compact structure. Type 3 (RS3) represents the most resistant form and are crystallized starches made by unique cooking and cooling of gelatinized starch. Type 4 (RS4) is a starch consisting of chemical bonds other than α-(1,4) and α-(1,6). The chemical modification is induced by esterification, crosslinking, or transglycosylation and is responsible for its limited digestibility in human gut [26].

Nondigestible oligosaccharides: They are found naturally in plants, fruits, and cereals and are synthesized by polymerization of monosaccharides or disaccharides.

They perform similar physiological functions as that of dietary fiber and are highly fermentable in intestine, e.g., fructo-oligosaccharides and galacto-oligosaccharides. Due to their prebiotic properties, its application in food systems is quite promising.

3 Analysis of Dietary Fiber: Methodologies and Modifications

The analytical methods of dietary fiber estimation is varied and have been modified with time. Weende system started way back in nineteenth century and is still adopted in many industries to determine fiber content. The proximate system of analysis relies on determining carbohydrate content by difference. Further through successive acid and alkaline digestion, the extraction of crude fiber was done, but the crude fiber makes up only very small part of whole dietary fiber. During acid–base treatment, hemicellulose and lignin loss occurs, and hence the total fiber is underestimated. Later, with the advancement of chemical analytical protocols, different solvent methods were employed for extraction and measurement of dietary fiber from foods [27]. The protein and starch are removed with various solvents, leaving behind undigested fiber. These methods are divided into many subtypes based on type of solvent used e.g., alcohol insoluble fiber extraction, neutral detergent method, and acid detergent method. The neutral detergent method measuring insoluble fractions and lignin provided a reliable methodology for estimating fiber fractions [28]. However, the relative disadvantage of solvent extraction methods is that there can be damage to the fiber during processing by solvent at many stages, leading to underestimation of total fiber. Currently, the estimation of dietary fiber in foods is carried out by three different methods, viz., nonenzymatic-gravimetric, enzymatic-gravimetric, and enzymatic-chemical. However, enzymatic-gravimetric Association of Official Analytical Chemists (AOAC) method and enzymatic-chemical method are predominately used [29, 30]. Enzymatic-gravimetric method was first introduced by Schaller [31] wherein he presented amylase treatment in preexisting methodology of Van Soest [32]. Later, Prosky et al. [29] adapted the method for insoluble and soluble fractions which is still in use. This method determines mainly a group of polysaccharides, lignin, and some of associated compounds like waxes. The enzymatic treatment removes starch and protein along with precipitation of soluble fiber components by organic solvents. The main disadvantage of this method is that oligosaccharides and some of resistant starches are not quantified, which has been rectified by McCleary et al. [33] in their modified method of fiber determination.

The enzymatic-chemical methods also involve removal of starch and protein as a first step. Thereafter, precipitation with ethanol or dialysis ensures separation of SDF fraction from hydrolyzed starch and sugars [34]. The neutral sugar contents are determined by GLC or HPLC, whereas, total sugars are computed spectrophotometrically. Filtration is carried out to get the residue after hydrolysis of total polysaccharides and quantified as Klason lignin. The nonstarch polysaccharide and Klason lignin in combination gives total dietary fiber value in addition to separate quantification of SDF and IDF contents.

Additionally, mixed treatment methodology for determination of total dietary fiber content yields better results than any single treatment. It involves chemical, mechanical, enzymatic, and microbial fermentation methods in proper combinations. Zong Cai et al. [35] reported that microbial fermentation improved the content of SDF by 15% when used alone, whereas, the yield increased to 35% when used in combination with microfluidization. So, the hybrid methodologies perform better in extraction and separation of dietary fiber constituents than a single treatment process [27].

4 Physiological Benefits and Health Implications of Dietary Fiber

A strong link has been established between dietary fiber consumption and human health through a number of studies carried out on human subjects. A correlational analysis through independent observational studies helped in zeroing down on concept of dietary fiber's role as a potential disease-preventing nutrient [36, 37]. However, the mechanism through which fiber exerts its effect depends mainly on type and composition of that fiber.

(a) **Dietary fiber and cardiovascular health:** Cardiovascular diseases are reported to be the most common cause of death around the globe [38]. Basically, it is a group of etiologies that includes coronary heart disease, hypertension, arrhythmias, strokes, and heart failure [39]. The role of fiber in preventing cardiovascular disease is multifaceted. It acts as reducing agent of hyperlipidemia and hypocholesterolemia through interfering with lipid or bile acid metabolism [2, 40]. Babio et al. [41] reviewed the different sources of dietary fiber on lipid profile and concluded that intake of highly viscous dietary fiber results in decreasing LDL-cholesterol. Highly fermentable dietary fiber like pectin, gums, mucilage, etc., produce short-chain fatty acids (butyric, acetic, propionic), and it further results in rejuvenation of microbiota and reduction in intestinal inflammation which leads to further lowering down of cholesterol in body. On similar lines, Zhang et al. [42] established cholesterol lowering effect of oat bran in ileostomized hypercholesterolemic individuals. Lowering of serum total cholesterol and LDL-cholesterol by 4% and 6%, respectively has been reported by supplementing diet with psyllium [43]. Other mechanisms that have been suggested include inhibition of hepatic lipoprotein production and cholesterol synthesis by fermentation products of dietary fiber. This may lead to increased insulin sensitivity [44]. The dietary fiber may also directly interact with lipase enzyme resulting in reduction of its activity [45], bind with bile salts, thereby preventing lipid emulsification and its absorption in small intestine [46], may act as a barrier between lipase enzyme and fat droplet by forming a protective covering around it [47] or by increasing aqueous phase viscosity leading to disturbance in lipid droplet coalescence and disruption in stomach and intestine [48]. Dietary fiber may also interact with bile acids in small intestine resulting in

lower reabsorption and higher excretion of fat including cholesterol [49]. Further, it may also alter micelle formation and simultaneously limit incorporation of cholesterol in that micelle [50].

(b) **Maintenance of gut health:** Major carbohydrates that reach the intestine and exert their beneficial action are nonstarch polysaccharides, some polyols, resistant starch, and nondigestible oligosaccharides [51]. Dietary fiber undergo fermentation in the large intestine particularly, colon resulting in production of favorable short-chain fatty acids (SCFA), primarily, acetate, propionate, and butyrate. They play a key role in maintaining gut health. Butyrate is preferential fuel for colonocytes that forms a protective lining in colon [52]. Also, SCFA result in lowering pH of colon leading to inhibition of growth of pathogenic microorganisms [53, 54]. The fermentation mass in colon further results in increase in fecal volume and output. Further, absorption of water by dietary fiber adds to fecal buildup [55, 56]. Increased bulk of feces decreases colonic transition time and prevents constipation and production of carcinogenic compounds [57]. Some of the fiber exhibit prebiotic properties, e.g., inulin and fructo-oligosaccharides (FOS) that encourage and stimulate growth of beneficial bacteria like lactobacilli and bifidobacteria in the gut [58]. Dietary fiber also plays a key role in the maintenance of gastrointestinal immunity through increasing T-cell mitogen response and stimulating gut-associated lymphoid tissue (GALT) [59].

(c) **Dietary fiber and its role in prevention and management of diabetes:** Prospective cohort epidemiological studies have concluded a direct link between consumption of fiber in diet and reduction in prevalence of diabetes. Management of type 2 diabetes majorly revolves around glycemic control. Intake of low-glycemic index (GI) diets will control hyperglycemia, and all the fiber-rich diets are low GI in nature. Several events leading to decrease in blood glucose level after consumption of fiber are explained by a number of workers. Lowering of postprandial glucose peak, which leads to decreased insulin demand, and over-exhaustion of the pancreas is facilitated through consumption of fiber. Delayed gastric emptying after consumption of soluble fiber results in lower postprandial glucose and insulin levels and higher consumption of insoluble fiber reduces absorption of carbohydrates in GI tract by increasing passage rate of foodstuffs in gut [60]. Kelley and Mandarino [61] reported that increase in free fatty acids in blood may result in alteration of glucose metabolism through inhibition of GLUT-4 transporters. However, the SCFA produced by dietary fiber in gut results in decrease in serum free fatty acid and may further reduce blood glucose levels. Dietary fiber could limit the transportation of glucose to intestinal absorptive surface by producing contractions and thus result in decreasing postprandial glucose peak [62]. Another mechanism through which dietary fiber can result in preventing diabetes is by stimulating postprandial insulin release via accelerating secretion of incretin hormones, i.e., GLP-1 and GIP and by increasing insulin sensitivity in the tissues [63, 64].

(d) **Dietary fiber and cancer:** Anticarcinogenic and antitumorigenic effects of dietary fiber are well-documented and supported by research on human subjects.

Fermentation of fiber by colonic bacteria results in the production of SCFA which are recognized to be potential players in slowing growth and increasing apoptosis in colon cells. Further, they have a role in decreasing mutations and cancer risk by activation of different drug-metabolizing enzymes [65]. Tang et al. [66] ascertained the relationship between dietary fiber intake and reduction in risk of esophageal cancer in Xingjiang, China, through case-control study. The regular intake of fiber was found to be inversely associated with esophageal cancer risk. Another mechanism proposed for role of fiber in countering cancer risk is that fiber adds to fecal bulk and thereby decreases the interaction between intestinal mucosa and cancer-risk agents present in feces [67]. Also, decrease in intestinal transit time will reduce the production of pathogenic bacteria in colon and eventually limit the production of carcinogens [68].

(e) **Dietary fiber and weight management:** A major predisposing factor for CVD and diabetes is excess weight and obesity. A number of studies have established a strong negative correlation between fiber intake practices and weight control in human subjects. Miller et al. [69] established effect of diet on fat content in body and reported that lean subjects had a higher fiber intake than their obese counterparts. The mechanism that have been proposed for role of fiber in decreasing weight is that it absorbs water and adds bulk resulting in satiety [70]. Hyperinsulinemia that leads to lipogenesis and increased incidence of obesity may also explain the role of fiber in weight management [2]. Fiber-rich diets delay gastric emptying, resulting in slower rate of absorption, and subsequently lesser gain of weight. Sakata [71] proposed that diets rich in fiber may increase chewing time and efforts resulting in various cephalic- and gastric-phase signals and early satiation. It is an established fact that fibers help in controlling and managing weight and subsequently root out the chances of associated ailments like CVD and diabetes.

(f) **Dietary fiber and mineral bioavailability:** Some fiber sources regulate the absorption of minerals in the gut which further affects overall ionic balance in the body. Soluble dietary fibers like pectin, certain gums, etc., helps in the absorption of minerals like calcium, iron, and magnesium [72, 73] by producing SCFA and decreasing intestinal luminal pH [74] and proliferation of epithelial cells in the caeco-colon [75]. Both these factors may lead to enhanced dissolution of insoluble minerals salts in colon. Further, fiber absorbs water and increases its content in colon, solubilize the minerals, and enhance absorption through permeable gut membrane [76].

5 Technological Functionality of Dietary Fiber

For the development of dietary fiber enriched meat products, the technological properties of incorporated fiber play a key role. Apart from providing all the possible health benefits discussed in previous section, fiber sources possess attributes that help in modifying the physicochemical characteristics of meat products. This may

pose a new way for designing and developing meat products for enhanced consumer acceptability.

(a) **Hydration properties and oil-binding capacity:** Water absorption, water-holding capacity (WHC), and swelling are the parameters that encompasses hydration properties of dietary fiber. Water absorption is dependent on substrate pore volume [77] and is determined by water uptake as measured by Baumann apparatus [78]. The amount of water retained by known weight of dietary fiber at specified time and temperature conditions refers to water-holding capacity of that fiber. Due to hydrophilic nature of polysaccharides, fiber holds water molecules in void spaces. This property of dietary fiber is largely dependent on its source, e.g., fiber from cereal by-products have lowest affinity whereas soluble fibers from algae have highest affinity. Other than this, the structure of dietary fiber, percentage of soluble fraction, and presence or absence of charged groups are major determinants of hydration properties. Also, fiber possesses capacity to hold oil that may help in designing novel functional meat products. Oil-holding capacity (OHC) is dependent on surface properties of fiber, overall charge density, and hydrophillicity [79, 80]. Dietary fibers with high OHC may be useful in stabilizing meat emulsion, whereas, superior WHC ensures higher product yield, juiciness, and calorie and salt control in meat products. Further, it may be used as a texture and viscosity modifier in emulsion-based meat products.

(b) **Solubility:** On the basis of degree of solubility in water, fiber may be categorized into insoluble and soluble dietary fractions. The regularity in the structure of polysaccharides decides its fate for enzymatic degradation. Higher degree of irregularity, e.g., β- glucan renders weak linkages in structure and fiber is prone to enzymatic degradation resulting in easy solubilization whereas, regular structure leads to insolubility of fiber, e.g., cellulose. The technological functionality differs for both fractions. Soluble fiber tends to increase viscosity, gel strength, and emulsification as compared to insoluble fiber. As a result, meat product incorporated with SDF is juicy, has a higher cooking yield, and emulsion stability with soft texture. Insoluble fraction tends to increase hardness in meat products with a concurrent reduced gel strength. So, as per the product attributes and demands, the type of fiber to be incorporated may be decided by processors.

(c) **Viscosity:** Ability of dietary fiber particularly SDF to form gels and resist flow behavior determines its viscosity. It is one of the most important technological functionality of fiber that determines its application in various categories of meat products. Water soluble fiber increases viscosity of solution and vice versa [81].

(d) **Antioxidant Properties:** Countering free radicals and delaying lipid peroxidation is one of the most important functionality of dietary fiber, particularly, nonstarch polysaccharides (NSP). It has been reported that several fractions of rice bran possess superior antioxidant properties [82] which are equally effective as ascorbate. This property may be utilized for the extension of storage life of meat products without any addition of synthetic antioxidants.

6 Effect of Fiber Addition on Physicochemical Properties of Meat Products

Effect of addition of dietary fiber in meat products can be visualized through series of change in their functional properties. Different sources of fibers and their possible effects on meat products is summarized in tabulated form (Table 3). Various physicochemical properties, viz., pH, water-holding capacity, cooking yield, etc., are profoundly affected by incorporation of dietary fiber. This in turn affects the processing characteristics of meat products, e.g., an increase in emulsion stability by addition of fiber sources in meat holds immense importance from technological and economic perspective. Further, the change in pH due to fiber source addition affects the overall quality characteristics.

The change in pH of the meat product after addition of fiber is largely dependent on the pH of fiber source. The citrus by-products have comparatively lower pH and when incorporated in meat products, the pH drops significantly. Alesson-Carbonell et al. [83] reported that addition of lemon albdeo in nonfermented dry cured sausages resulted in a decrease in pH owing to the organic acids present in raw albedo. Similarly, with the addition of peach dietary fiber suspensions in low-fat frankfurters, viscosity of meat batter increased. Further, a decline in pH was observed and the decrease was prominent as the level of inclusion of peach dietary fiber increased [84]. Incorporation of *kinnow* rind powder in goat meat patties resulted in lower pH of products as compared to control [85]. On the contrary, Fernandez-Lopez et al. [86] found that there was no decrease in pH in Spanish dry-fermented sausages incorporated with orange dietary fiber. Apart from citrus by-products as a source of fiber in meat products, many other sources have resulted in pH change in different classes of meat products. Yilmaz [87, 88] added different levels (5, 10, 15, and 20%) of rye and wheat bran in low-fat meatballs and observed an increase in pH value as compared to control. Similar findings have been reported by Rao and Reddy [89] and Talukdar and Sharma [90] in chicken loaves and chicken meat patties incorporated with gram flour and wheat and oat bran, respectively. However, Mehta et al. [91, 92] reported that addition of psyllium husk in chicken meat patties and rolls had no significant effect on pH. Similarly, Caceres et al. [93] and Wu and Lin [94] reported that soluble fiber didn't affect the pH of meat products and it was similar to control.

Water-holding capacity is another parameter that is technologically significant in the development of functional meat products. Ability to retain its own or added water ensues increase in emulsion stability and cooking yield. Tunisian beef sausages incorporated with three dietary fibers, namely, VITACEL LC 200, barley beta-glucan concentrate, and VITACEL KF500 potato fiber were developed and its physicochemical properties were studied [95]. The fiber displayed high water and oil-binding capacity resulting in an overall increase in water-holding capacity (WHC) of beef sausages. However, no significant effects ($P > 0.05$) on a_w values was observed. A significant decrease in cooking loss was also observed with an increase in amount of fiber in beef sausages. The reduction in diameter of sausages in

Table 3 Various fiber sources in formulation of meat products and their effects

Type/source of fiber	Type of meat product	Major effects on meat products	References
Highly soluble fiber sources			
Fructo-oligosaccherides	Fermented cooked sausages	40% reduction in fat content Improvement in technological and sensory properties of sausages	[104]
	Sausages	Cooked sausages with 35% less energy value and high amount of soluble dietary fiber without adverse impact on sensory properties Fat reduction close to 40% was obtained	[93]
	Dry-fermented sausages	Fiber had no effect on physicochemical parameters and microbiota evolution during ripening Decrease in lightness value for color and hardness value for texture was observed	[105]
Psyllium husk	Chicken patties	Significant increase in dietary fiber content Increase in emulsion stability and cooking yield Cholesterol content of meat product was decreased significantly	[91]
	Chicken meat rolls	4% level of incorporation of psyllium husk was found to be optimum Decrease in moisture, protein, and fat content of chicken rolls with fiber Fall in texture and tenderness scores on sensory evaluation	[92]
Inulin	Low-fat bologna sausage	With addition of fiber, increase in moisture and decrease in fat content Significant change in textural attributes than control	[106]
	Pork loaves	Improvement in processing quality and functionality of loaves Significant increase in cooking yield and emulsion stability Crude fiber increased by 250% in treated products	[107]
	Sausages	Addition of inulin did not increase crude fiber and textural properties in sausages	[108]
	Mortadella	Textural properties revealed increased hardness values even at lowest concentration of 2.5% Improvement in sensory scores as compared to control	[109]

(*continued*)

Table 3 (continued)

Type/source of fiber	Type of meat product	Major effects on meat products	References
	Veal meatballs	Lower concentration of fat and total trans fatty acids as compared to control Meatballs with 20% inulin had lowest moisture, salt, and redness value but highest ash and protein contents	[110]
	Water-boiled sausage	Reduction of fat from 43 to 52% without any loss to sensory quality	[111]
	Cooked meat batters	Better emulsion stabilization with creamy and softer texture	[112]
	Low-fat dry-fermented sausages	Decrease in total calorie counts without any change in sensory acceptability	[113]
Pectin	Cooked ham	Increase in sensory acceptability with addition of fiber	[114]
Alginate	Sausages	Reduction in fat without compromising sensory attributes	[115]
Carrageenan	Mutton kofta	Reduction in fat content with an increase in protein, ash, and carbohydrate content Soft texture with low free fatty acid content	[116]
Konjac flour	Frankfurters	Treated products were better on sensory acceptability scores Better shelf life than high fat control	[117]
Cereals and bran			
Wheat bran	Meatballs	Product with lighter color than control High ratio of unsaturated to saturated fatty acid content	[88]
	Beef patties	Increase in hardness and gumminess of product but decreased springiness and cohesiveness Decrease in protein and increased fat content of cooked beef patties	[118]
	Chicken patties	Increase in water-holding capacity and emulsion stability Higher cooking yield, firmness, total dietary fiber, and unsaturated fatty acid content, whereas, lower sensory attributes, moisture, protein, fat, and cholesterol contents	[90]
Rice bran	Kung-wan: an emulsified pork meatball	Decrease in protein and fat content, however, an increase in carbohydrate content	[119]

(*continued*)

Table 3 (continued)

Type/source of fiber	Type of meat product	Major effects on meat products	References
		Decrease in textural parameters of hardness, gumminess, and chewiness Sensory acceptability up to 10% level of inclusion	
	Meat batter	Increase in emulsion pH, emulsion stability, and cooking yield Viscosity of batters containing fiber was higher than control Hardness, cohesiveness, gumminess, and chewiness increased in fiber-added meat batter	[120]
	Emulsion-type sausages	Increase in moisture, fat, and pH Sausages with 3% rice bran had lowest cooking loss and 4% rice bran were hardest in textural analysis	[121]
	Pork meat batter	Increase in emulsion stability and cooking yield Increase in moisture, protein, ash, pH values, yellowness value, textural parameters, viz., hardness, gumminess, chewiness, cohesiveness, and viscosity of meat batter	[122]
	Meat emulsion	Increase in moisture and ash content and yellowness value, cohesiveness, gumminess, chewiness, and sarcoplasmic protein solubility Reduction in total fat content in product	[123]
	Low-fat frankfurters	Increase in moisture and ash content Reduction in fat, cholesterol, energy, and trans-fat levels Increased pH, cooking yield, and TBA values	[124]
	Pork protein gel	Addition of rice bran fiber at 1% level increased moisture, myofibrillar protein solubility, and water-holding capacity Decrease in lightness, redness, and textural values No change in protein on SDS gel electrophoresis	[125]
	Chicken rolls and patties	Decrease in sensory scores of meat rolls and patties with addition of rice bran and psyllium husk combination	[126]
Rye bran	Meatballs	Decrease in total fat, total trans fatty acids, moisture, salt content, weight losses, and redness value	[87]

(*continued*)

Table 3 (continued)

Type/source of fiber	Type of meat product	Major effects on meat products	References
		Increase in protein, ash, lightness, and yellowness values	
	Meatballs	Due to particulate nature, rye bran was found to be the best fiber source in meatballs as compared to oat bran and barley fiber No significant difference in firmness as compared to reference after pan frying	[127]
Oat bran	Low-fat beef burgers	Decrease in shear force values with increase in level of fiber	[128]
	Chevon patties	With the increasing level of oat bran, decrease in moisture, protein, and fat content but an increase in ash and total dietary fiber content Increase in concentration of unsaturated fatty acids and decrease in cholesterol, cooking loss, and shear force	[129]
	Light bologna and fat-free frankfurters	Increase in product yield, lightness, and hardness value Decrease in redness value and purge at 3% concentration	[130]
	Meatballs	Substitution of fat by fiber source and it decreased concentration of total fat and trans fatty acids With the increase in amount of oat bran (5%–20%), moisture and fat content decreased and protein and ash content increased No significant effect on sensory properties by fiber addition	[131]
	Beef patties	Significant improvement in cooking yield, fat retention, and moisture content Higher juiciness perception as compared to control	[132]
Oat flour	Beef patties	Decrease in moisture content of raw patties but increased after cooking No effect on protein, ash, and fat content Improvement in cooking characteristics with simultaneous decrease in lightness value No adverse effects on sensory properties	[133]
	Mutton kofta	Decrease in fat content whereas, an increase in moisture, protein, ash, and carbohydrates.	[134]

(*continued*)

Table 3 (continued)

Type/source of fiber	Type of meat product	Major effects on meat products	References
		Significant increase in cooking yield No significant detrimental effect on physicochemical and sensory attributes at 8% oat flour inclusion level	
	Chicken kofta	Increase in product yield and decrease in fat Increase in hardness, gumminess, cohesiveness, and chewiness	[135]
Ragi millet/finger millet	Chicken patties	No significant effect on fat and protein content Reduction in shrinkage of patties after cooking Decrease in lightness and yellowness values	[136]
	Chevon patties	Increase in emulsion stability and cooking yield Increase in pH, moisture, water activity, ash, and fat retention whereas, decrease in protein and fat content Significant increase in fiber and calcium content Decrease in instrumental texture profile attributes	[137]
Barley	Pork bologna sausages	Firmer texture and better sensory properties Better purge control and water-holding capacity during storage	[138]
Corn bran	Chevon rolls	Decrease in moisture and protein content while no significant effect on fat and ash content Increase in crude fiber, water-holding capacity, and emulsion stability Increase in polyphenolic content in fiber-enriched rolls	[139]
Fruits, vegetables, and their by-products			
Apple pulp	Fermented sausages	Reduction in energy value of products to 35% and fiber content Sensory and textural properties decreased with inclusion of fiber	[140]
	Chicken nuggets	Decrease in batter stability and cooking yield Reduction in texture and overall acceptability scores Decrease in pH, moisture, dietary fiber, and color parameters	[100]

(*continued*)

Table 3 (continued)

Type/source of fiber	Type of meat product	Major effects on meat products	References
Grape fiber	Minced fish muscle	Delay in oxidation in minced horse mackerel muscle during the first 3 months of frozen storage	[141]
	Minced fish muscle	Direct correlation of bound water after thawing and cooking with amount of fiber used Increase in softness and loss of cohesiveness Delay in lipid oxidation parameters	[142]
	Chicken hamburgers	Significant decrease in redness and yellowness values Improvement in oxidative stability and radical scavenging activity Sensory acceptability was not affected by addition of fiber	[6]
	Frankfurters	Increase in protein, dietary fiber, and water-holding capacity Decrease in color values and oxidation levels Frankfurters up to 2% level of inclusion were sensory acceptable	[143]
Tomato fiber	Chopped cooked chicken products	Reduction in pH, increase in water-holding capacity, and increase in product hardness proportional to fiber addition Reduction in lipid oxidation	[144]
	Chicken sausages	Decrease in moisture and an increase in ash content Increase in emulsion stability, cooking yield, and crude fiber content	[145]
Orange	Fermented sausages	Reduction in energy value of products Decrease in sensory and textural properties with inclusion of fiber Best organoleptic quality at 10% pork back fat ad 1.5% orange fiber	[139]
	Dry-cured sausage	Decrease in lipid oxidation parameter, i.e., TBARS value in samples with orange fiber Significant reduction in residual nitrite level	[146]
	Salachichon (Spanish dry-fermented sausage)	pH, water activity, residual nitrite level, and Micrococcus count were affected during dry curing	[147]
	Bologna	Decrease in TBARS values along with aerobic and lactic acid bacteria counts	[148]

(*continued*)

Table 3 (continued)

Type/source of fiber	Type of meat product	Major effects on meat products	References
		Decrease in juiciness and increase in hardness perception on sensory analysis	
	Sucuk (Turkish dry-fermented sausage)	Significant effect on lactic acid bacteria, Micrococcus, and pH values Decrease in residual nitrite level and increase in TBARS value Decrease in cooking loss and increase in lightness and yellowness values No statistical difference in sensory scores of texture, color, odor, taste, and general acceptability scores than control	[149]
Carrot dietary fiber	Sobrasaada (dry-fermented sausage)	Decline in all sensory attributes with an inclusion above level of 3% Significant effect on pH and hardness value	[150]
	Chicken nuggets	Improvement in cooking yield and emulsion stability Lower protein, fat, and ash content but higher moisture and fiber than control Hunter color values were higher in treated products, whereas, no change in textural parameters as compared to control	[151]
	Low-salt pork sausages	Improvement in water-binding capacity and marked effects on textural properties Marked increase in whitening with increasing content of fiber	[152]
Peach dietary fiber	Frankfurters	Increase in viscosity of meat batters and water retention Reduction in pH values and no change in protein and collagen content	[84]
	Fermented sausages	Sensory and textural properties decreased with inclusion of fiber	[140]
Lemon albedo	Bologna sausages	Increase in moisture, protein, and fiber content whereas, decrease in fat content Increase in hardness and decrease in juiciness perception	[153]
	Breakfast sausages	Decrease in shrinkage and cooking loss Increased value for lightness	[154]
Citrus fiber	Bologna sausages	Enhancement of oxidative stability, color, and dietary fiber content Negative effect on textural	[155]

(*continued*)

Table 3 (continued)

Type/source of fiber	Type of meat product	Major effects on meat products	References
		properties, whereas, no effect on sensory parameters below 2% level	
Sugarbeet fiber	Frankfurters	Slight lowering of sensory scores	[156]
	Frankfurters	Increase in water-holding capacity and total dietary fiber content No significant effect on sensory scores	[157]
	Turkish-type salami	Increase in water-holding capacity and total dietary fiber content with incorporation of fiber No significant changes in appearance, color, texture, or any other sensory scores	[158]
Sugarcane dietary fiber	Low-fat meat batter	Replacement of fat up to 10% level Improved texture and sensory scores Increase in water- and fat-binding properties, dynamic rheology, and reduction of cholesterol contents	[159]
Oyster mushroom	Chicken patties	Decrease in hardness, cohesiveness, gumminess, and chewiness but increase in springiness value as compared to control	[160]
Legume hulls and flour			
Peanut and cowpea flour	Chicken nuggets	Decrease in moisture loss, fat gain, and protein content Decrease in shear force values and energy as compared to control Unacceptable flavor scores at 20% level	[161]
Soy hull	Camel meat patties	Reduction in thickness, fat, water retention, and shear force values Significant effect on chemical composition, cooking yield, flavor, and cooking yield	[162]
Inner pea fiber	Beef patties	Increase in cooking yield and tenderness without adverse effect on flavor	[163]
Bengal gram and black gram flour	Chicken loaves	Reduction in cooking loss and extract release volume and increase in emulsion stability and pH values	[89]
Soybean, Bengal gram, green gram, and black gram flours	Buffalo meat burger	Increase in yield and protein content with simultaneous decrease in shrinkage and fat absorption Roasted flours registered lower TBA values, i.e., enhancement of oxidative stability during storage	[164]

(*continued*)

Table 3 (continued)

Type/source of fiber	Type of meat product	Major effects on meat products	References
Blackeye bean, chickpea, lentil flours, and rusk	Meatballs	Improvement in cooking yield, fat retention, and moisture retention Lower penetration values and higher sensory acceptability scores	[98]
Hazelnut pellicle	Low-fat beef burger	Increase in cooking yield, diameter, and thickness of beef burgers Decreased sensory scores of appearance, flavor, and juiciness as compared to control	[97]
Roasted pea flour	Chicken nuggets	Nonsignificant increase in cooking yield up to 10% level	[165]
Pea fiber	Beef burgers	Improvement in water-holding capacity, cooking yield, and decrease in shrinkage Minimization of production cost without compromising sensory attributes	[166]
Soy flour and moong bean powder	Buffalo meat patties	Reduction of moisture and fat content and increase in protein and fiber content Increase in water-holding capacity and decrease in cooking loss	[167]
Pea flour	Low-fat bologna	Improvement in texture profile values with lower cooking and purge loss Increase in water-holding capacity without compromising sensory attributes	[168]
Bambara groundnut seed flour	Beef patties	No significant effect on proximate composition of raw patties but moisture, protein, and carbohydrate content differed significantly in cooked products Reduction in shrinkage and increase in cooking yield, moisture, and fat retention	[169]
Tiger nut fiber	Pork burgers	Increase in cooking yield, fiber content, fat, and moisture retention Increase in textural parameters like chewiness but negative effects on color attributes	[170]
Chickpea hull flour	Chicken nuggets	Decrease in moisture, protein, ash, color values, total cholesterol, emulsion stability, and cooking yield Improvement in dietary fiber content and textural parameters	[101]
Black gram hull flour	Chicken rolls and patties	Fall in organoleptic quality with an increasing level of black gram hull	[171]

control was reported to be higher as compared to fiber-treated products. Addition of carrageenan or oat fiber increased cooking yield by increasing emulsion stability and water-holding capacity in low-fat frankfurters [96]. Decreasing fat content from 30% to 5% resulted in increased loss during cooking but addition of 2% oat fiber led to reduction in total expressible fluid from 9.6% to 7.6% and from 4.8 to 4.3% in frankfurters with 5 and 12% targeted fat, respectively. Turhan et al. [97] observed improvement in reduction of cooking loss and thickness of beef burgers by addition of hazel nut pellicle and on similar lines increase in cooking yield was observed on addition of bean and lentil flour in meatballs by Serdaroglu et al. [98]. Carrot fiber and dietary fiber suspensions from olive mill wastewater was tried as dietary fiber source in low-fat meatballs by Galanakis et al. [99] and they too were found to be in agreement with other authors for improvement in cooking properties and restriction of oil uptake leading to sustained reduced fat content. But on the contrary, a decrease in emulsion stability and cooking yield was recorded in low-salt and low-fat chicken nuggets incorporated with apple pulp and chickpea hull flour which could be explained by replacement of lean by fiber sources [100, 101]. Similarly, Morin et al. [102] also found an increase in cooking loss in breakfast sausages on addition of carboxymethyl cellulose.

Various other sources of fibers like psyllium husk have been used for increasing fiber content in meat products and a concurrent increase in emulsion stability and cooking yield had been observed. It was attributed due to the presence of soluble dietary fiber in husk that might have entrapped moisture and lead to the formation of a gel during cooking [91, 92]. Similar finding have been reported by Luruena-Martınez et al. [103] who recorded the effect of addition of locust bean/xanthan gum on various quality characteristics of low-fat frankfurters. They observed a significant increase in emulsion stability, cooking yield, and lower jelly and fat separation. Lemon albedo as a source of dietary fiber was used in emulsions by Saricoban et al. [172]. Two types of albedo at five different concentrations, viz., 0, 2.5, 5, 7.5, and 10% were added in emulsions and their functional properties were studied. An increase in emulsion capacity with highest value at 5% level was observed. Similar increasing trend was observed in emulsion stability and viscosity values; however, no change in flow behavior of emulsions was observed other than pseudoplasticity. Addition of fiber as a fat replacer was tried by Pinero et al. [132] and compared with high-fat control (20% fat). They found an increase in cooking yield, fat retention, and moisture content in fiber-added meat products as compared to high-fat control. A decrease in shrinkage of cooked patties from 9.13 to 6.76% with addition of Bambara groundnut seed flour (BGSF) and an increase in cooking yield, moisture retention, and fat retention from 79.1 to 87.2%, 67.5 to 78.05%, and 73.51 to 88.34%, respectively in beef patties was observed [169]. Thus, addition of fiber in various meat products is generally associated with improvement of physicochemical properties, viz., emulsion stability, cooking yield, pH, water-holding capacity, reduction of shrinkage, etc. The change is largely dependent on fiber source and processing characteristics.

7 Fiber Incorporation and Proximate Composition of Meat Products

The overall composition of meat products can be changed with incorporation of fiber. Fiber generally reduces fat content and increases carbohydrate fraction along with dietary fiber component. This has somehow generated a new class of functional meat products with enhanced health benefits. Hydrated oat bran was used by Chang and Carpenter [173] in frankfurters and an increase in moisture and carbohydrate content and a decrease in total fat had been reported by them. This can be attributed to increase water-binding capacity of soluble fiber in oats. On the contrary, formulation of chevon patties with oat bran at different levels, viz., 15, 20, 35, and 50%, resulted in decrease in moisture, protein, and fat content [129]. Soluble and insoluble fiber content in product increased and authors concluded that nutritional value of chevon patties could be enhanced with 15 or 20% oat bran addition. Similar studies were carried out by Yilmaz and Daglioglu [131] and Yilmaz [87] who reported an increase in protein and ash content but a decrease in moisture and fat content with addition of oat bran and rye bran, respectively in meatballs. Rice bran as a source of dietary fiber was used in emulsified meatballs known as Kung-wan in Taiwan by Huang et al. [119]. They reported a significant decline in protein and fat content of meatballs with increasing level of rice bran above 5%. However, an increase in fiber and carbohydrate content was also observed. Similarly, Choi et al. [123] estimated values for protein, moisture, fat, and ash content in meat batter formulations designed with varying levels of grape seed oil and rice bran fiber and found that protein, fat, and ash content in treatments with 2% rice bran fiber were higher than control. The rice bran incorporation in chicken meat rolls and patties at three different levels, viz., 5, 10, and 15% was tried by Mehta [174]. A significant decrease in moisture and protein level was observed in treated products, whereas, an increase in fat, ash, and dietary fiber content was recorded. Utilization of oat flour at three different concentrations in beef patties resulted in decrease in moisture content of raw products and an increase was observed in cooked ones. However, no change in protein, fat, or ash content with addition of oat flour was reported irrespective of cooking or not [133]. Bilek and Turhan [175] used flaxseed flour at 3–15% levels in beef patties and reported an increase in fat and ash content but a decrease in moisture and protein percentage in products. Cereal brans at 5–20% levels in meatballs were utilized as a fiber source by Yasarlar et al. [176] and recorded a gradual decrease in moisture and fat percent while an increase in protein, ash, and dietary fiber with increasing levels of wheat and oat bran. An increase in protein and fiber content of various meat products with the use of legume flours have been reported. Kenawi et al. [167] studied the effect of incorporation of mung bean powder and/or low fat soy flour on proximate composition of buffalo meat patties. They observed that at a level of 10%, both the fiber sources led to reduction in moisture and fat content whereas an increase in the fiber and protein contents. However, a lower protein content in beef patties incorporated with common bean flour [177] and Bambara groundnut seed flour [169] was observed. The change in proximate composition is dependent on the type of fiber incorporated in meat products. Soluble fibers like psyllium husk, oat bran, etc. tend to increase moisture content due to enhanced water-binding capacity, whereas, protein-rich legume flours result in increase

of protein content in final product. A modification can be designed as per product and consumer requirement by the processor.

8 Dietary Fiber and Textural Properties of Meat Products

Texture of meat product is an important attribute that holds technological as well as sensorial importance. Human perception is a key factor in deciding optimum textural quality of meat products; however, to objectify, various instruments are used to measure texture of meat products, viz., Instron Texture Analyzer, Warner Bratzler shear press, and Kramer Shear apparatus. The fiber addition in meat products modifies the texture to a great extent and ultimately affects chewability. There can be both negative as well as positive influence of fiber addition on textural parameters. Addition of tapioca starch, oat fiber, and whey protein in low-fat beef burgers resulted in decrease in all textural parameters [128]. Garcia et al. [109] reported an increase in hardness value of *mortadella*, a Spanish cooked meat product incorporated with inulin when added at 2.5% level; however, in form of gel, it produced same effect at 7.5%. Similarly, an increase in hardness value was reported in pig liver pate developed using three enzymatically extracted fibers along with dry potato pulp and commercial potato fiber (potex) as reference [178]. A gradual decrease in hardness, gumminess, and chewiness of pork sausage with incorporation of hydrated oatmeal was observed by Yang et al. [179]. On the other hand, addition of 2% kimchi powder in breakfast sausages resulted in increased value of harness, gumminess, and chewiness as compared to control [180]. Similar trend has been observed in a number of meat products due to the addition of soy fiber, plasma protein, and various kinds of fiber sources [181, 182]. Incorporation of fiber has resulted in modified textural properties in fish-based products too. A low-fat fish sausage added with swelite (a dietary fiber obtained from inner pea) and fibruline (a dietary fiber obtained from chicory root) was developed by Cardoso et al. [183] and addition of swelite resulted in improved gel strength and hardness whereas, the products from fibruline were poorer in texture than control in terms of cohesiveness and chewability. Wheat bran as a source of dietary fiber was utilized in the formulation of cooked beef patties by adoption of a Box-Behnken design wherein three different variables were studied simultaneously. Wheat bran resulted in higher hardness and gumminess value but a lower springiness, resilience, and cohesiveness without any influence on adhesiveness [118]. Nonconventional fiber sources like grey oyster mushroom at 0, 25, or 50% level were used in chicken patties by Wan Rosli et al. [160] and they found that hardness, cohesiveness, gumminess, and chewiness of chicken patties decreased with incorporation of oyster mushroom whereas, the value for springiness increased.

9 Sensory Properties of Meat Products in Relation with Added Fiber Sources

The consumer acceptability is center to any product formulation and development. It is a complex phenomenon which is judged by sensory evaluation. The different subsets for meat products are generally color and appearance, flavor, texture,

juiciness, and overall acceptability. Tender and juicy meat products attract focus of consumers. Utilization of various fibers has been associated with a change in sensory properties, particularly juiciness and texture. An increase in hardness and decrease in juiciness by the addition of lemon albedo in bologna sausages has been reported by Fernandez-Gines et al. [153]. Similarly, addition of chickpea flour at 2.5 and 5% levels in low-fat pork bologna had no adverse effects on sensory perception. However, textural properties of cohesiveness and graininess increased but juiciness was rated much lower scores than control [184]. However, many fiber sources produced much better sensory perception than control itself, viz., hull-less waxy barley and normal starch barley in pork bologna sausages [138]. According to Kenawi et al. [167], 5% of both low-fat soy flour and mung bean powder resulted in the product with highest values for color, taste, odor, juiciness, and overall acceptability as compared to control. The extent of change in organoleptic quality is largely dependent on the concentration of fiber used in the meat product, e.g., carrot dietary fiber, if added in a concentration above 3% in *sobrassada* resulted in poorer sensory scores than control [150]. In concordance with these observations, cooked beef patties formulated with Bambara groundnut flour at 5% level had no significant effect on sensory attributes but at higher level, i.e., 7.5%, a significant fall in sensory scores for appearance, flavor, and overall acceptability was observed by Alakali et al. [169].

10 Conclusions

With the advancement of technology and reliance on processed products, meat industry is expanding its horizon with introduction of novel value-added products in market. Lack of essential components like dietary fiber can be a great hazard for health of consumers. Thus, its inclusion is needed for portraying meat as a complete food. Further, numerous functional properties like water retention, viscosity, etc. help in formulating the products with higher yield and better sensory acceptability. The technological advancements in developing low-fat, low-salt, high-fiber, and functional meat products can be realized by integrating fiber sources in meat. It is a novel area wherein lot of unconventional fiber sources can be tried for gelling up in meat products.

References

1. Mehta N, Ahlawat SS, Sharma DP, Dabur RS (2015) Novel trends in development of dietary fiber rich meat products – a critical review. J Food Sci Technol 52(2):633–647
2. Kendall CW, Esfahani A, Jenkins DJ (2010) The link between dietary fibre and human health. Food Hydrocoll 24(1):42–48
3. Mudgil D, Barak S (2013) Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: a review. Int J Biol Macromol 61:1–6
4. Biesalski HK (2005) Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet? Meat Sci 70:509–524

5. WHO/FAO (2003) Diet, nutrition and prevention of chronic diseases. WHO technical report series 916, Geneva
6. Sáyago-Ayerdi S, Brenes A, Goñi I (2009) Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburgers. LWT-Food Sci Technol 42(5):971–976
7. Desmond E, Troy DJ, Buckley J (1998a) Comparative studies on non-meat ingredients used in the manufacture of low-fat burgers. J Muscle Food 9:221–241
8. Mansour EH, Khalil AH (1999) Characteristics of low fat beef burgers as influenced by various types of wheat fibers. J Sci Food Agric 79:493–498
9. Hipsley EH (1953) Dietary fiber and pregnancy toxaemia. Br Med J 2:420–442
10. Burkitt DP (1975) Large-bowel cancer: an epidemiological jigsaw puzzle. J Natl Cancer Znst 54:3–6
11. Trowell H (1976) Definition of dietary fiber and hypotheses that it is a protective factor in certain diseases. Am J Clin Nutr 29(4):417–427
12. AACC (2001) Cereal Food World 46:112–113
13. Codex Alimentarius Commission (CAC). Report of the 27th session of the Codex Committee on nutrition and foods for special dietary uses, Bonn, 21–25 November 2005. ALINORM 06/29/26, 2006
14. Dhingra D, Michael M, Rajput H, Patil RT (2012) Dietary fibre in foods: a review. J Food Sci Technol 49(3):255–266
15. Tungland BC, Meyer D (2002) Non-digestible oligo- and polysaccharides (dietary fiber): their physiology and role in human health and food. Compr Rev Food Sci Food Saf 1(3):90–109
16. Esposito F, Arlotti G, Bonifati AM, Napolitano A, Vitale D, Vincenzo F (2005) Antioxidant activity and dietary fiber in durum wheat bran by-products. Food Res Int 38:1167–1173
17. Chawla R, Patil GR (2010) Soluble dietary fiber. Comp Rev Food Sci F 9:178–196
18. BeMiller JN (2001) Classification, structure and chemistry of polysaccharides of food. In: Cho SS, Dreher ML (eds) Handbook of dietary fibre. Marcel Dekker, Inc., New York, pp 603–611
19. Aspinall GO (1970) Polysaccharides. Pergamon Press, Oxford, pp 130–144
20. Kay RM (1982) Dietary fibre. J Lipid Res 23:221–242
21. Schubert WJ (1956) Lignin biochemistry. Academic, New York, pp 2–6
22. Theander O, Aman P (1979) The chemistry, morphology and analysis of dietary fiber component. In: Inglett G, Falkehag (eds) Dietary fibres: chemistry and nutrition. Academic, New York, pp 214–244
23. Brown L, Rosner B, Willett WW, Sacks FM (1999) Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr 69:30–42
24. Jenkins DJA, Leeds AR, Gassull MA, Cochet B, Alberti KGMM (1977) Decrease in post-prandial insulin and glucose concentrations by guar and pectin. Ann Intern Med 86:20–23
25. Sajilata MG, Singhal RS, Kulkarni PR (2006) Resistant starch – a review. Compr Rev Food Sci Food Saf 5(1):1–7
26. Haub MD, Hubach KL, Al-Tamimi EK (2010) Different types of resistant starch elicit difference glucose responses in humans. J Nutr Metab 2010:30501
27. Yang YY, Ma S, Wang XX, Zheng XL (2017) Modification and application of dietary fiber in foods. J Chem. https://doi.org/10.1155/2017/9340427
28. Goering HK, Van Soest PJ (1970) Forage fibre analysis. US Department of Agriculture, Washington, DC, p 379
29. Prosky L, Asp NG, Schweizer TF, DeVries JW, Furda I (1988) Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. J Assoc Off Anal Chem 71(5):1017–1023
30. Englyst HN, Quigley ME, Hudson GJ (1994) Determination of dietary fiber as non-starch polysaccharides with gas-liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst 119:1497–1509
31. Schaller D (1977) Analysis of dietary fiber. Food Prod Devel 11(9):70–72
32. Van Soest PU (1967) Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. J Assoc Off Anal Chem 50:50–55

33. McCleary BV, De Vries JW, Rader JI, Cohen G, Prosky L, Mugford DC, Champ M, Okuma K (2010) Determination of total dietary fiber (CODEX definition) by enzymatic-gravimetric method and liquid chromatography: collaborative study. J AOAC Int 93(1):221–233
34. Manas E, Bravo L, Saura-Calixto F (1994) Sources of error in dietary fibre analysis. Food Chem 50(4):331–342
35. Zong-cai TU, Jin-lin LI, Roger R, Cheng-mei L, Hui W, Xue-chun Z (2006) Study on production of high activity dietary fiber from soybean dregs. Food Sci 27(7):144–147
36. Burkitt DP, Walker AR, Painter NS (1974) Dietary fiber and disease. JAMA 229(8):1068–1074
37. Trowell HC, Burkitt DP (1981) Western diseases, their emergence and prevention. Cambridge, MA: Harvard University Press
38. Goyal A, Yusuf S (2006) The burden of cardiovascular disease in the Indian subcontinent. Indian J Med Res 124:235–244
39. Viuda-Martos M, L'opez-Marcos MC, Fernandez–Lopez J, Sendra E, Lo'pez-Vargas JH, Perez–Alvarez JA (2010) Role of fiber in cardiovascular diseases. Food Control 21:436–443
40. Chau CF, Huang YL, Lin CY (2004) Investigation of the cholesterol-lowering action of insoluble fiber derived from the peel of *Citrus sinensis* L. cv. Liucheng. Food Chem 87(3):361–366
41. Babio N, Balanza R, Basulto J, Bulló M, Salas-Salvadó J (2010) Dietary fibre: influence on body weight, glycemic control and plasma cholesterol profile. Nutr Hosp 25:327–340
42. Zhang JX, Hallmans G, Andersson H, Bosaeus I, Aman P, Tidehag P, Stenling R, Lundin E, Dhlgren S (1992) Effect of oat bran on plasma cholesterol and bile acid excretion in nine subjects with ileostomies. Am J Clin Nutr 56:99–105
43. Anderson JW, Allgood LD, Lawrence A, Altringer LA, Jerdack GR, Hengehold DA, Morel JG (2000) Cholesterol-lowering effects of psyllium intake adjunctive to diet therapy in men and women with hypercholesterolemia: meta-analysis of 8 controlled trials. Am J Clin Nutr 71:472–479
44. Theuwissen E, Mensink RP (2008) Water-soluble dietary fibers and cardiovascular disease. Physiol Behav 94:285–292
45. Klinkesorn U, McClements DJ (2009) Influence of chitosan on stability and lipase digestibility of lecithin-stabilized tuna oil-in-water emulsions. Food Chem 114(4):1308–1315
46. Thongngam M, McClements JD (2005) Isothermal titration calorimetry study of the interactions between chitosan and a bile salt (sodium taurocholate). Food Hydrocoll 19(5):813–819
47. Mun S, Decker EA, Park Y, Weiss J, McClements DJ (2006) Influence of interfacial composition on in vitro digestibility of emulsified lipids: potential mechanism for chitosan's ability to inhibit fat digestion. Food Biophys 1(1):21–29
48. Gallaher D, Schneeman BO (2003) Fibra alimentaria. In: Bowman B, Russel R (eds) Conocimientos actuales sobre nutrición. Organizacion Panamericana de la Salud. Publicaci on Tecnica n°59
49. Dongowski G, Huth M, Gebhardt E (2003) Steroids in the intestinal tract of rats are affected by dietary fibre-rich barley-based diets. Br J Nutr 90:895–906
50. Carr TP, Jesch ED (2006) Food components that reduce cholesterol absorption. Adv Food Nutr Res 51:165–204
51. Elia M, Cummings JH (2007) Physiological aspects of energy metabolism and gastrointestinal effects of carbohydrates. Eur J Clin Nutr 61(Suppl. 1):40–74
52. Buttriss JL, Stokes CS (2008) Dietary fibre and health: an overview. Nutr Bull 33(3):186–200
53. Gray J (2006) Dietary fibre: definition, analysis, physiology and health. ILSI Europe dietary fibre concise monograph series. Available from: http://www.ilsi.org/Europe/Publications/C2006Diet_FibEng.pdf.
54. Scott KP, Duncan SH, Flint HJ (2008) Dietary fibre and the gut microbiota. Nutr Bull 33:201–211
55. Cummings JH (1993) The effect of dietary fiber on faecal weight and composition. In: Spiller GA (ed) Handbook of dietary fibre in human nutrition. CRC Press, Boca Raton, p 263

56. Lefebvre AC, Thebaudin J (2002) Fibras extraidas de las hortalizas. In: Tirilly Y, Bourgeois C (eds) Tecnologia de las Hortalizas. Acribia, Zaragoza, pp 459–481
57. Gibson GR (2004) Fibre and effects on probiotics (the prebiotic concept). Clin Nutr Suppl 1:25–31
58. Nugent AP (2005) Health properties of resistant starch. Nutr Bull 30:27–54
59. Field CJ, McBurney MI, Massimino S, Hayek MG, Sunvold GD (1999) The fermentable fiber content of the diet alters the function and composition of canine gut associated lymphoid tissue. Vet Immunol Immunopathol 72:325–341
60. Jenkins DJ, Wolever TM, Leeds AR, Gassull MA, Haisman P, Dilawari J, Goff DV, Metz GL, Alberti KG (1978) Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. Br Med J 1(6124):1392–1394
61. Kelley DE, Mandarino LJ (2000) Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 49(5):677–683
62. Mälkki Y (2001) Oat fiber. Production, composition, physicochemical properties, physiological effects, safety, and food applications. In: Cho SS, Dreher ML (eds) Handbook of dietary fiber. Marcel Dekker, Inc., New York, pp 497–517
63. Weickert MO, Pfeiffer AF (2008) Metabolic effects of dietary fiber consumption and prevention of diabetes. J Nutr 138(3):439–442
64. Weickert MO, Mohlig M, Koebnick C, Holst JJ, Namsolleck P, Ristow M, Osterhoff M, Rochlitz H, Rudovich N, Spranger J, Pfeiffer AF (2005) Impact of cereal fibre on glucose-regulating factors. Diabetologia 48(11):2343–2353
65. Scharlau D, Borowicki A, Habermann N, Hofmann T, Klenow S, Miene C, Munjal U, Stein K, Glei M (2009) Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. Mutat Res Rev Mutat Res 682(1):39–53
66. Tang L, Xu F, Zhang T, Lei J, Binns CW, Lee AH (2013) Dietary fibre intake associated with reduced risk of oesophageal cancer in Xinjiang, China. Cancer Epidemiol 37(6):893–896
67. Harris PJ, Ferguson LR (1993) Dietary fibre: its composition and role in protection against colorectal cancer. Mutat Res 290:97–110
68. Rumney C, Rowland IR (1995) Nondigestible oligosaccharides-potential anti-cancer agents? BNF Nutr Bull 20:194–203
69. Miller WC, Niederpruem MG, Wallace JP, Lindeman AK (1994) Dietary fat, sugar, and fiber predict body fat content. J Am Diet Assoc 94(6):612–615
70. Ludwig DS (2000) Dietary glycemic index and obesity. J Nutr 130(2):280S–283S
71. Sakata T (1995) A very-low-calorie conventional Japanese diet: its implications for prevention of obesity. Obesity 3(S2):233s–239s
72. Morais MB, Feste A, Miller RG, Lifschitz CH (1996) Effect of resistant and digestible starch on intestinal absorption of calcium, iron, and zinc in infant pigs. Pediatr Res 39(5):872–876
73. Lopez HW, Coudray C, Bellanger J, Younes H, Demigné C, Rémésy C (1998) Intestinal fermentation lessens the inhibitory effects of phytic acid on mineral utilization in rats. J Nutr 128(7):1192–1198
74. Manning TS, Gibson GR (2004) Prebiotics. Best Pract Res Clin Gastroenterol 18(2):287–298
75. Conway PL (2001) Prebiotics and human health: the state-of-the-art and future perspectives. Näringsforskning 45(1):13–21
76. Kaur N, Gupta AK (2002) Applications of inulin and oligofructose in health and nutrition. J Biosci 27(7):703–714
77. Guillon F, Champ M (2000) Structural and physical properties of dietary fibres, and consequences of processing on human physiology. Food Res Int 33(3):233–245
78. Elleuch M, Bedigian D, Roiseux O, Besbes S, Blecker C, Attia H (2011) Dietary fibre and fibre-rich by-products of food processing: characterization, technological functionality and commercial applications: a review. Food Chem 124(2):411–421
79. Caprez A, Arrigoni E, Amadò R, Neukom H (1986) Influence of different types of thermal treatment on the chemical composition and physical properties of wheat bran. J Cereal Sci 4(3):233–239

80. Fleury N, Lahaye M (1991) Chemical and physico-chemical characterisation of fibres from *Laminaria digitata* (kombu breton): a physiological approach. J Sci Food Agr 55(3):389–400
81. Abdul-Hamid A, Luan YS (2000) Functional properties of dietary fibre prepared from defatted rice bran. Food Chem 68(1):15–19
82. Zha XQ, Wang JH, Yang XF, Liang H, Zhao LL, Bao SH, Luo JP, Xu YY, Zhou BB (2009) Antioxidant properties of polysaccharide fractions with different molecular mass extracted with hot-water from rice bran. Carbohydr Polym 78(3):570–575
83. Alesson-Carbonell L, Fernandez-Lopez J, Sendra E, Sayas Barbera E, Perez-Alvarez JA (2004) Quality characteristics of non-fermented dry cured sausage formulated with lemon albedo. J Sci Food Agric 84:2077–2084
84. Grigelmo-Miguel N, Abadias-Seros MI, Martin-Belloso O (1999) Characterisation of low-fat high-density fibre frankfurters. Meat Sci 52(3):247–256
85. Devatkal SK, Narsaiah K, Borah A (2010) Anti-oxidant effects of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties. Meat Sci 85(1):155–159
86. Fernández-López J, Sendra E, Sayas-Barberá E, Navarro C, Pérez-Alvarez JA (2008) Physico-chemical and microbiological profiles of "salchichón"(Spanish dry-fermented sausage) enriched with orange fiber. Meat Sci 80(2):410–417
87. Yilmaz I (2004) Effect of rye bran addition on fatty acid composition and quality characteristics of low fat meatballs. Meat Sci 67:245–249
88. Yilmaz I (2005) Physicochemical and sensory characteristics of low fat meatballs with added wheat bran. J Food Eng 69:369–373
89. Rao BJ, Reddy KP (2000) Influence of binders and refrigerated storage on quality of chicken meat loaves. Indian. J Poult Sci 35(3):302–305
90. Talukdar S, Sharma DP (2010) Development of dietary fiber rich chicken meat patties using wheat and oat bran. J Food Sci Technol 47(2):224–229
91. Mehta N, Ahlawat SS, Sharma DP, Yadav S, Arora D (2013) Development and quality evaluation of chicken patties incorporated with psyllium husk. Haryana Vet 52(2):6–11
92. Mehta N, Ahlawat SS, Sharma DP, Dabur RS, Yadav S (2016) Optimization and quality evaluation of dietary fiber rich chicken meat rolls incorporated with psyllium husk. Fleischwirtschaft Int 3:65–70
93. Caceres E, Garcia ML, Toro J, Selgas MD (2004) The effect of fructooligosacharides on the sensory characteristics of cooked sausages. Meat Sci 68:87–96
94. YB W, Lin KW (2011) Influences of xylooligosaccharides on the quality of Chinese-style meatball (kung-wan). Meat Sci 88(3):575–579
95. Ktari N, Smaoui S, Trabelsi I, Nasri M, Salah RB (2014) Chemical composition, techno-functional and sensory properties and effects of three dietary fibers on the quality characteristics of Tunisian beef sausage. Meat Sci 96(1):521–525
96. Hughes E, Calfrades S, Troy DJ (1997) Effect of fat level, oat fiber and carrageenan on frankfurters with 5, 12 or 30% fat. Meat Sci 45(3):273–281
97. Turhan S, Sagir I, Ustun NS (2005) Utilization of hazelnut pellicle in low–fat beef burgers. Meat Sci 71:312–316
98. Serdaroglu M, Yildiz-Turp G, Abrodimov K (2005) Quality of low fat meatballs containing legume flours as extenders. Meat Sci 70:99–105
99. Galanakis CM, Tornberg E, Gekas V (2010) Dietary fiber suspensions from olive mill wastewater as potential fat replacements in meatballs. LWT-Food Sci Technol 43(7):1018–1025
100. Verma AK, Sharma BD, Banerjee R (2010) Effect of sodium chloride replacement and apple pulp inclusion on the physico-chemical, textural and sensory properties of low fat chicken nuggets. LWT-Food Sci Technol 43(4):715–719
101. Verma AK, Banerjee R, Sharma BD (2012) Quality of low fat chicken nuggets: effect of sodium chloride replacement and added chickpea (*Cicer arietinum* L.) hull flour. Asian-Australas J Anim Sci 25(2):291–298
102. Morin LA, Temelli F, McMllen L (2004) Interaction between meat proteins and barley Hordeurm spp. β-glucan within a reduced-fat breakfast sausage system. Meat Sci 68:419–430

103. Luruena-Martınez MA, Vivar-Quintana AM, Revilla I (2004) Effect of locust bean/xanthan gum addition and replacement of pork fat with olive oil on the quality characteristics of low-fat frankfurters. Meat Sci 68(3):383–389
104. dos Santos BA, Campagnol PC, Pacheco MT, Pollonio MA (2012) Fructooligosaccharides as a fat replacer in fermented cooked sausages. Int J Food Sci Tech 47(6):1183–1192
105. Salazar P, García ML, Selgas MD (2009) Short-chain fructooligosaccharides as potential functional ingredient in dry fermented sausages with different fat levels. Int J Food Sci Tech 44(6):1100–1107
106. Barretto AC, Pacheco MT, Pollonio MA (2015) Effect of the addition of wheat fiber and partial pork back fat on the chemical composition, texture and sensory property of low-fat bologna sausage containing inulin and oat fiber. Food Sci Technol (Campinas) 35(1):100–107
107. Verma AK, Chatli MK, Mehta N, Kumar P, Malav OP (2016) Quality attributes of functional, fiber-enriched pork loaves. Agric Res 5(4):398–406
108. Huang SC, Tsai YF, Chen CM (2011) Effects of wheat fiber, oat fiber and inulin on sensory and physico-chemical properties of Chinese-style sausages. Asian-Aust J Anim Sci 24(6):875–880
109. Garcia ML, Caceres E, Selgas MD (2006) Effect of inulin on the textural and sensory properties of mortadella, a Spanish cooked meat product. Int J Food Sci Technol 41:1207–1215
110. Yilmaz I, Gegel U (2009) Effect of inulin addition on physico chemical and sensory characteristics of meatballs. J Food Sci Technol 46:473–476
111. Hadorn R, Piccinali P, Suter M (2007) Fat reduction with inulin in water-boiled sausages. Revue suisse d'agriculture 39:244–248
112. Álvarez D, Barbut S (2013) Effect of inulin, β-glucan and their mixtures on emulsion stability, color and textural parameters of cooked meat batters. Meat Sci 94(3):320–327
113. Mendoza E, Garcia ML, Casas C, Selgas MD (2001) Inulin as fat substitute in low fat, dry fermented sausages. Meat Sci 57:387–393
114. Cardoso JB, Henry FD, Almeida SB, Ferreira KS, Ladeira SA (2013) Characterization of cooked ham containing pectin and potassium chloride. J Food Process Preserv 37(2):100–108
115. Beriain MJ, Gómez I, Petri E, Insausti K, Sarriés MV (2011) The effects of olive oil emulsified alginate on the physico-chemical, sensory, microbial, and fatty acid profiles of low-salt, inulin-enriched sausages. Meat Sci 88(1):189–197
116. Modi VK, Yashoda KP, Mahendrakar NS (2009) Low-fat mutton kofta prepared by using carrageenan as fat replacer: quality changes in cooked product during storage. J Food Sci Technol 46:316–319
117. Lin KW, Huang HY (2003) Konjac/gellan gum mixed gels improve the quality of reduced-fat frankfurters. Meat Sci 65(2):749–755
118. Saricoban C, Yilmaz MT, Karakaya M (2009) Response surface methodology study on the optimization of effects of fat, wheat bran and salt on chemical, textural and sensory properties of patties. Meat Sci 83:610–619
119. Huang SC, Shiau CY, Liu TE, Chu CL, Hwang DF (2005) Effects of rice bran on sensory and physico chemical properties of emulsified pork meatballs. Meat Sci 70:613–619
120. Choi YS, Jeong JY, Choi JH, Han DJ, Kim HY, Lee MA et al (2007) Quality characteristics of meat batters containing dietary fiber extracted from rice bran. J Korean Soci Food Sci Anim Resour 27(3):228–234
121. Choi YS, Jeong JY, Choi JH, Han DJ, Kim HY, Lee MA et al (2008) Effects of dietary fiber from rice bran on the quality characteristics of emulsion type sausages. J Korean Soc Food Sci Anim Resour 28(1):14–20
122. Choi YS, Choi JH, Han DJ, Kim HY, Lee MA, Kim HW, Jeong JY, Kim CJ (2009) Characteristics of low fat meat emulsion systems with pork fat replaced by vegetable oils and rice bran fibers. Meat Sci 82(2):266–271
123. Choi YS, Choi JH, Han DJ, Kim HY, Lee MA, Kim HW, Lee JW, Chung HJ, Kim CJ (2010) Optimization of replacing pork back fat with grape seed oil and rice bran fiber for reduced-fat meat emulsion systems. Meat Sci 84(1):212–218

124. Choi YS, Choi JH, Han DJ, Kim HY, Lee MA, Jeong JY, Chung HJ, Kim CJ (2010) Effects of replacing pork back fat with vegetable oils and rice bran fiber on quality of reduced fat frankfurters. Meat Sci 84(3):557–563
125. Choi YS, Choi JH, Han DJ, Kim HY, Lee MA, Kim HW, Jeong JY, Kim CJ (2011) Effects of rice bran fiber on heat induced gel prepared with pork salt soluble meat proteins in model system. Meat Sci 88:59–66
126. Mehta N, Ahlawat SS, Sharma DP, Yadav S, Arora D (2013) Sensory attributes of chicken meat rolls and patties incorporated with the combination levels of rice bran and psyllium husk. J Anim Res 3(2):179–185
127. Petersson K, Godard O, Eliasson AC, Tornberg E (2014) The effects of cereal additives in low-fat sausages and meatballs. Part 2: rye bran, oat bran and barley fibre. Meat Sc 96(1):503–508
128. Desmond E, Troy DJ, Buckley J (1998b) The effects of tapioca starch, oat fiber and whey protein on the physical and sensory properties of low-fat beef burgers. LWT-Food Sci Technol 31(7):653–657
129. Dawkins NL, Phelps O, Mcmillan KW, Forrester IT (1999) Composition and physicochemical properties of chevon meat patties containing oat bran. J Food Sci 64(4):597–600
130. Steenblock RL, Sebranek JG, Olson DG, Love JA (2001) The effects of oat fiber on the properties of light bologna and fat-free frankfurters. J Food Sci 66:1409–1415
131. Yilmaz I, Daglioglu O (2003) The effect of replacing fat with oat bran on fatty acid composition and physicochemical properties of meatballs. Meat Sci 65:819–823
132. Pinero MP, Parrak Leidenz NH, deMoreno LA, Ferrer M, Araujo S, Barboza Y (2008) Effect of oat's soluble fiber (β-glucan) as a fat replacer on physical, chemical, microbiological and sensory properties of low fat beef patties. Meat Sci 80:675–680
133. Serdaroglu M (2006) The characteristics of beef patties containing different levels of fat and oat flour. Int J Food Sci Technol 41:147–153
134. Modi VK, Yashoda KP, Naveen SK (2009) Effect of carrageenan and oat flour on quality characteristics of meat kofta. Int J Food Prop 12:228–242
135. Prasad B, Rashmi MD, Yashoda KP, Modi VK (2011) Effect of casein and oat flour on physicochemical and oxidative processes of cooked chicken kofta. J Food Proc Pres 35(3):359–368
136. Naveena BM, Muthukumar M, Sen AR, Babaji Y, Murthy TRK (2006) Quality characteristics and storage stability of chicken patties formulated with finger millet flour (Eleusina coracana). J Muscle Foods 17:92–104
137. Kumar D, Chatli MK, Mehta N, Verma AK, Kumar P (2015) Quality evaluation of chevon patties fortified with dietary fibre. Indian J Small Rumin 21(1):85–91
138. Shand PJ (2000) Textural, water holding and sensory properties of low fat pork bologna with normal or waxy starch hull-less barley. J Food Sci 65:101–107
139. Parkash J, Yadav S, Sharma DP, Pathera AK, Raut S (2016) Development of dietary fibre enriched Chevon rolls by incorporating corn bran and dried apple pomace. J Anim Res 6(4):367–370
140. Garcia ML, Dominguez R, Garlvez MD, Casas C, Selgas MD (2002) Utilization of cereal and fruit fibers in low-fat dry fermented sausage. Meat Sci 60:227–236
141. Sánchez-Alonso I, Jiménez-Escrig A, Saura-Calixto F, Borderías AJ (2007) Effect of grape antioxidant dietary fibre on the prevention of lipid oxidation in minced fish: evaluation by different methodologies. Food Chem 101(1):372–378
142. Sánchez-Alonso I, Borderías AJ (2008) Technological effect of red grape antioxidant dietary fibre added to minced fish muscle. Int J Food Sci Tech 43(6):1009–1018
143. Özvural EB, Vural H (2011) Grape seed flour is a viable ingredient to improve the nutritional profile and reduce lipid oxidation of frankfurters. Meat Sci 88(1):179–183
144. Cava R, Ladero L, Cantero V, Rosario Ramírez M (2012) Assessment of different dietary fibers (tomato fiber, beet root fiber, and inulin) for the manufacture of chopped cooked chicken products. J Food Sci 77(4):C346–C352

145. Yadav S, Malik A, Pathera A, Islam RU, Sharma D (2016) Development of dietary fibre enriched chicken sausages by incorporating corn bran, dried apple pomace and dried tomato pomace. Nutri Food Sci 46(1):16–29
146. Fernandez-Lopez J, Sendra E, Sayas-Barbera E, Navarro C, Perez-Alvarez JA (2008) Physico-chemical and microbiological profiles of "salchichon" (Spanish dry – fermented sausage) enriched with orange fiber. Meat Sci 80:410–417
147. Fernandez-Lopez J, Viuda-Martos M, Sendra E, Sayas-Barbera E, Navarro C, Perez Alvarez JA (2007) Orange fiber as potential functional ingredient for dry cured sausages. Eur Food Res Technol 226:1–6
148. Viuda-Martos M, Ruiz–Navajas Y, Fernandez–Lopez J, Perez–Alvarez JA (2010) Effect of orange dietary fibre, oregano oil and packaging conditions on shelf-life of bologna sausages. Food Control 21:436–443
149. Yalınkılıç B, Kaban G, Kaya M (2012) The effects of different levels of orange fiber and fat on microbiological, physical, chemical and sensorial properties of sucuk. Food Microbiol 29(2):255–259
150. Eim VS, Small S, Rossello C, Femenia A (2008) Effect of addition of carrot dietary fibre on the ripening process of a dry fermented sausage (Sobressada). Meat Sci 80:173–182
151. Bhosale SS, Biswas AK, Sahoo J, Chatli MK, Sharma DK, Sikka SS (2011) Quality evaluation of functional chicken nuggets incorporated with ground carrot and mashed sweet potato. Food Sci Technol Int 17(3):233–239
152. Grossi A, Søltoft-Jensen J, Knudsen JC, Christensen M, Orlien V (2012) Reduction of salt in pork sausages by the addition of carrot fibre or potato starch and high-pressure treatment. Meat Sci 92(4):481–489
153. Fernandez-Gines JM, Fernandez-Lopez J, Sayas-Barbera ME, Sendra E, Perez Alvarez JA (2004) Lemon albedo as a new source of dietary fibre: application to bologna sausage. Meat Sci 67:7–13
154. Aleson-Carbonell L, Fernandez-Lopez J, Perez-Alvarez JA, Kuri V (2005) Functional and sensory effects of fibre rich ingredients on breakfast fresh sausages manufacture. Food Sci Technol Int 11:89–97
155. Fernandez-Gines JM, Fernandez-Lopez J, Sayas-Barbera ME, Sendra E, Perez Alvarez JA (2003) Effect of storage conditions on quality characteristics of bologna sausages made with citrus fiber. J Food Sci 68(2):710–714
156. Ozboy-Ozbas O, Vural H, Javidipour I (2003) Effects of sugarbeet fiber on the quality of frankfurters. Zucker Ind 128:171–175
157. Vural H, Javidipour I, Ozbas Ozen O (2004) Effects of interesterified vegetable oils and sugarbeet fiber on the quality of frankfurters. Meat Sci 67:65–72
158. Javidipour I, Vural H, Ozbas OO, Tekin A (2005) Effect of interestified vegetable oils and sugar beet fibre on the quality of Turkish-type salami. Int J Food Sci Technol 40(2):177–185
159. Zhuang X, Han M, Kang ZL, Wang K, Bai Y, XL X, Zhou GH (2016) Effects of the sugarcane dietary fiber and pre-emulsified sesame oil on low-fat meat batter physicochemical property, texture, and microstructure. Meat Sci 113:107–115
160. Wan Rosli WI, Solihah MA, Aishah M, Nik Fakrudin NA, Mohsin SSJ (2011) Colour, textural properties, cooking characteristics and fibre content of chicken patty added with oyster mushroom (*Pleurotus sajor-caju*). Int Food Res J 18:621–627
161. Prinyawiwatkul W, McWatters KH, Beuchat LR, Philips RD (1997) Physicochemical and sensory properties of chicken nuggets extended with fermented cowpea and peanut flours. J Agric Food Chem 45:1891–1899
162. Al-Khalifa A, Atia M (1997) Effect of soy hull and fat on camel meat patties. Alexandria Sci Exch 18:303–311
163. Anderson ET, Berry BW (2000) Sensory, shear and cooking properties of low-fat beef patties made with inner pea fibre. J Food Sci 65(5):805–810
164. Modi VK, Mahendrakar NS, Narsimha Rao D, Sachindra NM (2003) Quality of buffalo meat burger containing legume flour as binders. Meat Sci 66:143–149

165. Singh OP, Singh JN, Bharti MK, Kumari S (2008) Refrigerated storage stability of chicken nuggets containing pea flour. J Food Sci Technol 45:460–462
166. Besbes S, Attia H, Deroanne C, Makni S, Blecker C (2008) Partial replacement of meat by pea fiber: effect on the chemical composition, cooking characteristics and sensory properties of beef burgers. J Food Qual 31:480–489
167. Kenawi MA, Abdelsalam SA, El-Sherif SA (2009) The effect of mung bean powder, and/or low-fat soy flour as meat extender on the chemical, physical and sensory quality of buffalo meat product. Biotechnol Anim Husb 25(5–6):327–337
168. Pietrasik Z, Janz JA (2010) Utilization of pea flour, starch-rich and fiber-rich fractions in low fat bologna. Food Res Int 43(2):602–608
169. Alakali JS, Irtwange SV, Mzer MT (2010) Quality evaluation of beef patties formulated with Bambara groundnut (*Vigna subterranean* L.) seed flour. Meat Sci 85:215–223
170. Sanchez-Zapata E, Munoz CM, Fuentes E, Fernandez-Lopez J, Sendra E, Sayas E, Navarro C (2010) Effect of tiger nut fibre on quality characteristics of pork burger. Meat Sci 85:70–76
171. Mehta N, Ahlawat SS, Sharma DP, Yadav S, Arora D (2013) Organoleptic quality of chicken meat rolls and patties added with the combination levels of black gram hull and psyllium husk. J Anim Res 3(2):237–243
172. Saricoban C, Oezalp B, Yilmaz MT, Oezen G, Karakaya M, Akbulut M (2008) Characteristics of meat emulsion systems as influenced by different levels of lemon albedo. Meat Sci 80(3):599–606
173. Chang HC, Carpenter JA (1997) Optimizing quality of frankfurters containing qat bran and added water. J Food Sci 62(1):194–197
174. Mehta N (2011) Designer chicken meat rolls and patties incorporated with fiber. Dissertation, Lala Lajpat Rai University of Veterinary and Animal Sciences
175. Bilek AE, Turhan S (2009) Enhancement of the nutritional status of beef patties by adding flax seed flour. Meat Sci 82:472–477
176. Yasarlar EE, Daglioglu O, Yilmaz I (2007) Effect of cereal bran addition on chemical composition, cooking characteristics and sensory properties of Turkish meatballs. Asian J Chem 19(3):2353–2361
177. Dzudie T, Scher J, Hardy J (2002) Common bean flour as an extender in beef sausages. J Food Eng 52:143–147
178. Kaack K, Laerke HN, Meyer AS (2006) Liver pate enriched with dietary fibre extracted from potato fibre as fat substitutes. Eur Food Res Technol 223(2):267–272
179. Yang HS, Choi SG, Jeon JT, Park GB, Joo ST (2007) Textural and sensory properties of low fat pork sausages with added hydrated oatmeal and tofu as texture modifying agents. Meat Sci 75:283–289
180. Lee MA, Han DJ, Jeong JY, Choi JH, Choi YS, Ki HY, Paik HD, Kim CJ (2008) Effect of kimchi powder level and drying methods on quality characteristics of breakfast sausage. Meat Sci 80:708–714
181. Confrades S, Guerra MA, Carballo J, Ferna'ndez-Marti'n F, Jime'nez-Colmenero F (2000) Plasma protein and soy fiber content effect on bologna sausage properties as influenced by fat level. J Food Sci 65(2):281–287
182. Colmenero FJ, Ayo MJ, Carballo J (2005) Physicochemical properties of low sodium frankfurter with added walnut: effect of transglutaminase combined with caseinate, KCl and dietary fiber as salt replacers. Meat Sci 69(4):781–788
183. Cardoso C, Mendes R, Nunes ML (2008) Development of a healthy low-fat fish sausage containing dietary fibre. Int J Food Sci Technol 43(2):276–283
184. Sanjeewa WGT, Wanasundara JPD, Shand PJ (2008) Physical, textural and sensory properties of low fat pork bologna with added chickpea flour. Presented at Institute of Food Technol annual meeting, from 28 June–1 July, New Orleans, Poster 134-12

Chemistry, Biological, and Pharmacological Properties of Gum Arabic

27

Hassan Hussein Musa, Abdelkareem Abdall Ahmed, and Taha Hussein Musa

Contents

H. H. Musa (✉)
Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan
e-mail: hassanhm@uofk.edu

A. A. Ahmed
Department of Physiology and Biochemistry, Faculty of Veterinary Sciences, University of Nyala, Nyala, Sudan
e-mail: kareemo151@gmail.com

T. H. Musa
Key Laboratory of Environmental Medicine, Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu, China
e-mail: taha.hm99@yahoo.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_11

Abstract

Gum Arabic (GA) is a natural branched-chain multifunctional hydrocolloid with a highly neutral or slightly acidic, arabino-galactan-protein complex containing calcium, magnesium, and potassium. Gum Arabic is dried exudate obtained from the stem and branches of Acacia trees manly *Acacia senegal* and *Acacia seyal*. GA was used by the Ancient Egyptians as an adhesive when wrapping mummies and in mineral paints when making hieroglyphs since the second millennium BC. In modern times, GA is used in foods, pharmaceutical, and many other industries. In this chapter, we describe the structure, chemical, and physical properties of Gum Arabic. In addition, biological properties include antioxidant properties of Gum Arabic, an effect of GA on renal function, blood glucose concentration, intestinal absorption, degradation of GA in the intestine, lipid metabolism, tooth mineralization, and hepatic macrophages. Similarly, pharmaceutical, food, and cosmetic properties of Gum Arabic are discussed.

Keywords

Gum Arabic · Chemical · Biological · Pharmacological · Food · Cosmetic · Properties

Abbreviations

AG	Arabinogalactan
AGP	Arabinogalactan protein
ATGL	Adipose triglyceride lipase
CAT	Catalase
CDC	Chenodeoxycholic acid
CRF	Chronic renal failure
GA	Gum Arabic
GP	Glycoprotein
GPx	Glutathione peroxidase
HDL	High-density lipoprotein
HSL	Hormone-sensitive lipase
LDL	Low-density lipoprotein
MDA	Malondialdehyde
MGL	Monoacylglycerol lipase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TAP	2,4,6-Triaminopyrimidine
TC	Total cholesterol
VLDL	Very low density lipoprotein

1 Introduction

Gum Arabic is a natural branched-chain multifunctional hydrocolloid with a highly neutral or slightly acidic, arabino-galactan-protein complex containing calcium, magnesium, and potassium [1]. According to the definition of the Joint Expert Committee for Food Additives (JECFA), Gum Arabic is a dried exudate obtained from the stem and branches of Acacia trees (Fig. 1) [2]. There is more than 1000 species of the genus Acacia Gums; only two are significant for commercial purposes *Acacia senegal* and *Acacia seyal* [3]. *Acacia senegal* is considered the best in quality due to a low quantity of tannins and comprises the majority of global trade [4], whereas *Acacia seyal* produces a lower grade of Gum [5]. Acacia trees are abundant in central Sudan, central and West Africa, and tropical and semitropical areas of the world [6, 7]. Sudan is the leading producer of Acacia Gums worldwide, followed by Nigeria, Chad, Mali, and Senegal [8]. Europe and USA are the most important GA markets, while Japan is the largest Asian consumer.

The use of GA dates back to the second millennium BC when the Ancient Egyptians used Gum Arabic as an adhesive when wrapping mummies and in mineral paints when making hieroglyphs [9]. In modern times, they are used in foods, pharmaceutical, and many other industries [10–13].

2 Structure of Gum Arabic

The chemical composition of GA is complex, and numerous papers have been published on this subject [14]. The backbone of GA is composed of 1,3-linked β-D-galactopyranosyl units. The side chains are composed of two to five 1,3-linked β-D-galactopyranosyl units, joined to the main chain by 1,6-linkages. Both the main and the side chains contain units of α-L-arabinofuranos, α-L-rhamnopyranosyl, β-D-glucopyranosyl, and 4-O-methyl-β-D-glucopyranosyl, the last two mostly as end units (Fig. 2).

Gum Arabic consists mainly of high-molecular weight polysaccharides and their calcium, magnesium, and potassium salts, which on hydrolysis, yield three main

Fig. 1 Gum Arabic formed on a wounded branch of *A. senegal*

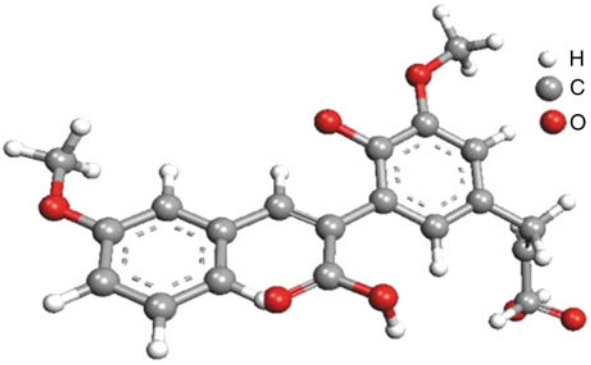


Fig. 2 Structure chemic of the Acacia Gum

fractions of polysaccharides and proteins, including arabinogalactan (AG), arabinogalactan protein (AGP), and glycoprotein (GP), which differ from their molecular weight and chemical composition [15]. The arabinogalactan fraction represents 88% of the total Gum weight has a low molecular weight (Mw, ~300 KDa) and associated little protein content below 1%. The arabinogalactan protein fraction (~10% of the total Gum) has a high molecular weight (Mw) (~1500 KDa) and protein content (~10%). The glycoprotein fraction (<2% of the total Gum) has the lowest molecular weight (Mw, ~250 KDa) and the highest protein content (~20%e50%). Among these fractions, AGP is the most interfacially active component [16, 17], and primarily responsible for the emulsifying properties of GA [18, 19]. This fraction can be adsorbed on the oil–water interface to form a viscoelastic film and reduce the interfacial tension between oil and water because of its amphiphilic characteristics, which is conferred by the hydrophobic protein chains combined to the hydrophilic polysaccharide fragments [17].

3 Chemical Properties of Gums

Chemically, GA is a complex mixture of macromolecules of different size and composition characterized by a high proportion of carbohydrates (D-galactose and L-arabinose) (~97%), and a low proportion of proteins (<3%) [14]. The chemical composition of GA varies slightly depending on its origin, climate, harvest season, tree age, and processing conditions, such as spray dying [20–23]. Many studies have shown some differences between the chemical composition of the GA from *Acacia senegal* and *Acacia seyal* [24–26], the most recent study was conducted by Lopez-Torrez et al. [29]. Both Acacia Gums contained the same amino acids with a higher content of protein in *A. senegal* (2.7%) than in *A. seyal* (1.0%) (Table 1).

Hydroxyproline, serine, leucine, and proline were the most abundant residues and represented more than 55% of the total amino acids in each variety (Table 2). Similar amino acid profiles were identified in previous studies on Acacia Gums from different origins [27–29]. The *A. senegal* Gum samples shown to contain approximately twice the amount of protein compared to the Gum obtained from *A. seyal* [28].

Table 1 Biochemical composition of *A. senegal* and *A. seyal* Gums in dry basis (mean ± standard deviation)

Component (mg g^{-1})	*A. senegal*	*A. seyal*
Total dry matter	889.0 ± 0.27	893.0 ± 0.02
Sugars[a]	940.0	950.0
Galactose (%)[b]	35.8 ± 1.20	36.9 ± 1.05
Arabinose (%)[b]	30.3 ± 2.50	47.6 ± 0.60
Rhamnose (%)[b]	15.5 ± 0.35	3.0 ± 0.30
Glucuronic acid (%)b	17.4 ± 1.15	6.7 ± 0.40
4-O-Me-glucuronic acid (%)[b]	1.0 ± 0.05	5.8 ± 0.55
Proteins	27.0 ± 0.01	10.0 ± 0.04
Minerals	33.0 ± 0.24	40.0 ± 0.07

[a]Total content of sugars was calculated by the difference of proteins and minerals from 1000 mg g^{-1} in dry basis
[b]Sugar composition was determined by GC-MS
Source: Lopez-Torrez et al. [29]

Table 2 Amino acid composition for *A. senegal* and *A. seyal* Gums in dry basis (mean ± standard deviation)

Amino acids (mg g^{-1})	Abbreviations	*A. senegal*	*A. seyal*
Alanine	Ala	0.49 ± 0.04	0.22 ± 0.01
Arginine	Arg	0.31 ± 0.05	0.12 ± 0.00
Aspartic acid	Asp	1.24 ± 0.04	0.49 ± 0.04
Glutamic acid	Glu	0.92 ± 0.01	0.28 ± 0.003
Glycine	Gly	0.79 ± 0.004	0.25 ± 0.07
Histidine	His	1.37 ± 0.01	0.27 ± 0.01
Hydroxyproline	Hyp	6.26 ± 0.52	2.13 ± 0.19
Isoleucine	Ile	0.31 ± 0.02	0.13 ± 0.01
Leucine	Leu	1.83 ± 0.04	0.60 ± 0.0
Lysine	Lys	0.63 ± 0.02	0.12 ± 0.03
Phenylalanine	Phe	0.82 ± 0.05	0.21 ± 0.01
Proline	Pro	1.61 ± 0.14	0.56 ± 0.001
Serine	Ser	2.50 ± 0.05	0.95 ± 0.03
Threonine	Thr	1.42 ± 0.02	0.34 ± 0.02
Tyrosine	Tyr	0.31 ± 0.04	0.14 ± 0.02
Valine	Val	0.71 ± 0.0001	0.31 ± 0.07
Total amino acids		21.5 ± 0.47	7.1 ± 0.16

Source: Lopez-Torrez et al. [29]

4 Physical Properties of Gums Arabic

The physical properties of GA may vary depending on the origin and age of trees, the exudation time, and climate. Treatment of Gums after collection such as washing, drying, and bleaching in the sun and storage conditions effected the physical properties of Gums [19]. Gum Arabic of excellent quality is tear-shaped,

Table 3 International specifications of Gum Arabic quality FAO [32]

Property	Value
Moisture (%)	13–15
Ash content (%)	2–4
Internal energy (%)	30–39
Volatile matter (%)	51–65
Optical rotation (degrees)	(−26)–(−34)
Nitrogen content (%)	0.26–0.39
Cationic composition of total ash at 550 °C	
Copper (ppm)	52–66
Iron (ppm)	730–2490
Manganese (ppm)	69–117
Zinc (ppm)	45–111

Source: Dauqan and Abdullah [35]

round, with an orange-brown color. After it is crushed or shattered, the pieces are paler in color and have a vitreous appearance. GA has high water solubility and a relatively low viscosity compared with other Gums. GA can get dissolved at water in a concentration of 50% w/v, forming a fluid solution with acidic properties (pH ~4.5). The resulting solution is colorless, tasteless, and does not interact easily with other chemical compounds [30, 31]. The viscosity of GA solutions can be modified by the addition of acids or bases as these change the electrostatic charge on the macromolecule.

The physical properties of Gum Arabic established as quality parameters include moisture, total ash, volatile matter, and internal energy, which were regarding with reference to Gums taken from *Acacia senegal* species in Sudan (Table 3). These parameters can be used to identify raw Gums mostly used as food additives [32, 34, 33]. Gum Arabic is a natural product complex mixture of hydrophilic carbohydrate and hydrophobic protein components [32]. Hydrophobic protein component functions as an emulsifier which adsorbs onto surface of oil droplets, while hydrophilic carbohydrate component inhibits flocculation and coalescence of molecules through electrostatic and steric repulsions in food additives [36, 37].

The moisture content facilitates the solubility of GA carbohydrate hydrophilic and hydrophobic proteins [38]. The total ash content is used to determine the critical levels of foreign matter, insoluble matter in acid, calcium, potassium, and magnesium [39]. The compositions of cations in the ash residue are used to determine the specific levels of heavy metals in the Gum Arabic quality [32, 40]. The volatile matter determines the nature and degree of polymerization of the compositions contained in sugar (arabinose, galactose, and rhamnose) which exhibits strong binding properties to act as emulsifiers and stabilizers in the manufacture of cough syrups in the pharmaceutical industry [2]. The GA internal energy is the required energy to produce an amount of carbon by heating at 500 °C to release carbon dioxide. Optical rotation is used to determine the nature of GA sugars as well as to identify the source of production. Nitrogen content in Gum Arabic determines the number of amino acid compositions with the range of 0.26–0.39% [32].

5 Biological Properties of Gum Arabic

5.1 Antioxidant Properties of Gum Arabic

Oxidative stress is largely occurred through the deregulation of oxidants/antioxidants injury or breakdown of macromolecules of biological nature such as proteins, carbohydrates, lipids, and nucleic acids, ensuing in the imbalances of intracellular homeostasis and consequently the production of several types of reactive oxygen species (ROS) which finally induce more oxidative injury or damage [41]. The antioxidant enzymes activity plays a vital role in the damage of hyperglycemia-related tissue [42]. Previous studies indicated that production of ROS in diabetes might commence the chronic diabetic lesions development on many tissues such as blood vessels [43], eye retina [44], kidneys [45], and neurodegenerative disorders [46].

Superoxide dismutase (SOD) [47], catalase (CAT) [48], and glutathione peroxidase (GPx) [49] are considered the main vital defense tools against reactive oxygen molecules, which are involved in the oxidative damage [50, 51]. In the diabetic patients, the oxidative stress was found to induce various unfavorable effects at the molecular levels in cell physiology [52]. The oxidative stress reduced the concentrations of glutathione (GSH) in patients with type 2 diabetes [52], decreased the activity of CAT [53], reduced the concentrations of renal SOD [54], and increased the level of heat shock protein 70 (HSP70) [55]. The reduction in antioxidant components can cause diabetic complications [56]. The administration of Alloxan by GA was cause a significant reduction of antioxidant enzymes including GPx, CAT, and SOD. The reduction of antioxidant enzymes activities was associated with observable augment in production of malondialdehyde (MDA). The elevation MDA levels ultimately cause oxidative stress, and consequently it mirrors a decreased antioxidant defense potentiality [50, 57].

Several studies indicated that GA is exert a nephron-protective effect against gentamicin (antibiotic) and cisplatin-induced nephrotoxicity in rat [58, 59], and doxorubicin-induced cardio toxicity in rat [60]. Trommer and Neubert [61] indicated that the treatment of GA together with polysaccharides reduced lipid peroxidation. In contrast, Ali [62] revealed that administration of GA to rats at concentrations of 2.5%, 5.0%, and 10.0% in the form of drinking water for eight successive days did not significantly change the levels of free radical scavenger's GSH, acid vitamin C (ascorbic acid, and SOD, or lipid peroxidation). The mechanism of action through which GA improves the antioxidant capacity may be due to the fact that GA contains several types of amino acid residues such as lysine, tyrosine, and histidine, which are commonly considered as antioxidants biomolecules [63, 64]. Moreover, the antioxidant properties of GA in biological systems require a more direct knowledge of the antioxidant capacity [65, 66]. Consequently, the antioxidant activity of GA in biological systems is still an unresolved issue, and therefore it requires a more direct knowledge of the antioxidant capacity of GA that can be obtained by in vitro experiments against different types of oxidant species.

5.2 Effect of GA on Renal Function

The administration of GA in the form of drinking water (15%, w/v) reduces the concentrations of plasma urea and creatinine in adenine-induced chronic renal failure (CRF) in rats. It also decreased the clearance of creatinine and induced significant increases in the inflammatory mediator's concentrations. Further, the treatment with GA significantly ameliorated all adverse effect that induced by adenine. The mechanism underlying the salutary effect of GA in adenine-induced CRF may associate with mitigation of the adenine-induced inflammation and generation of free radicals [67].

Pretreatment of GA (7.5 g/kg/day per oral administration), starting 5 days before mercuric chloride injection, resulted in a complete reversal of Hg-induced increase in blood urea nitrogen, creatinine, thiobarbituric acid reactive substances, and total nitrate/nitrite to control values. Pretreatment of GA prevented Hg-induced degenerative changes of kidney tissues. Thus indicated that GA is an efficient cytoprotective agent against Hg-induced nephrotoxicity [68]. The protective effect of GA on renal function was also confirmed to significantly reduce blood creatinine and urea nitrogen concentrations in diabetic nephropathy patients [69]. Recent studies have shown that GA significantly reduced serum urea and urinary protein. In addition, GA significantly decreased α-SAM and TGF-β1 gene expression in kidney and increased kidney cytokeratin19 and E-cadherin gene expression when compared to STZ-treated group. Therefore, GA may attenuate the development of nephropathy in type I diabetes rat [70].

5.3 Effect of GA on Blood Glucose Concentration

In India, GA is one of the indigenous medicines, and it has many medicinal uses. In Tunisia, the use of GA as hypoglycemic medicinal plants to treat diabetic patients was found to reach more than 70% compared to other medicinal plants [71].

A clinical trial used 40 participants with a daily supplement of powdered GA (10 g/day) for 16 weeks in healthy individuals, prediabetics patients, type 2 DM patients, and diabetic nephropathy patients. The results showed that supplementation of GA significantly decreased fasting blood glucose levels and glycosylated hemoglobin (HbAc1) together with significant reduction of blood uric acid and total protein concentrations [72]. Moreover, supplementation of GA in different forms to the normal or diabetic or fed-high fat diet rat/mice significantly reduced blood glucose levels [83, 85]. The blood glucose lowering propriety of GA may be because GA inhibits absorption of glucose in the intestine via interaction with membrane abundance of sodium-glucose transporter 1 (SGLT1) in experimental mice [69].

5.4 Effect of GA on Intestinal Absorption

The small intestine is a major part of the gastrointestinal tract (GIT) where almost all organic nonelectrolytes and electrolytes absorbed in it different mechanisms which

are operating at the cellular and molecular levels [73]. Concurrent intestinal secretion is a physiological occurrence which is tightly controlled by various mechanisms. This process maintains within the intestinal lumen a condition of variability, dilution, and solubilization that is indispensable to the usual intestinal function of digestion and absorption. The net effects of those mechanisms play a significant role to maintain the normal mammalian small intestine in an absorptive manner. On the other hand, under a certain condition, secretion forces exceed absorption, and a net secretory condition ensues resulting in diarrhea and dehydration. It has been reported that GA enhances small intestinal absorption of sodium in normal experimental rats [74, 75]. GA is also found to increase the absorption of sodium and water in two animal models of diarrheal disease [76]. In normal young rats, the addition of 5 and 10 g/L of GA increased sodium removal rates from the intestinal lumen perfused with oral rehydration solutions containing either 60 or 90 mM sodium. Although GA tended to ease bidirectional fluid movement in those experiments, the net absorption of water was not influenced [77]. At high concentration, GA was associated with increases and expansion of the basolateral intercellular space. Experimental diarrhea was induced in rats by either 1 week of drinking cathartic (magnesium citrate-phenolphthalein) solution to produce chronic osmotic-secretory effects or by jejunal perfusion of theophylline to induce jejunal secretion. The positive influences of the GA on electrolyte and fluids absorption were shown in jejunal perfusion studies in experimental rats that were recovering from chronic osmotic diarrhea induced by cathartic agents [73]. In free living rats, the administration of GA in the form of drinking supplemented ad libitum revealed accelerated recovery when compared to those receiving either water or oral rehydration solutions (ORS) without GA [73]. GA was reported to increase water and electrolytes movement, e.g., water and sodium, from the intestinal lumen to the blood stream [78].

5.5 Degradation of GA in the Intestine

Gum Arabic is mostly indigestible to both animals and humans as it is not degraded in the small intestine. However, it fermented in the large intestine particularly in colon due to the enzymatic action of microorganisms [79]. Several studies have reported that intestinal bacteria can ferment GA to short-chain fatty acid, mainly propionate [80].

5.6 Effects of GA on Lipid Metabolism

Gum Arabic is considered as a dietary supplement that reduces the deposition of body fat. Ingestion of GA was revealed to decrease body mass index (BMI) and percentage of body fat in healthy adult human females [81]. GA has many anti-obesity properties as a dietary supplement in both humans and experimental animals. GA serves as a dietary fiber which helps to reduce body weight and fat deposition. The property of lowering caloric density of the diet of GA is the most potential mechanism involved in the reduction of body weight [82]. In addition, our recent

reports suggest that other complex mechanisms might be involved [83]. The property of lowering glucose and fat absorption of GA is an additional proposed mechanism; nevertheless, this mechanism was debated in earlier reports [84]. GA suppressed diet-induced obesity by altering the expression of mRNA levels of genes involved in lipid metabolism in mouse liver [83]. In addition, GA consumption in the form of drinking water decreased visceral adipose tissue (VAT) associated with downregulation of 11β-hydroxysteroid dehydrogenase type I in liver and muscle of mice [85].

Administration of GA reduced plasma total cholesterol, triglyceride, and low density lipoprotein (LDL) concentrations in human [86] and mice [85]. In agreement with these findings, we reported that GA supplementation decreased plasma LDL, very low density lipoprotein (VLDL), and total cholesterol concentrations, whereas increased HDL concentrations. Numerous mechanisms have been proposed to reveal the hypocholesterolemic effects of dietary fiber [87]. One potential clarification is that dietary fiber increases the viscosity of the intestinal contents, and therefore, interfering nutrient with absorption and micelle formation, which, sequentially, decreases intestinal lipid absorption [88]. Another mechanism suggested that soluble fibers act by disrupting the enterohepatic circulation of bile acids, consequential to increased bile acid excretion, and subsequently reduces plasma cholesterol concentrations [89]. Moreover, the viscosity of fermentable dietary fibers is found to contribute substantially to the lipid lowering effects in rat [90].

Supplementation of GA significantly downregulated hepatic adipose triglyceride lipase (ATGL) mRNA and conserved receptor expressed in brain 2 (SREB2) mRNA expression in mice fed with high-fat diet (HFD) [83, 91]. HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis mRNA expression, was significantly lowered in the GA-treated mice [83]. The dietary fibers have pertained to the hepatic mRNA expression of HMGR in rat [92, 93]. Thus, these results provided insight into how dietary fibers affect lipid metabolism at the gene level. Adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and mono-acyl-glycerol lipase (MGL) are tightly regulated by a nutritional factor. However, under a condition of energy intake, imbalance leads to failure to efficiently control their activity. Consequently, serious metabolic disorders will occur and is believed to be a key mechanism in the development of type 2 diabetes in obesity [94].

5.7 The Effect of GA on Tooth Mineralization

Dental caries is found to occur when the tooth enamel is lost due to an imbalance of the demineralization and demineralization phases, and prevention can be accomplished if the demineralization phase is enhanced [95]. A number of agents have been used for that purpose, including fluoride [96]. Lately, using histopathological methods, it is found that GA can promote demineralization possibly by sustaining other demineralization activities [97]. This supporting function was approved to the rich content of $Mg2+$, $Ca2+$, and $K+$ salts of polysaccharides in GA, and to the

influence of the Gum on Ca2+ metabolism and possibly phosphate. It is also reported that GA contains cyanogenetic glycosides and other numerous types of enzymes such as peroxidases, oxidases, and pectinases that exhibit antimicrobial properties against certain microorganisms such as *Prevotella intermedia* and *Prophyromonas gingivalis* [98].

5.8 Effect of GA on Hepatic Macrophages

Macrophages are known to play a vital role in the regulation of immunological process in all vertebrates. It was reported that the GA activates macrophage by its ability to produce superoxide anions in vitro [99]. While other studies report that GA was competent to blocking the macrophage function completely [100, 101]. Therefore, the scientist inferred that such influences of GA would promise the consideration in the treatment of chronic liver disease, as a disturbed function of Kupffer cells and hepatic macrophages occurs in this kind of disease and is involved in its complications, such as end toxemia [102].

6 Pharmaceutical Properties of Gum Arabic

In recent years, plant-derived polymers have evoked tremendous interest due to their diverse pharmaceutical applications such as diluents, binders, disintegrants in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels, and bases in suppository [103].

In the pharmaceutical industry, GA is used as a carrier of drugs since it is considered a physiologically harmless substance. GA has some biological properties as an antioxidant [61, 104, 105] on the metabolism of lipids [106, 107] and in treating many diseases such as kidney [102, 108], cardiovascular [109], and gastrointestinal diseases [110].

Gum Arabic reduces glucose absorption, increases fecal mass and bile acids, and has the potential to beneficially modify the physiological state of humans [111]. GA is slowly fermented by the bacterial flora of the large intestine producing short-chain fatty acids [112]. In addition, GA is able to selectively increase the proportion of lactic acid bacteria and bifidus bacteria in healthy subjects. Previous studies have shown that a daily intake of 25–30 g of GA for 21–30 days reduced total cholesterol by 6% and 10.4%, respectively. The decrease was limited only to LDL and VLDL, with no effect on HDL and triglycerides [113, 114].

7 Food and Cosmetic Properties of Gum Arabic

Gum Arabic is used in textiles, ceramics, lithography, cosmetic, paints, and papermaking [9, 116]. In the food industry, GA is primarily used in confectionery, bakery, dairy, beverage, and as a microencapsulating agent. Acacia Gums are unique among

the various hydrocolloids; they are used notably in food industry because they modify and control the rheological properties of aqueous food systems acting as thickeners, stabilizers, film formers, suspending agents, flocculants, and emulsifiers. *A. senegal* Gum is most widely used in food applications mainly because of its better emulsifying properties than *A. seyal* Gum [115]. In addition, Gum solutions of *A. senegal* are generally less colorful than *A. seyal*. These properties explain differences in the higher price of *A. senegal* Gum compared to *A. seyal* in the international market. Gum Arabic is well recognized as emulsifier used in essential oil and flavor industries such as production of citrus and cola flavor oils for soft drinks [31, 34]. In dairy products, Gum Arabicis is used as a stabilizer in frozen products like ice and ice cream, absorbing water and producing a finer texture. In cosmetic industry, Gum Arabic is used as smoothener in lotions and protective creams, and adhesive in facial masks or face powders [9].

8 Conclusion

Although there are more than 1000 species of the genus *Acacia* Gums, only two are significant for commercial purposes: *Acacia senegal* and *Acacia seyal*. The chemical composition of GA varies slightly depending on its origin, climate, harvest season, tree age, and processing conditions, while the physical properties of Gum Arabic established as quality parameters including moisture, total ash, volatile matter, and internal energy.

GA improves the antioxidant capacity because it contains several types of amino acid residues such as lysine, tyrosine, and histidine, which are commonly considered as antioxidants biomolecules. GA effects renal function through reduction in blood creatinine and urea nitrogen concentrations in diabetic nephropathy patients. GA significantly decreased fasting blood glucose levels and glycosylated hemoglobin (HbAc1) together with a significant reduction in blood uric acid and total protein concentrations. GA increases water and electrolytes movement from the intestinal lumen to the blood stream. GA can be fermented by intestinal bacteria to short-chain fatty acid, mainly propionate. GA, as a dietary fiber, helps to reduce body weight and fat deposition.

In the pharmaceutical industry, GA is used as a carrier of drugs since it is considered a physiologically harmless substance. GA has some biological properties as antioxidant on the metabolism of lipids, and in treating many diseases such as kidney, cardiovascular, and gastrointestinal diseases.

In the food industry, GA is used in confectionery, bakery, dairy, beverage, and as a microencapsulating agent. In dairy products, it is used as the stabilizer in frozen products like ice and ice cream, absorbing water and producing a finer texture. In cosmetic industry, Gum Arabic is used as smoothener in lotions and protective creams, and adhesive in facial masks or face powders.

Acknowledgment Authors acknowledge all researchers whom conducted studies on Gum Arabic.

References

1. Renard D, Lavenant-Gourgeon L, Ralet MC, Sanchez C (2006) Acacia Senegal gum: continuum of molecular species differing by their protein to sugar ratio, molecular weight, and charges. Biomacromolecules 11(7):2637–2649. https://doi.org/10.1021/bm060145j
2. Phillips GO, Williams P (2001) Tree exudate gums: natural and versatile food additives and ingredients. Food Ingred Anal Int 23:26–28
3. Abuarra A, Hashim R, Bauk S, Kandaiya S, Tousi ET (2014) Fabrication and characterization of gum Arabic bonded *Rhizophora* spp. particleboards. Mater Des 60:108–115. https://doi.org/10.1016/j.matdes.2014.03.032
4. Egadu SP, Mucunguzi P, Obua J (2007) Uses of tree species producing gum arabic in Karamoja, Uganda. Afr J Ecol 45:17–21. https://doi.org/10.1111/j.1365-2028.2007.00732.x
5. Ibrahim OB, Osman ME, Hassan EA (2013) Characterization and simple fractionation of *Acacia senegal*. J Chem Acta 2:11–17
6. Hadi AH, Elderbi MA, Mohamed AW (2010) Effect of gum arabic on coagulation system of albino rats. Int J PharmTech Res 2:1762–1766
7. Wyasu G, Okereke NZ-J (2012) Improving the film forming ability of gum arabic. J. Nat. Prod. Plant Resour 2:314–317
8. Vanloot P, Dupuy N, Guiliano M, Artaud J (2012) Characterisation and authentication of *A. senegal* and *A. seyal* exudates by infrared spectroscopy and chemometrics. Food Chem 135:2554–2560. https://doi.org/10.1016/j.foodchem.2012:06.125
9. Verbeken D, Dierckx S, Dewettinck K (2003) Exudate gums: occurrence, production, and applications. Appl Microbiol Biotechnol 63:10–21
10. Glicksman M, Line Back DR, Ingett JE (eds) (1982) Food carbohydrates. Avi, CO., West port, CT
11. Walker B (1984) In: Phillips GO, Wedlock DJ, Williams TA (eds) Gum and stabilizers for the food industry, vol 2. Tergamon Press, Oxford
12. FAO (1996) A review of production, markets and quality control of gum Arabic in Africa. FAO, Rome. Forestry Dept, 191 p
13. Rodge AB, Sonkamble SM, Salve RV, Hashmi SI (2012) Effect of hydrocolloid (guar gum) incorporation on the quality characteristics of bread. J Food Process Technol 3:136
14. Islam AM, Phillips GO, Sljivo A, Snowden MJ, Williams PA (1997) A review of recent developments on the regulatory, structural and functional aspects of gum arabic. Food Hydrocolloids 11:493–505. https://doi.org/10.1016/S0268-005X (97)80048-3
15. Desplanques S, Renou F, Grisel M, Malhiac C (2012) Impact of chemical composition of xanthan and acacia gums on the emulsification and stability of oil-in-water emulsions. Food Hydrocoll 27:401–410. https://doi.org/10.1016/j.foodhyd.2011.10.015
16. Ray AK, Bird PB, Iacobucci GA, Clark BC (1995) Functionality of gum arabic. Fractionation, characterization and evaluation of gum fractions in citrus oil emulsions and model beverages. Food Hydrocoll 9:123–131. https://doi.org/10.1016/S0268-005X (09)80274-9
17. Castellani O, Guibert D, Al-Assaf S, Axelos M, Phillips GO, Anton M (2010) Hydrocolloids with emulsifying capacity. Part 1–emulsifying properties and interfacial characteristics of conventional (*Acacia senegal* (L.) Willd. var. *senegal*) and matured (*Acacia* (sen) SUPER GUM™) *Acacia senegal*. Food Hydrocoll 24:193–199. https://doi.org/10.1016/j.foodhyd.2009.09.005
18. Randall RC, Phillips GO, Williams PA (1989) Fractionation and characterization of gum from *Acacia senegal*. Food Hydrocoll 3:65–75. https://doi.org/10.1016/S0268-005X(89)80034-7
19. Al-Assaf S, Phillips GO, Aoki H, Sasaki Y (2007) Characterization and properties of *Acacia senegal* (L.) Wild. Var. *senegal* with enhanced properties (*Acacia* (sen) SUPER GUM™): part 1-controlled maturation of *Acacia senegal* var. *senegal* to increase viscoelasticity, produce a hydrogel form and convert a poor into a good emulsifier. Food Hydrocoll 21:319–328. https://doi.org/10.1016/j.foodhyd.2006.04.011

20. Al Assaf S, Phillips GO, Williams PA (2005) Studies on acacia exudate gums. Part I: the molecular weight of *Acacia senegal* gum exudate. Food Hydrocoll 9:647–660
21. Flindt C, Al-Assaf S, Phillips GO, Williams PA (2005) Studies on acacia exudate gums. Part V. Structural features of *Acacia seyal*. Food Hydrocoll 9:687–701
22. Hassan EA, Al-Assaf S, Phillips GO, Williams PA (2005) Studies on acacia gums: part III molecular weight characteristics of *Acacia seyal* var. seyal and *Acacia seyal* var fistula. Food Hydrocoll 19:669–677
23. Siddig NE, Osman ME, Al-Assaf S, Phillips GO, Williams PA (2005) Studies on acacia exudate gums, part IV. Distribution of molecular components in *Acacia seyal* in relation to *Acacia senegal*. Food Hydrocoll 19:679–686
24. Osman ME, Williams PA, Menzies AR, Phillips GO (1993) Characterization of commercial samples of gum arabic. J Agric Food Chem 41:71–77. https://doi.org/10.1021/jf00025a016
25. Williams PA, Phillips GO (2000) Handbook of Hydrocolloids. CRC Press, Cambridge, pp 155–168
26. Mahendran T, Williams PA, Phillips GO, Al-Assaf S, Baldwin TC (2008) New insights into the structural characteristics of the arabinogalactan − protein (AGP) fraction of gum arabic. J Agric Food Chem 56:9269–9276
27. Menzies AR, Osman ME, Malik AA, Baldwin TC (1996) A comparison of the physicochemical and immunological properties of the plant gum exudates of *Acacia senegal* (gum arabic) and *Acacia seyal* (gum tahla)∗. Food Addit Contam 13:991–999
28. Idris OH, Haddad GM (2012) Gum Arabic's (Gum Acacia's) journey from tree to end user. In: Kennedy JF, Phillips GO, Williams PA (eds) Gum Arabic. RSC Publishing, Cambridge, p 3e19
29. Lopez-Torrez L, Nigen M, Williams P, Doco T, Sanchez C (2015) *Acacia senegal* vs. *Acacia seyal* gums–part 1: composition and structure of hyperbranched plant exudates. Food Hydrocoll 51:41–53
30. ITC, International Trade Centre, 2008. Gum Arabic. Market News Service (MNS), Quarterly Edition
31. Hassan EA (2000) Characterization and fractionation of *Acacia seyal* gum. Doctoral dissertation, Ph. D. Thesis, University of Khartoum, Khartoum
32. FAO (1990) Specifications for identity and purity of certain food additives. Food and Nutrition Paper, 49. FAO, Rome
33. Larson BA, Bromely DW (1991) Natural resources prices, export policies, and deforestation: the case of Sudan. World Dev 19:1289–12897
34. Karamalla KA (1999) Gum arabic production, chemistry and applications. University of Khartoum, Khartoum
35. Dauqan E, Abdullah A (2013) Utilization of gum arabic for industries and human health. Am J Appl Sci 10:1270–1279. https://doi.org/10.3844/ajassp.2013.1270.1279
36. Anderson DM, Weiping W (1990) The characterization of *Acacia paolii* gum and four commercial Acacia gums from Kenya. Food Hydrocoll 3:475–484. https://doi.org/10.1016/S0268-005X (09)80225-7
37. Lelon JK, Jumba IO, Keter JK, Chemuku W, Oduor FD (2010) Assessment of physical properties of gum arabic from *Acacia senegal* varieties in Baringo District, Kenya. African J Plant Sci 4:95–98
38. Elmqvist B (2003) The vulnerability of traditional agroforestry systems: a comparison of the Gum Arabic livelihood strategy before the 1984 drought to that of the present in Kordofan-Sudan. Paper presented at the Environment, Place and Sustainable Natural Resource Management Conference, Uppsala
39. Mocak J, Jurasek P, Phillips GO, Varga S, Casadei E, Chikemai BN (1998) The classification of natural gums. X. Chemometric characterization of exudate gums that conform to the revised specification of the gum arabic for food use, and the identification of adulterants. Food Hydrocoll 12:141–150

40. FAO (1996) A review of production, markets and quality control of gum Arabic in Africa. FAO, Forestry Dept, Rome, 191 p
41. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5:9–19
42. Wu Y, Tang L, Chen B (2014) Oxidative stress: implications for the development of diabetic retinopathy and antioxidant therapeutic perspectives. Oxidative Med Cell Longev 10: 752387
43. Son SM (2012) Reactive oxygen and nitrogen species in pathogenesis of vascular complications of diabetes. Diabetes Metab J 36:190–198
44. Zhao Y, Yang K, Wang F, Liang Y, Peng Y, Shen R, Wong T, Wang N (2012) Associations between metabolic syndrome and syndrome components and retinal microvascular signs in a rural Chinese population: the Handan eye study. Graefes Arch Clin Exp Ophthalmol 250:1755–1763
45. Bondeva T, Wolf G (2014) Reactive oxygen species in diabetic nephropathy: friend or foe? Nephrol Dial Transplant 29:1998–2003. https://doi.org/10.1093/ndt/gfu037
46. Borza LR (2014) A review on the cause-effect relationship between oxidative stress and toxic proteins in the pathogenesis of neurodegenerative diseases. Rev Med Chir Soc Med Nat Iasi 118:19–27
47. Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930
48. Tiedge M, Lortz S, Munday R, Lenzen S (1998) Complementary action of antioxidant enzymes in the protection of bioengineered insulin-producing RINm5F cells against the toxicity of reactive oxygen species. Diabetes 47:1578–1585. https://doi.org/10.2337/diabetes.47.10.1578
49. Tsai CJ, Hsieh CJ, Tung SC, Kuo MC, Shen FC (2012) Acute blood glucose fluctuations can decrease blood glutathione and adiponectin levels in patients with type 2 diabetes. Diabetes Res Clin Pract 98:257–263
50. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI (2013) Markers of oxidative stress during diabetes mellitus. J Biomarkers 2013:8 p. https://doi.org/10.1155/2013/378790
51. Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Botany 24:26. https://doi.org/10.1155/2012/217037
52. Dinçer Y, Akçay T, Alademir Z, İlkova H (2002) Assessment of DNA base oxidation and glutathione level in patients with type 2 diabetes. Mutat Res 505(1):75–81
53. Góth L (2000) Lipid and carbohydrate metabolism in acatalasemia. Clin Chem 46:560–576
54. Wang C, Li S, Shang DJ, Wang XL, You ZL, Li HB (2011) Antihyperglycemic and neuroprotective effects of one novel Cu–Zn SOD mimetic. Bioorg Medicinal Chem Lett 21:4320–4324
55. Nakhjavani M, Morteza A, Khajeali L, Esteghamati A, Khalilzadeh O, Asgarani F, Outeiro TF (2010) Increased serum hsp70 levels are associated with the duration of diabetes. Cell Stress Chaperones 15:959–964
56. Nakhjavani M, Morteza A, Nargesi AA, Mostafavi E, Esteghamati A (2013) Appearance of leptin–HSP70 correlation, in type 2 diabetes. Meta Gene 1:1–7
57. Fujita H, Fujishima H, Chida S, Takahashi K, Qi Z, Kanetsuna Y, Breyer MD, Harris RC, Yamada Y, Takahashi T (2009) Reduction of renal superoxide dismutase in progressive diabetic nephropathy. J Am Soc Nephrol 20:1303–1313
58. Al-Majed AA, Abd-Allah AR, Al-Rikabi AC, Al-Shabanah OA, Mostafa AM (2003) Effect of oral administration of arabic gum on cisplatin-induced nephrotoxicity in rats. J Biochem Mol Toxicol 17:146–153
59. Al-Majed AA, Mostafa AM, Al-Rikabi AC, Al-Shabanah OA (2002) Protective effects of oral arabic gum administration on gentamicin-induced nephrotoxicity in rats. Pharmacol Res 46:445–451. https://doi.org/10.1016/S1043661802001251

60. Abd-Allah AR, Al-Majed AA, Mostafa AM, Al-Shabanah OA, Din AG, Nagi MN (2002) Protective effect of arabic gum against cardiotoxicity induced by doxorubicin in mice: a possible mechanism of protection. J Biochem Mol Toxicol 16(5):254–259
61. Trommer H, Neubert RH (2005) The examination of polysaccharides as potential antioxidative compounds for topical administration using a lipid model system. Int J Pharm 298:153–163. https://doi.org/10.1016/j.ijpharm.2005.04.024
62. Ali BH (2004) Does gum Arabic have an antioxidant action in rat kidney. Ren Fail 26:1–3. https://doi.org/10.1081/JDI-120028536
63. Marcuse R (1960) Antioxidative effect of amino-acids. Nature 186:886–887
64. Park EY, Murakami H, Matsumura Y (2005) Effects of the addition of amino acids and peptides on lipid oxidation in a powdery model system. J Agric Food Chem 53:8334–8341. https://doi.org/10.1021/jf058063u
65. Ali BH, Ziada A, Blunden G (2009) Biological effects of gum arabic: a review of some recent research. Food Chem Toxicol 47:1–8
66. Liu Y, Hou Z, Yang J, Gao Y (2015) Effects of antioxidants on the stability of β-carotene in O/W emulsions stabilized by Gum Arabic. J Food Sci Technol 52:3300–3311
67. Ali BH, Al-Qarawi AA, Haroun EM, Mousa HM (2003) The effect of treatment with Gum Arabic on gentamicin nephrotoxicity in rats: a preliminary study. Ren Fail 25:15–20. https://doi.org/10.1081/JDI-120017439
68. Gado AM, Aldahmash BA (2013) Antioxidant effect of Arabic gum against mercuric chloride-induced nephrotoxicity. Drug Des Dev Ther 7:1245
69. Nasir O, Babiker S, Salim AM (2016) Protective Effect of Gum Arabic Supplementation for Type 2 Diabetes Mellitus and its Complications. Int. J. Multidiscip Curr Res 4:288–294
70. Musa HH, Ahmed AA, Fedail JS, Musa TH, Sifaldin AZ (2016) Gum Arabic attenuates the development of nephropathy in type 1 diabetes rat. In: Gums and stabilisers for the food industry. Royal Society of Chemistry, Cambridge, pp 245–255
71. Othman RB, Ibrahim H, Mankai A, Abid N, Othmani N, Jenhani N, Tertek H, Trabelsi N, Trimesh A, Mami FB (2013) Use of hypoglycemic plants by Tunisian diabetic patients. Alexandria J Med 49:261–264
72. Nasir O, Artunc F, Wang K, Rexhepaj R, Föller M, Ebrahim A, Kempe DS, Biswas R, Bhandaru M, Walter M, Mohebbi N (2010) Downregulation of mouse intestinal Na+-coupled glucose transporter SGLT1 by Gum Arabic (*Acacia senegal*). Cell Physiol Biochem 25:203–210
73. Teichberg S, Wingertzahn MA, Moyse J, Wapnir RA (1999) Effect of gum arabic in an oral rehydration solution on recovery from diarrhea in rats. J Pediatr Gastroenterol Nutr 29:411–417
74. Rehman KU, Wingertzahn MA, Teichberg S, Harper RG, Wapnir RA (2003) Gum arabic (GA) modifies paracellular water and electrolyte transport in the small intestine. Dig Dis Sci 48:755–760
75. Codipilly CN, Wapnir RA (2004) Proabsorptive action of gum arabic in isotonic solutions orally administered to rats. II. Effects on solutes under normal and secretory conditions. Dig Dis Sci 49:1473–1478
76. Wapnir RA, Wingertzahn MA, Moyse JE, Teichberg SA (1997) Gum arabic promotes rat jejunal sodium and water absorption from oral rehydration solutions in two models of diarrhea. Gastroenterology 112:1979–1985
77. Wapnir RA, Teichberg S, Go JT, Wingertzahn MA, Harper RG (1996) Oral rehydration solutions: enhanced sodium absorption with gum arabic. J Am Coll Nutr 15:377–382
78. Turvill JL, Wapnir RA, Wingertzahn MA, Teichberg S, Farthing MJ (2000) Cholera toxin-induced secretion in rats is reduced by a soluble fiber, gum arabic. Dig Dis Sci 45:946–951
79. Phillips GO (1998) Acacia gum (gum arabic): a nutritional fibre; metabolism and calorific value. Food Addit Contam 15:251–264
80. Kishimoto A, Ushida K, Phillips GO, Ogasawara T, Sasaki Y (2006) Identification of intestinal bacteria responsible for fermentation of gum arabic in pig model. Curr Microbiol 53:173–177

81. Babiker R, Merghani TH, Elmusharaf K, Badi RM, Lang F, Saeed AM (2012) Effects of gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial. Nutr J 11:111
82. Schneeman BO (1987) Dietary fiber: comments on interpreting recent research. J Am Diet Assoc 87:1163
83. Ahmed AA, Musa HH, Fedail JS, Sifaldin AZ, Musa TH (2016) Gum arabic suppressed diet-induced obesity by alteration the expression of mRNA levels of genes involved in lipid metabolism in mouse liver. Bioact Carbohydr Diet Fibre 7:15–20
84. Ushida K (2011) Gum arabic and its anti-obese effect. In: Gum Arabic, pp 285–290
85. Ahmed AA, Fedail JS, Musa HH, Kamboh AA, Sifaldin AZ, Musa TH (2015) Gum Arabic extracts protect against hepatic oxidative stress in alloxan induced diabetes in rats. Pathophysiology 22:189–194
86. Mohamed RE, Gadour MO, Adam I (2015) The lowering effect of gum Arabic on hyperlipidemia in Sudanese patients. Front Physiol 6:160
87. Dvir I, Stark AH, Chayoth R, Madar Z, Arad SM (2009) Hypocholesterolemic effects of nutraceuticals produced from the red microalga *Porphyridium* sp. in rats. Forum Nutr 1:156–167
88. Lattimer JM, Haub MD (2010) Effects of dietary fiber and its components on metabolic health. Forum Nutr 2:1266–1289
89. Parnell JA, Reimer RA (2010) Effect of prebiotic fibre supplementation on hepatic gene expression and serum lipids: a dose–response study in JCR: LA-cp rats. Br J Nutr 103:1577–1584
90. Brockman DA, Chen X, Gallaher DD (2014) High-viscosity dietary fibers reduce adiposity and decrease hepatic steatosis in rats fed a high-fat diet. J Nutr 144:1415–1422
91. Park JA, Tirupathi Pichiah PB, JJ Y, SH O, Daily JW, Cha YS (2012) Anti-obesity effect of kimchi fermented with *Weissella koreensis* OK1-6 as starter in high-fat diet-induced obese C57BL/6J mice. J Appl Microbiol 113:1507–1516
92. Kishida T, Nogami H, Ogawa H, Ebihara K (2002) The hypocholesterolemic effect of high amylose cornstarch in rats is mediated by an enlarged bile acid pool and increased fecal bile acid excretion, not by cecal fermented products. J Nutr 132:2519–2524
93. Rideout TC, Harding SV, Jones PJ, Fan MZ (2008) Guar gum and similar soluble fibers in the regulation of cholesterol metabolism: current understandings and future research priorities. Vasc Health Risk Manag 4:1023
94. Nielsen TS, Jessen N, Jørgensen JO, Møller N, Lund S (2014) Dissecting adipose tissue lipolysis: molecular regulation and implications for metabolic disease. J Mol Endocrinol 52: R199–R222
95. Aoba T (2004) Solubility properties of human tooth mineral and pathogenesis of dental caries. Oral Dis 10:249–257
96. Cheng KK, Chalmers I, Sheldon TA (2007) Adding fluoride to water supplies. BMJ 335:699–702
97. Onishi T, Umemura S, Yanagawa M, Matsumura M, Sasaki Y, Ogasawara T, Ooshima T (2008) Remineralization effects of Gum Arabic on caries-like enamel lesions. Arch Oral Biol 53:257–260
98. Clark DT, Gazi MI, Cox SW, Eley BM, Tinsley GF (1993) The effects of *Acacia arabica* Gum on the in vitro growth and protease activities of periodontopathic bacteria. J Clin Periodontol 4:238–243. ISSN: 0303-6979
99. Mochida S, Ohno A, Arai M, Tamatani T, Miyasaka M, Fujiwara K (1996) Role of adhesion molecules in the development of massive hepatic necrosis in rats. Hepatology 23:320–328
100. Mochida S, Ogata I, Hirata K, Ohta Y, Yamada S, Fujiwara K (1990) Provocation of massive hepatic necrosis by endotoxin after partial hepatectomy in rats. Gastroenterology 99:771–777
101. Fujiwara K, Mochida S, Nagoshi S, Iijima O, Matsuzaki Y, Takeda S, Aburada M (1995) Regulation of hepatic macrophage function by oral administration of xiao-chai-hu-tang (sho-saiko-to, TJ-9) in rats. J Ethnopharmacol 46:107–114

102. Ali AA, Ali KE, Fadlalla A, Khalid KE (2008) The effects of GA oral treatment on the metabolic profile of chronic renal failure patients under regular haemodialysis in Central Sudan. Nat Prod Res 22:12–21
103. Zatz JL, Kushla GP (1989) In: Reiger MM, Banker GS (eds) Pharmaceutical dosage forms: Disperse systems. Marcel Dekker Inc., New York, p 508
104. Hinson JA, Reid AB, McCullough SS, James LP (2004) Acetaminophen-induced hepatotoxicity: role of metabolic activation, reactive oxygen/nitrogen species, and mitochondrial permeability transition. Drug Metab Rev 36(3–4):805–822
105. Ali BH, Al Moundhri MS (2006) Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: a review of some recent research. Food Chem Toxicol 44:1173–1183
106. Evans AJ, Hood RL, Oaken full DG, Sidhu GS (1992) Relationship between structure and function of dietary fibre: a comparative study of the effects of three galactomannans on cholesterol metabolism in the rat. Br J Nutr 68:217–229
107. Tiss A, Carrière F, Verger R (2001) Effects of gum Arabic on lipase interfacial binding and activity. Anal Biochem 294(1):36–43
108. Matsumoto N, Riley S, Fraser D, Al-Assaf S, Ishimura E, Wolever T, Phillips GO, Phillips AO (2006) Butyrate modulates TGF-beta1 generation and function: potential renal benefit for *Acacia* (sen) SUPERGUM (G.A.)? Kidney Int 69:257–265
109. Glover DA, Ushida K, Phillips AO, Riley SG (2009) *Acacia* (sen) SUPERGUMTM (Gum Arabic): an evaluation of potential health benefits in human subjects. Food Hydrocoll 23:2410–2415
110. Wapnir RA, Sherry B, Codipilly CN, Goodwin LO, Vancurova I (2008) Modulation of rat intestinal nuclear factor NF-kappaB by Gum Arabic. Dig Dis Sci 53:80–87
111. Adiotomre J, Eastwood MA, Edwards CA, Brydon WG (1990) Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans. Am J Clin Nutr 52:128–134
112. Annison G, Trimble RP, Topping DL (1995) Feeding Australian acacia gums and gum Arabic leads to non-starch polysaccharide accumulation in the cecum of rats. J Nutr 125(2):283–292
113. Ross AH, Eastwood MA, Brydon WG, Anderson JR, Anderson DM (1983) A study of the effects of dietary Gum Arabic in humans. Am J Clin Nutr 37:368–375
114. Sharma RD (1985) Hypocholesterolaemic effect of Gum acacia in men. Nutr Res 5(12):1321–1326
115. Jani GK, Shah DP, Prajapati VD, Jain VC (2009) Gums and mucilages: versatile excipients for pharmaceutical formulations. Asian J Pharm Sci 4:309–323
116. Elmanan M, Al-Assaf S, Phillips GO, Williams PA (2008) Studies on *Acacia* exudate gums: part VI. Interfacial rheology of *Acacia senegal* and *Acacia seyal*. Food Hydrocoll 22: 682e–6689

Resistant Starch in Food

28

Pinky Raigond, Som Dutt, and Brajesh Singh

Contents

P. Raigond (✉) · S. Dutt · B. Singh
Division of Crop Physiology, Biochemistry and Post Harvest Technology, ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India
e-mail: jariapink@gmail.com; sd_bio@yahoo.com; birju16@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_30

Abstract

Consumers' awareness on benefits of intake of low carbohydrate foods is increasing day by day. Carbohydrate-rich foods are generally blamed for causing/worsening diabetes and weight gain. However, the presence of resistant starch in carbohydrate-rich foods makes them somewhat suitable for health conscious persons. Resistant starch is of utmost importance to nutritionists and food processors. Resistant starch has assumed great importance due to its unique functional properties and health benefits. Resistant starch provides health benefits such as glycemic control, control of fasting plasma triglyceride and cholesterol levels, and absorption of minerals. Native quality of starch, processing techniques, and storage temperatures affect the resistant starch content in food. Commercial preparations of resistant starch are available in the market. These RS preparations are being used by food industries as an ingredient to lower the caloric value of the food products and also to improve textural and organoleptic characteristics of food. This book chapter will give insights into classification, structure, properties, production, applications, and health benefits of resistant starch.

Keywords

Resistant starch · Classification · Properties · Production technology · Applications · Health benefits

Abbreviations

ANN	Annealing treatment
GI	Glycemic index
HAS	High amylose starch
HHP	High hydrostatic pressure
HMT	Heat moisture treatment
HPT	High pressure treatment
NSP	Non-starch polysaccharides
RDS	Rapidly digestible starch
RS	Resistant starch
SCFA	Short chain fatty acids
SDS	Slowly digestible starch

1 Introduction

Carbohydrates are the main energy source for our body and their energy is used first in the body before protein and fat. Starch is the main form of carbohydrate present in most of the foods. Starch is made up of two major components,

i.e., amylose and amylopectin. These components are present in different ratios in different plants/foods. Quality of starch depends on the ratio and organization of these two molecules in starch granules [1]. Starch is indigestible in its raw form and its digestibility improves to great extent during cooking. Most of the part of starch becomes digestible after cooking; however, some parts remain resistant to digestion. A part of starch present in the diet that escapes digestion and absorption in the small intestine and is fermented in the large intestine of humans, with the production of short chain fatty acids (SCFA), is termed as "resistant starch" (RS) [2]. Resistant starch is present in most of the starchy foods. Resistant nature of the starch depends upon the quality of native starch such as its amylose: amylopectin ratio, compactness, crystallinity, etc. Along with these, behavior and nature of food, cooking methods, temperatures, storage after cooking, and interaction of starch with proteins, lipids, and other carbohydrates are also known to influence the digestibility of starch [3]. American Association of Cereals Chemists and the Food Nutrition Board of Institute of Medicine of the National Academies have defined RS as a type of dietary fiber. There are five different types of RS based on their origin and characteristics. RS is known to positively influence the functioning of digestive tract, gut microbial flora, blood cholesterol, and glycemic index (GI) level and assists in the control of diabetes, protects from colon cancer, diverticulitis, and hemorrhoids through production of SCFA [4, 5].

Due to increasing awareness about healthy and nutritious foods, consumers are now concerned with supplementary health merits of a food and are ready to pay more for healthy food products. International food industry is working on development of innovative functional food products with additional health benefits to fulfill the growing demands for functional foods. Most of carbohydrate-rich foods are known to be high glycemic. Glycemic index (GI) ranks food according to their effect on blood glucose level and high GI food cause fatal health problems such as diabetes and obesity. Development of carbohydrates-based functional foods with low GI is need of the hour. Due to the inverse relation between GI and RS, food nutritionists are looking for use of RS as food fortificant to lower the GI of food. For development of functional foods, fortification is an economically feasible way to deliver the desired component such as RS, in targeted population group. RS is naturally available in different botanical sources, which provides opportunity of its use as a functional ingredient. Food products fortified with RS are becoming popular among consumers. RS is already being incorporated in variety of products such as breads, cookies, muffins, pizza crust, tortillas, breakfast cereals, and snack products. For production of RS at a large scale, various techniques/treatments such as heat, enzymatic, heat with enzyme and chemical treatments are being practiced. Literature/reports on RS have appeared in the past but there is continuous accumulation of newer information as resistant starch continues to attract the attention of researchers. Therefore, it became necessary to update the available information on RS, and this chapter attempts to analyze the information published, especially in the recent past on classification, structure, properties, production technologies, applications, and health benefits of resistant starch.

2 Starch

Starch is the most abundant storage polysaccharide present in form of granules in cereals or legume seed endosperm, tubers (potato and sweet potato), unripe fruits (banana and mango), and in many other plant reserve organs. Depending on the botanical source starch is present in diverse shapes such as round, oval, lenticular, and angular and the granule size generally range between 1 and 100 μm. A starch granule is made up of a number of monosaccharide or glucose molecules that are linked together with α 1–4 and α 1–6 linkages [6]. Starch is made up of two components namely amylose and amylopectin. Amylose is a linear chain of glucose with 6000 degree of polymerization, whereas, amylopectin is highly branched and its degree of polymerization is up to two million. Carbohydrates are stored in insoluble and tightly packed manner in starch granules [7]. Based on the X-ray diffraction spectrum, starches are divided into two forms, i.e., "A type" and "B type." These two types differ in the granule crystalline structures. A-type starches are present in cereals, whereas, B-type starches are present in tubers and other amylose-rich starches. In case of legumes, mixture of both A-type and B-type crystalline structures are present and this mixture is termed as "C-type" structure [8].

Enzymes such as α-amylase, glucoamylase, and sucrose-isoamylase can hydrolyze the gelatinized/digestible starches in small intestine. B-type starches resist enzymatic digestion, whereas A-type starches are slowly digested [9, 10]. Digestibility of starch is affected by a number of factors such as supramolecular structure (packing of crystallites inside starch granules), ratio of amylose to amylopectin, fine structure of amylose, as well as surface characteristics of starch granule [11]. Starch granules being compact structures are insoluble in cold water. Starch gelatinizes at high temperatures (generally above 40 °C) and starches from different sources have different gelatinization temperature. Gelatinized starch contains dissolved carbohydrates, amylose, and low polymerized chains of amylopectin that binds considerable amount of water upon cooling. Amylose and amylopectin chains in gelatinized starch realign upon cooling of starch paste and this process is termed as "retrogradation." Retrogradation occurs at higher rate if the starch paste is stored at lower temperatures. Retrograded starches contain both crystalline and semi-crystalline structures and are hence semi-crystalline in nature. Products of amylopectin retrogradation are less thermostable compared to amylose retrogradation products and their rehydration occurs at the temperatures over 60 °C and 120 °C, respectively.

3 Classification of Starch

Starch is classified based on its rate and extent of digestion and physiological properties. There are three types of starches viz. rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (Table 1) [12, 13].

Table 1 Classification of starch [13]

Starch fraction	RDS	SDS	RS (Type 1–4)
Digestion timeline (in vitro)/place	Within 20 min, mouth and small intestine	20–120 min, small intestine	>120 min, not in the small intestine, main action in colon
Examples	Freshly cooked food	Native waxy maize starch, millet, legumes	Raw potato, stale bread
Amount (g/100 g dry matter)	Boiled hot potato: 65	Boiled millet: 28	Raw potato starch: 75
Main physiological property	Rapid source of energy	Slow and sustained source of energy and sustained blood glucose	Effects on gut health (e.g., prebiotic, fermentation to butyrate with hypothesized anticarcinogenic effects)
Structure	Mainly amorphous	Amorphous/crystalline	Depending on the type, mainly crystalline

3.1 Rapidly Digestible Starch

Rapidly digestible starch is defined as a "type of starch which is rapidly (within 20 minutes) converted to glucose molecules by the enzymatic digestion" [13]. High levels of RDS are present in starchy foods cooked under moist heat such as potatoes and breads. Major portion of dietary starch is rapidly digestible. More RDS in food is detrimental to health, as it releases glucose to blood at a fast rate which elevates blood glucose level and insulin response [14]. RDS is significantly correlated to GI in literature [15–17].

3.2 Slowly Digestible Starch

Slowly digestible starch is defined as a "type of starch which is converted to glucose after 120 minutes of enzymatic digestion" [13]. SDS is a physically inaccessible amorphous starch that takes long time for digestion and is completely digested in the small intestine. Englyst's in vitro test revealed that raw cereal starches are rich in SDS. However, human testing of normal corn starch (A type) revealed the slow and prolonged postprandial glucose release profile [18]. Slow digestion property of native starches is generally lost during processing of starchy food under moisture [19]. SDS-rich foods as well as low GI foods confer similar health benefits. Such foods delay the occurrence of metabolic syndrome, diabetes, and cardiovascular diseases [20–22]. Retrogradation of partially debranched amylopectin after isoamylase treatment generates SDS [23, 24].

3.3 Resistant Starch

The term "resistant starch" was coined by Englyst et al. to describe a small fraction of starch that resist hydrolysis by α-amylase and pullulanase treatment in vitro [12]. RS resists digestion and absorption in the small intestine and is fermented in the colon. When compared to RDS and SDS, RS is not hydrolyzed to glucose in the small intestine within 120 minutes of being consumed. Amylose amylopectin ratio influences the resistant nature of starch. Digestion of amylose is slow and that of amylopectin is fast after retrogradation. RS is a linear molecule of α 1, 4 D-glucan and is mainly derived from retrograded amylose in cooked starchy food. Large number of factors influences the rate and extent of starch digestion and all of these factors are interlinked which complicate the understanding of resistant nature of starch. With some exceptions such as pea starch, RS content of granular starch is directly correlated with its amylose content [25–27]. Due to this positive correlation as well as other functional and nutritional properties of amylose, agricultural researchers started working on development of crops containing high amylose starches (HAS) [28]. High amylose potato, barley, and wheat have already been developed by some researchers [29–32].

4 Relationship between RDS, SDS, and RS

All the three starch fractions are related to each other. RDS shows inverse relationship with SDS and RS. SDS and RS are structurally more similar in terms of retrogradation and coexist in processed foods [33]. SDS can be made from RDS by several physical, chemical, and physiological methods [34].

5 Classification and Structure of Resistant Starch

Resistant starch is classified into five sub-types viz. RS1, RS2, RS3, RS4, and RS5 (Table 2). Earlier reports described about only three types of RS, i.e., RS1, RS2, RS3, however, over the period of time two more types, i.e., RS4 and RS5, were added to the classification [13, 35–38].

RS1: This type of starch is physically inaccessible to digestion due to its entrapment within whole or partly milled grains or seeds and due to presence of intact cell walls in grains, seeds, or tubers. This type of RS escapes digestion due to inaccessibility of amylolytic and digestive enzymes and passes to small intestine as such [39]. Only proper grinding/milling can make this RS to digest completely in small intestine. Its heat stable nature does not allow it to break down or open up during normal cooking.

RS2: Native starch granules which are protected from digestion due to their conformation/structure are termed as RS2. Raw potato and green banana contain this type of starch. RS2 being crystalline and compact in nature has no access to digestive enzymes and amylases and hence is poorly susceptible to hydrolysis [40].

Table 2 Classification of types of resistant starch (RS), food sources, and factors affecting their resistance to digestion in the colon [59]

RS type	Description	Food sources	Resistance minimized by	Digestion in small intestine
RS1	Physically protected	Whole- or partly milled grains and seeds, legumes	Milling, chewing	Slow rate, partial degree, totally digested if properly milled
RS2	Ungelatinized resistant granules with type B crystallinity, slowly hydrolyzed by α-amylase	Raw potatoes, green bananas, some legumes, high amylose corn	Food processing and cooking	Very slow rate, little degree, totally digested when freshly cooked
RS3	Retrograded starch	Cooked and cooled potatoes, bread, cornflakes, food products with repeated moist heat treatment	Processing conditions	Slow rate, partial degree, reversible digestion, digestibility improved by reheating
RS4	Chemically modified starches due to cross-linking with chemical reagents	Foods in which modified starches have been used (e.g., breads, cakes)	Less susceptible to digestibility in vitro	A result of chemical modification, can resist hydrolysis
RS5	Amylose-lipid complexes	Foods with high amylose content	Not susceptible to hydrolysis by α-amylase	Can resist digestion

Both RS1 and RS2 show slow and incomplete digestion in small intestine. Nowotny observed the resistant nature of raw potato starch in 1937 while working on the enzymatic hydrolysis of raw starch from numerous plant species [41]. He observed the small extent of enzymatic hydrolysis of raw potato starch. Later his results were confirmed by various researchers [42, 43]. Fine-grain high amylose maize starch and coarse grain potato starch showed similar resistant nature. The structure as well as resistance of high amylose maize starch is retained even during processing/preparation of food. Starches from different sources vary in the size of granule. Granule size affects the extent of enzyme adsorption on its surface. However, no relationship was found between the extent of enzyme adsorption and degree of starch hydrolysis [44]. Later the extent of enzymatic hydrolysis was linked to degree of starch crystallization. Starch hydrolytic enzymes first degrade the amorphous region, therefore, resistant nature was attributed to the crystalline region of starch; however, resistant nature of starch is not always linked to its degree of crystallinity. High amylose starches as well as B-type starches are more resistant to enzymatic hydrolysis [43, 45].

In B-type starches only surface of granule is hydrolyzed, whereas in A-type starches deep enzymatic hydrolysis takes place. Starch resistance depends upon the size of blocklets in the granule. Potato starch contains large blocklets formed due to the double helices and are incorporated in crystalline layers of granules and hence potato starch is more resistant compared to cereal starches which contains small blocklets [46–48]. Resistant nature of starch is not dependent on any one factor, but a large number of factors such as granule size, shape, amylose content, crystallinity, and also size of pores/holes in the granule affects the starch resistance [39].

RS3: Physically modified starches are termed as RS3. RS3 is formed during moist-heat processing of food and is basically a retrograded starch or more precisely a retrograded or recrystallized amylose found during cooking of gelatinized starch and in cooked starchy foods that are stored at low or room temperature. RS3 is highly thermostable and is used as an ingredient in a wide variety of conventional foods [40]. Most common examples of RS3 are cooked and cooled potatoes and corn flakes [49]. RS3 is nutritionally important type of RS and has good quality due to high water holding capacity compared to granular starch [50]. During storage of gelatinized starch at low temperature for some time, amylose double helices realign and aggregate to form a highly thermostable B-type crystalline structure. Amylose retrogradation renders α-1 → 4 glucosidic linkages inaccessible to amylase which is responsible for resistant nature of RS3. Being thermostable these aggregates can be rehydrated only at high temperatures, i.e., above 150 °C. Formation of RS3 is highly influenced by storage temperature and duration. Storage of starch paste at low temperature results in more RS3 formation compared to storage at high temperature [51]. Starch crystallinity is also affected by storage temperature. Storage of gelatinized starch at low temperature results in B-type crystallinity, whereas storage at boiling temperature results in A-type crystallinity [52]. Both amorphous as well as crystalline fractions are present in starch gels, wherein amorphous fraction is hydrolyzed by amylolytic enzymes and crystalline fraction remains resistant [53]. Formation of RS3 is hampered by formation of amylose-lipid complexes, which results in less production of RS3 [54]. When starch gels are stored at low temperatures, amylopectin also participates in formation of partly crystallized gels. However, crystallization by amylopectin is quite slow and amylopectin crystallization structures are less thermostable compared to amylose crystallization structures and can be rehydrated at 55–70 °C temperature [36]. Amylopectin crystallization structures are also resistant to amylolytic enzyme activity. Formation of RS3 depends on the botanical origin of starch, amylose content, and procedure used for its preparation. Preparation of amylopectin retrogradation products is a lengthy process; however, this process can be hastened with repeated heating and cooling of starch paste.

RS4: Chemically modified starches are known as RS4 [55]. RS4 is a group of starches in which digestibility is decreased with chemical procedures such as etherization, esterification, or cross-bonding with chemicals. Based on water solubility of RS4 as well as analysis method, RS4 is further divided into four sub-categories [4]. Chemical modification mainly involves conversion, substitution, and cross-linking. During chemical modification enzyme access to starch is blocked by formation of atypical linkages which decrease its digestibility by hydrolytic enzymes [56].

The resistance of acetylated and hydroxypropylated starches is directly proportional to degree of substitution [57]. When compared to native starches, hydroxypropyl distarch phosphate and acetylated distarch phosphate are more resistant to enzyme hydrolysis [58]. The level of resistance in RS4 is directly proportional to the degree of chemical modification. For example, higher the degree of substitution with phosphoric acid, more will be the resistance of monostarch phosphate. The structure as well as composition of starch granules is changed during chemical modification, which in turn increase their resistance to amylolytic enzymes.

RS5: Amylose chains are penetrated by lipids in starch from many plant sources. RS5 is a type of RS which is formed due to amylose-lipid complexes. Generally, these complexes are formed during food processing; however, they can also be prepared under controlled conditions. These type of complexes appeared in high amylose starches, hence formation of amylose-lipid complexes is influenced by the amylose-amylopectin ratio of starch and botanical source. RS5 comprises polysaccharides of water insoluble linear poly α-1,4 glucan that are resistant to hydrolysis by α-amylase [38]. These polysaccharides promote the formation of SCFA, mainly butyrate, the most important SCFA [59].

6 Properties of RS

RS has gained importance because of its nutritional aspects as well as functional properties. RS can be used in a variety of foods due to numerous desirable properties such as swelling, viscosity increase, gel formation, and water-binding capacity (Table 3) [60]. Due to these properties RS can replace flour on one-to-one basis without affecting dough rheology significantly. Other properties of RS include its bland flavor, which upon incorporation in some other food does not change taste of

Table 3 Functional properties and advantages of commercial sources of RS2 and RS3 [59]

Natural sources
Bland in flavor
White in color
High gelatinization temperature
Fine particle size (which causes less interference with texture)
Good extrusion and film-forming qualities
Lower water properties than traditional fiber products
Lowering the calorific value of foods
Increase coating crispness of products
Increase bowl life of breakfast cereals
Functional food ingredients
Useful in products for celiacs as bulk laxatives and in products for oral rehydration therapy
Allow the formation of low-bulk high-fiber products with improved texture, appearance, And mouth feel (such as better organoleptic qualities) compared with traditional high-fiber products

food. RS appear white in color and have fine particle size. Like taste, texture of food also remains unaltered due to its fine particle size. Due to low calorie content of RS (1.6–2.8 Kcal g^{-1}) it can complement reduced fat and low sugar food formulations and can impart special characteristics along with dietary fiber fortification in high fiber food [61]. Low water holding capacity of RS provides good handling during processing, improves crispiness, expansion, and texture in the end product. Other qualities of RS include high gelatinization temperature, good extrusion, and film forming property. Incorporation of RS improves coating crispiness of the product and also enhances the bowl life of breakfast cereals. Food industry is successfully using RS in a range of baked and extruded products. RS is highly suitable in grain-based, low moisture to moderate moisture food systems [59].

7 RS Production Technologies

To fulfill the growing demands for functional foods, food industry is investigating ways to produce innovative functional food products with additional health benefits. With increasing awareness about health and nutrition among consumers, researchers and producers are aiming to develop functional foods with supplementary health merits [62]. Researchers and nutritionists are working together for development of functional foods with low GI. Due to various health benefits such as positive influence on digestive tract functioning, gut microbial flora, blood cholesterol, GI as well as on diabetes control, and being low calorie ingredient RS can be used for fortification of high GI foods to convert them to low GI [63]. Presence of natural sources of RS makes it suitable functional ingredient for fortification purposes [64]. In order to increase the intake of dietary fiber, consumers are ready to pay more for RS-fortified food products. Several techniques are available to alter the GI as well as rate of starch digestion. These techniques involve modification of key functional ingredients using low or no calorie sugars, formation of starch lipid-complexes, or through processing techniques such as heat moisture treatment and extrusion. RS is included in the food to combine physical characteristics of the food such as texture, water holding capacity, processing stability, and nutritional functionality. To preserve the nutritional functionality of RS-containing food, stability of RS during processing is of utmost importance [65]. Various techniques such as heat, enzymatic, heat with enzyme, and chemical treatment are available for manufacture of RS.

7.1 Heat Treatment

7.1.1 Heating Cooling Cycles

Repeated heating and cooling cycles for RS production are being used since long. Another method for RS production involves starch gelatinization followed by enzymatic debranching of gelatinized polymer, deactivation of the debranching enzyme, and isolation of the resultant product by drying/extrusion/cocrystallization. RS produced by using heating-cooling cycles is RS3. Various researchers have

reported the increase in RS3 content by using different combinations of storage duration and heating and cooling temperatures. More RS3 yield is obtained with gelatinization in high pressure autoclave as compared to water bath (90 °C) [66]. High RS3 can be prepared by autoclaving of starch (30% w/v) at 121 °C for 1 h and cooling at room temperature followed by storage at 48 °C for 1 day. Same steps are repeated several times to obtain high RS3 yield. Heating of wheat starch suspension (20% w/w) at 100 °C for 16 h, followed by air drying results in 12.9% RS3 as compared to 10.4% tested as dietary fiber [67]. Boiling and cooling of potatoes at 4 °C for overnight results in 2.8-fold increase in RS yield [68]. RS content increased up to 63% after boiling and cooling of potatoes at 4 °C for 48 h compared to boiled hot potatoes [49]. Gelatinization of starch at 120 °C for 20 min followed by cooling to room temperature yielded high amount of RS [69]. Starch gelatinization at 110 °C, 121 °C, 127 °C, 134 °C, and 148 °C for 30 min to 1 h can increase the RS3 yield even in starches containing normal amylose level [70–72]. Gelatinization of water-starch suspension in 1:3.5 ratio at 134 °C with four repeated heating cooling cycles results in good yield of RS3 [73, 74].

7.1.2 Hydrothermal Treatment

Hydrothermal treatment involves physical modifications that change the physico-chemical properties of starch without altering its granular structure. Annealing (ANN) and heat moisture treatment (HMT) are the main hydrothermal treatments where the temperature and heating time need to be controlled. These treatments are based on the starch to moisture ratio. In ANN treatment, a combination of excess of water (>40%) and temperature below gelatinization is used, whereas HMT takes place under controlled moisture content (10–30%) with high temperature (90–120 °C) [75]. Partial acid hydrolysis enhances the effect of hydrothermal treatment and can result in heat stable granular RS [76]. HMT is natural physical modification technique that is safer than chemical modification of starch. In ANN treatment temperature must be held below gelatinization temperature to retain the initial granular structure of starch. Granular structure is lost due to combination of gelatinization and melting at 40–60% moisture level. Enhanced granular stability due to hydrothermal treatment results in high RS content [65].

ANN treatment is known to enhance the degree of starch crystallinity, strengthen crystalline form of granules, and order starch chains in crystalline as well as amorphous layers. All of this increases granule stability with decreased solubility and swelling capability and hence, in turn, increase resistance of starch granules to amylolytic enzyme [77]. Lee et al. [78] used different combinations of moisture (20–25%) treatment, duration (1, 5, and 9 h), and temperatures (110 °C, 130 °C, and 150 °C) to treat waxy potato starch (0% amylose) and achieved maximum RS yield, i.e., 66.8% with the combination of 20% moisture, 110 °C temperature, and 5-h treatment.

Starch treated with HMT has increased resistance to amylolytic enzymes. HMT of high amylose maize starch at 100 °C temperature and moisture content below 35% leads to crystallite formation along with ordering and binding of amylose chains in the amorphous region which results in lower degree of swelling and hence results in

resistant nature of starch [79]. Chung et al. [80] reported 7.7%, 20.3%, and 5.6% increase in RS yield after HMT of corn, pea, and lentil starches, respectively. Chung et al. [81] compared the effect of ANN and HMT on RS yield and observed that HMT results in higher RS yield compared to ANN. ANN increased RS to 0.3%, 1.3%, and 1.6% whereas increase with HMT was 1.7, 4.7, and 5% in starches of pea, lentil, and navy bean, respectively. An extensive range of botanical sources and a range of HMT conditions were studied by various researchers and they observed numerous changes on granular surface, swelling factor, amylose leaching, gelatinization temperature, X-ray diffraction pattern, crystallization, and gelatinization parameters of starch [82–85].

Most widely used methods to increase RS3 yield are ANN, acid hydrolysis of amylomaize starch, and repeated freeze-thawings [65, 86]. Generally high amylose corn starch is used for preparation of RS3 [87]. ANN treatment (50 °C for 24 h at 50% moisture) increased RS content in pinto bean and black bean to 39.7% and 19.7%, respectively. Jiranuntakul et al. [88] observed that different starches respond differently to HMT. They observed that HMT (25% moisture, 100 °C temperature, and 16 h) increases the RS from 27–40.3% in waxy corn starch, whereas same conditions decrease the RS from 4.7% to 29.7% in normal corn, rice, and potato starch. Zero to seven cycles of boiling and freezing at −18 °C for 23 h to whole wheat flour in water (1:15% w/v) increased the RS content from 1.03% to 8.07% [89].

7.1.3 Extrusion

Extrusion technique is widely used in food processing industry to prepare food products with different shapes. This is a high temperature short time process (HTST) and has the potential to increase the RS content of food product up to certain extent. During extrusion high shear force causes depolymerization followed by thermal cleavage of the starch molecule. As a result straight chains are produced which are more prone for retrogradation into RS3 [90]. RS content of normal corn starch increased from 11% to 20% after acid hydrolysis followed by low or high shear extrusion [91]. Extrusion conditions such as barrel temperature, screw speed, and shear force have more impact on RS yield as compared to starch moisture content [92]. Extrusion of maize starch with 12–18% moisture did not show significant increase in RS content, whereas at 20% moisture level, RS content increased significantly. Origin of starch affects the RS yield during extrusion. Because of high gelatinization temperature and amylose content banana starch is most efficient for production of RS through extrusion, compared to other starches [64]. RS content of wheat starch increase from 0.8% to 2.8%, normal maize starch from 1.5% to 2.1% [93], barley from 2% to 3% [94] after extrusion. Reports on increase in RS after extrusion are conflicting as many studies reported decrease in RS content after extrusion and attributed this to degradation of starch granules under high temperature, pressure, and shear forces [95]. Possible reason for this conflict could be the interaction of starch with other components available in various food matrix.

7.1.4 Heat and Enzyme Treatment

RS production can be increased by using a combination of heat and enzymatic or chemical and enzymatic modification. Enzymes or chemicals can be used to remove amorphous region of retrograded starch. Example of simultaneous heat and enzyme treatment is pullulanase treatment of gelatinized starch and isolation of the product by drying/extrusion. RS production is also achieved by controlled heat treatment of starch, followed by debranching with the help of enzymes, annealing, and drying [96]. Treatment of gelatinized starch with debranching enzyme such as isoamylase or pullulanase results in debranched amylopectin starch. Debranched amylopectin starch is used in formation of reduced-fat food products and can be prepared from any starch containing amylopectin such as common corn and waxy maize starch. In high amylose maize starch, RS content is increased by enzymatic debranching followed by extrusion or drying, where the addition of an inorganic salt or debranched starch before the isolation further increases the RS content [74, 97]. Debranching of potato amylopectin with pullulanase before repeated heating-cooling cycles as well as maize starch debranching increase the RS3 yield [70, 98]. However, to get high yield of RS3 from maize starch autoclaving of starch at 121 °C for 1 h before debranching is required.

7.2 Enzymatic Treatment

Lower molecular mass of starch and amylopectin debranching play a role in enhanced RS production and enzymes are used for both of these purposes [99]. Debranching enzymes such as pullulanase and isoamylase act only on α 1,6 glycosidic bonds at branch points of amylopectin and break these bonds. As a result amylose content of the starch increases which in turn forms tightly packed crystalline structures which is responsible for the resistant nature of starch. Enzymes such as α- and β-amylase can also be used to break the α-1,4 glycosidic bonds. Both enzymes act on different areas of starch molecules, where α- amylase breaks all α-1,4 glycosidic bonds leaving those near the branch points and releases glucose monomers. Whereas β-amylase breaks every other α-1,4 glycosidic bond from nonreducing end of amylopectin or amylose and releases maltose units. In practice, pullulanase and isoamylase are commonly used for RS production compared to α- and β-amylase. As α-amylase breaks almost all the α-1 → 4 glycosidic bonds of starch, it lowers the starch paste viscosity and as a result adversely affects crystal formation. In low viscosity starch pastes fast movement of linear chains causes difficulty in crystal formation [100]. Optimization of α-amylase is required to obtain sufficient yield of RS. Measurement of total dietary fiber content of RS samples through enzyme treatment revealed that the concentration of α-amylase is more critical compared to amyloglucosidase [101]. The α-amylase activity showed inverse relationship with RS content. Poly 1,4-α-D-glucan can also be used for production of readily fermentable heat stable RS of optimal chain length [102]. Pullulanase enzyme can also be used to produce RS with the same cooking quality as that of untreated rice starch or flour. Starches

from potato, oat, barley, sago, corn, wheat, tapioca, and arrow root can be used to produce RS through pullulanase treatment [74].

7.3 Chemical Treatment

Chemical reagents are used to block the enzyme access and as a result modified starch escapes digestion after chemical treatment. Chemical treatment changes the molecular structure of starch which results in high RS production. Acidification, esterification, and cross-linking are the major forms of chemical modification of starch.

7.3.1 Acidification

The purpose of acidification is to first hydrolyze the amorphous parts of starch granules followed by hydrolysis of crystalline region. This process generates short chains of amylopectin which are disorganized by autoclaving followed by acidification. During retrogradation, these chains reorient to form more ordered double helix structures which resist enzyme hydrolysis [103]. Acid modification followed by autoclaving and retrogradation to increase RS yield is reported by several researchers [104, 105]. Acids such as hydrochloric acid, orthophosphoric acid, and sulfuric acid can be used for starch modification [106]. Tester et al. [107] obtained 49.5% RS by treatment of lima bean (*Phaseolus lunatus*) with hydrochloric acid at 1/60 parts of native starch at 90 °C for 1 h. Xie and Liu [108] obtained 68.3% RS yield with chemical treatment of normal corn starch with citric acid followed by dry heating at 140 °C for 7 h and heating at 100 °C in boiling water bath. During acidification by citric acid, citric anhydride substitutes the hydroxyl glucans of starch chains that resist digestion by amylolytic enzymes. Thermal-acid treatment of starch for production of RS needs optimization, as high intensity of this treatment may destroy the resistant structure of starch. Heat and citric acid treatment of normal maize starch increased RS yield to 35.62% [109].

7.3.2 Cross-Linking

Food industry is using cross-linking to improve functional property, freeze thaw stability, and cold storage stability of starch pastes. Cross-linking stabilizes and strengthens starch by randomly adding inter- and intramolecular bonds [110, 111]. Seib and Woo [112] and Woo and Seib [113] have described the use of various cross-linking techniques for increasing RS yield in normal starches derived from several botanical sources. Starches are chemically modified by treating with multifunctional reagents that form ether or ester linkage between hydroxyl groups of starch molecule [111]. Chemically modified starches show resistance to enzyme hydrolysis in both raw as well as gelatinized form [114]. During chemical modification, starch resistance is improved by substitution of starch hydroxyl group with citryl, acetyl, octenylsuccinyl, and hydroxypropyl [108, 115–118]. Cross-linking of starch with phosphate showed contradictory results as some authors [113, 119] reported decrease in digestibility of starch and some authors [58, 118, 120] reported either slight or no change in the digestibility of starch. These contradictions

could be due to the difference in the origin and property of starch and the conditions used to modify starch. For production of cross-linked starches bi-or-polyfunctional reagents such as sodium trimetaphosphate, phosphorous oxychloride, or mixed anhydrides of acetic acid and dicarboxylic acid like adipic acid are used. Kahraman et al. [121] reported use of reagents such as sodium triphosphate or its mixture with sodium tripolyphosphate for cross-linking glucans for RS production. Factors such as source of starch, reaction conditions like time, temperature, pH, and type and concentration of cross-linking reagent affect the chemical and functional properties of cross-linked starches [111, 122]. Woo and Seib [113] reported positive correlation between RS content and reaction time. Temperature and pH both increase the RS content of cross-linked corn and wheat starch, where cross-linking is more subjective to pH than temperature-induced modifications. Cross-linking of corn and wheat starch yield 80.4% and 83.9% RS, respectively [123]. Wheat and corn starch has different optimum conditions for production of cross-linked RS. For production of cross-linked wheat starch 38 °C temperature and pH 12 and for cross-linked corn starch 70 °C temperature and pH 12 are optimum treatment conditions [121]. Carlos-Amaya et al. [124] reported increase in RS from 21.49% to 29.14% in banana starch with dual modification using cross-linking and esterification. Starch may appear as swollen granules, combination of starch fragments, as well as dispersed starch molecules or completely dispersed starch molecules based on the degree of gelatinization. Ratio of starch fractions viz. RDS, SDS, and RS is determined by the degree of starch gelatinization and the structural changes in starch caused by physical or chemical treatment [125].

Chemically modified starches have been utilized as food additives, thickening or gelling agents, and fat replacers. Hydrothermal treatments increase the accessibility of chemically modified starches to the amylolytic enzymes. However, the level of digestion is determined by the origin of starch and degree of substitution with chemical groups [120]. Di-starches are modified RS with high dietary fiber content ($\geq$ 70% w/w) and their resistance to the activity of amylase show direct correlation with degree of chemical substitution [113]. Acetylated retrograded starches also come under chemically modified starches and their quality is influenced by degree of substitution and raw material used for esterification [126]. Hydroxypropylation, roasting with glycine, and cross-linking with epichlorohydrin also increase starch resistance to amylolytic enzymes [125].

7.4 Hydrostatic Pressure Treatment

Hydrostatic pressure treatment (HPT) is a nonthermal food processing method where food is processed at high hydrostatic pressure (HHP) ranging from 200 to 600 MPa. Water is used as a pressure transmitting medium in this process [127]. During HPT, starch microstructure is influenced by factors such as pressure level, method of pressure application, time, temperature, constitution of food, and phase state of food [128–132]. High pressure treatment at 120 to 600 MPa for 30 min converted the C-type X pattern of starch to B-type in mung bean [131]. Potato starch being more resistant to pressure than rice, corn, or tapioca starch yields low RS at equal

HHP level [133]. Among the continuous and two-cycle (200 and 600 MPa) HPT, HHP at 600 MPa significantly alters the microstructure and lowers the RS content of rice starch in comparison to HHP level 200 MPa [134]. Two 15-min cycle of high hydrostatic pressure treatment at 200 MPa could be beneficial to increase RS content. Zhang et al. [135] reported 11.7% RS yield of high amylose maize starch (50% amylose content) gelatinized at high pressure and temperature (10.3 MPa, 110 °C) and retrograded for 24 h.

8 Improving RS Production in Plants

In view of the industrial application and the nutritional benefits of resistant starch, researchers around the globe have been working to increase the RS content of the plants. The approaches for increasing the RS content in plants includes natural selection, conventional breeding, as well as transgenic. All these approaches are based on biosynthetic pathways of starch metabolism. The key enzymes for starch biosynthesis are AGPase, starch synthases, and branching enzymes. Generation of the sugar nucleotide ADP-glucose is catalyzed by AGPase. Starch synthases catalyze the polymerization of glucose residues resulting in formation of α-1,4 glucans. Branching enzymes cleave α-1,4 glucans and reattach the cleaved chain to an α-1,4 glucan chain by an α-1,6 glycosidic linkage thereby forming a branch.

In 2000, Gerhard et al. developed very-high-amylose potato starch by manipulating starch branching enzymes through genetic engineering [29]. They simultaneously inhibited two isoforms of starch branching enzyme to below 1% of the wild-type activities which resulted in altered starch granule morphology and composition. In these potatoes amylopectin was found to be absent, whereas the amylose content increased to levels comparable to the highest commercially available maize starches. Slade et al. used TILLING (Targeting Induced Local Lesions in Genomes) approach and identified mutations in the form of single nucleotide polymorphisms (SNPs) in starch branching enzyme IIa genes (SBEIIa) [136]. They combined these new alleles of SBEIIa through breeding which resulted in the development of high amylose durum and bread wheat varieties containing 47–55% amylose and having elevated resistant starch levels compared to wild-type wheat. Recently in 2014, Sparla et al. applied the TILLING approach on barley and identified 29 new alleles in five genes related to starch metabolism known to be expressed in the endosperm during grain filling: BMY1 (Beta-amylase 1), GBSSI (Granule Bound Starch Synthase I), LDA1 (Limit Dextrinase 1), SSI (Starch Synthase I), SSIIa (Starch Synthase IIa). A line having a nonsense mutation in SSIIa exhibited a twofold increased amylose/amylopectin ratio [137].

9 Commercially Available RS Products

Starch Australia Ltd. introduced the first commercial RS, i.e., Hi-maize. Later other companies introduced new commercial starches to market using different preparation technologies. The commercial RS prepared by different companies vary in

percent RS content. Other commercial RS are CrystaLean® (RS3), Novelose®240 (RS2), Novelose®260 (RS2), Novelose®330 (RS3), Eurylon® (RS2), Amylomaize VII (RS2), and Neo-amylose (RS3) (Table 4). CrystaLean is RS3 produced by starch retrogradation of high amylose maize starch ae-VII hybrid. National Starch and Chemical Co. (USA) introduced Hylon-VII, a natural high amylose maize starch. Most of the above RS3 products are prepared by amylose retrogradation of high amylose corn starch using repeated heating and cooling cycles under controlled moisture and temperature conditions. These processes lead to manufacture of granular form of concentrated RS containing up to 47–60% RS content. A highly crystalline RS3 namely Actistar Act*-RS3 has also been prepared using maltodextrins as starting material. Due to the starting material and process used for the production, Act*-RS3 tastes very natural. High amylose corn starch is also being used for the production of Fibersym HA, which is being used in a wide array of lower-net-carbohydrate food products. Fibersym HA provides more than 70% dietary fiber and is used in the preparation of food products such as pizza crust, breads, tortillas, cookies, muffins, breakfast cereals, snack products, and nutritional bars. Potato starch is used for the production of Fibersym 80ST. Fibersym 80ST has slightly higher water holding property which influences the properties of finished food products like cookie spread and muffin volume. Nutriose FB06 and Fibersol-2 also contain high RS content and provide 85% and 90% fiber content, respectively. Fibersym 80ST, Fibersym RW, Fibersym HA, and Fibersol-2 are all RS4 preparations and are available in the market. RS preparations without altering the organoleptic properties of food products reduce the availability of some saccharides. RS fortification does not alter the quality of product and organoleptic properties of extruded, baked products and confectionary remains unchanged [59, 138].

10 Applications of RS

Potential physiological benefits and unique functional properties of RS have attracted the attention of nutritionists and food processors. With ever increasing awareness of consumers towards health and nutritious food, they are concerned with supplementary health merits derived from regular ingestion of RS along with traditional nutritional aspects of food [139]. Looking into this, food processors, researchers, and producers are working on development of improved foods with slow digestion and additional health benefits. RS is one such ingredient which is being used for fortification of food products to enhance the nutritional value and health merits of food. RS being naturally present in broad range of starchy foods makes it convenient to use it as a functional ingredient for fortification purposes. RS-fortified food products are gaining popularity among consumers and consumers are even ready to pay more for such food products to increase their dietary fiber intake [140]. At commercial level, RS containing starch ingredients are available in name of "resistant starch." Most of these RS-enriched products are fully digestible and act as RS supplier [108]. In mid-1990s the first ever commercially available RS product was reported. However, nowadays RS-rich powders are prepared by large number of

Table 4 Commercially manufactured resistant starches commonly used in various foods [59].

Brand name of commercial RS	Type	RS/TDF% content	Physiological and/or health benefits	Manufacturer
Hi-maize	RS2	30–60% TDF	Prebiotic properties. Lowers fecal pH. Increases the level of SCFA (in particular butyrate which may reduce cancer risk). Increases bowel action with its mild laxative effect. Increases the bowels' beneficial microflora	National Starch and Chemicals co., USA
CrystaLean	RS3	19.2–41% RS	Prebiotic effect. Increases proportion of butyrate. Increases cell proliferation in proximal colon (in rats). Provides soluble dietary fiber and prebiotic effects. Low glycemic index	Opta food ingredients Inc., USA
Novelose 240	RS2	47% RS	Lowers glycemic response when used as a substitute for flour and other rapidly digested carbohydrates	National Starch and chemicals co., USA
Novelose 260	RS2	60% RS	Lowers glycemic response when used as a substitute for flour and other rapidly digested carbohydrates	National Starch and chemicals co., USA.
Novelose 300	RS3	<30% TDF	Lowers glycemic response when used as a substitute for flour and other rapidly digested carbohydrates	National Starch and chemicals co., USA
C*Actistar	RS3	53% RS	Health benefit potential. Prebiotic effect. Source of butyrate. Supports the immune system. Reduced glycemic response. Low calorific value. Easily fermentable RS. Very well tolerated.	Cerestar (Cargill company)
Fibersym™ HA	RS4	>70% TDF	Acts as prebiotic, reduces the glycemic and insulin response of healthy individuals as well as type 2 diabetics	MGP ingredients, Inc. (Atchison, Kans.) and Cargill
Fibersym™ 80ST	RS4	80% TDF	Acts as prebiotic, reduces the glycemic and insulin response of healthy individuals as well as type 2 diabetics	MGP ingredients, Inc. (Atchison, Kans.) and Cargill
Nutriose FB	–	85% TDF	Low calorific value	Roquette, Freres, France
Fibersol 2	–	90% TDF	Probiotics effect, intestinal regularity, and blood sugar regulation	ADM/Matsutani

(*continued*)

Table 4 (continued)

Brand name of commercial RS	Type	RS/TDF% content	Physiological and/or health benefits	Manufacturer
Hylon^{R} VII	RS2	23% TDF	Increases level of SCFA	National Starch and chemicals co., USA.
Neo-amylose	RS3	87 or 95% RS	Prebiotic. Protects against inflammatory intestinal disease. May protect against colorectal cancer. May help control blood glucose levels in diabetics	Protos-biotech. (Celanese ventures GmbH)

companies employing various technologies. RS cannot be replaced by traditional insoluble fiber in a RS-fortified food product, due to the high quality of RS-enriched final product [141]. RS is being used in the preparation of moisture-free food products. Cross-linked RS prepared from starches of maize, tapioca, and potato are being used for formulation requiring pulpy texture, smoothness, flowability, and low pH and high temperature storage [142]. Baked products, pasta products, as well as beverages are fortified with RS to improve their textural properties and nutritional quality. Most of the fat in imitation cheese was successfully replaced with RS, without adversely affecting the meltability or hardness of RS. In such cases, RS provides dual benefits, one being reduction of fat in a food product and another being health benefits conferred by RS itself. Nowadays large number of RS/fiber-fortified products such as high fiber breads, biscuits, and breakfast cereals are available in the market. The availability of techniques to prepare RS tolerant to processing made it possible to prepare RS-rich food products. Dry pasta products containing up to 15% RS can be prepared, without affecting dough rheology during extrusion. In comparison to unfortified pasta, RS-fortified pasta appears light in color and has a firm texture in the same time as that of unfortified pasta [74]. RS added opacity to the beverages. It is being used in thickened opaque health drinks in which insoluble fiber is desirable. RS is superior to other fibers, due to its bland taste, which imparts less gritty mouthfeel and masks flavor to much lesser extent. However, other fibers have a strong flavor, coarse texture, and poor and dry mouth feel.

11 Health Benefits of RS Consumption

Health awareness is increasing at a fast rate among consumers. To meet the growing demand of consumers for functional foods, international food industry is investigating ways to produce innovative food products with additional health benefits. Carbohydrate-rich foods are main part of our diet and most of the carbohydrate-rich foods are high glycemic. Therefore, development of carbohydrate-rich foods with low GI is need of the hour. High GI foods adversely affect health by spiking

blood glucose level and cause insulin disturbance. Carbohydrate products with low GI can improve the control of obesity and diabetes and subsequently can reduce the risk of cardiovascular diseases [62]. However, all RS types are not effective to control cholesterol level. The composition and properties of RS determine the extent of production of SCFA by bacterial fermentation of RS in the large intestine [143]. RS confers physiological benefits of soluble fiber and has a positive impact on colonic health by increasing crypt cell production rate or decreasing colonic epithelial atrophy in comparison with no fiber diet. Consumption of carbohydrate-rich foods was highly recommended, until recently. However, due to change in the viewpoint on nutrition, in developed countries, nutritional quality of food is expressed by the tendency of reducing calorific value of food. Civilizational changes also contributed to increase in dietary fiber intake that is required for proper functioning of body. RS being a natural food component, attracted much attention. Dietary fiber including RS with little calorific value confers several health benefits like laxation, blood cholesterol attenuation, and blood glucose attenuation.

11.1 RS: A Type of Dietary Fiber

Dietary fibers are "the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine" [144]. Champ et al. [145] defined dietary fiber as "a part of plant present in the form of a chemical substance which has indigestibility in the small intestine and/or has beneficial digestive and physiological effects and metabolic fate." Dietary fiber comprises of non-starch polysaccharides (NSP), lignin, RS, and nondigestible oligosaccharides. Dietary fiber majorly consists of NSP and lignin. NSPs include majorly cellulose and hemicelluloses (glucan, gums, and pectin). NSP are completely resistant to amylolytic enzymes. In the small intestine, soluble dietary fiber such as β-glucan and arabinoxylan leads to the formation of viscous solution and hence, increase viscosity in the intestine, which in turn slows intestinal transit, delays gastric emptying leading to slow glucose and sterol absorption [146]. Lignin, cellulose, and hemicelluloses are insoluble dietary fibers with high water-holding capacity which contributes to increased fecal bulk. Dietary fibers that are not fermented in the body are excreted in the feces. Dietary fibers are essential for body and they help in regular bowel movement, blood cholesterol attenuation, and blood glucose attenuation.

11.2 RS Improves Probiotic Bacteria

Prebiotic property of RS is of great interest. Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth as well as activity of one or more number of bacterial species residing in the colon and as a result improve host health. Prebiotic and probiotic have a symbiotic relationship. One of the best example of prebiotic is fructo-oligosaccharide, others being inulin,

oligofructose, RS, and inulin type-fructans [140, 148]. RS plays a role as prebiotic as well as symbiotic [148]. Probiotics enhance the epithelial barrier, increase adhesion to intestinal mucosa, and concomitantly inhibit the pathogen adhesion, competitive exclusion of pathogenic microorganisms, production of anti-microorganism substances, as well as modulation of the immune system [149]. RS is known to promote the growth and activity of probiotic bacteria in the colon and also can interact with other prebiotic dietary fibers such as β glucan [145, 150, 151]. RS ingestion helps in extending the viability of some probiotic organisms in the colon. RS acts by protecting some of the ingested organisms on their path to colon and increase the initial levels of bacterial species once they reach the colon. RS acts as a substrate for probiotics in the colon [147]. High amylose starch (HAS) being source of RS2 also acts as prebiotic and prevents the development of nonreversible insulin resistance, lower plasma cholesterol, and triglyceride concentration compared to diet rich in amylopectin starch [152]. RS affects fecal bulk and SCFA metabolism, hence has direct impact on colonic health [74]. RS3 has a high rate of fermentation by intestinal microflora which leads to production of SCFA containing high concentration of butyrate, which has positive impact on colon health [153]. In comparison to RS3, RS4 is inaccessible to enzymes due to chemical modification of starch. Large number of microorganisms and enzymes residing in the colon digest and metabolize most of the biopolymers. RS is hydrolyzed to glucose by bacterial amylase, which is further metabolized into organic acid (e.g., lactic acid) and gases (CO_2, H_2, CH_4) [65, 154]. Among the SCFA, butyrate acts as a main nutrient for colonocytes and deficiency/lack of butyrate increase the risk of colonic diseases such as colon cancer. Compared to all other dietary fibers, RS produces high concentration of butyrate, hence is very much important for human health. RS being source of butyrate in colon prevents the risk of colorectal cancer. Butyrate acts as a source of energy for epithelial cells and inhibits the malignant transformation of such cells in vitro. The rate of fermentation of RS in the colon determines the site of production of SCFA [144]. RS can also be beneficial to adults suffering from colonic lesions. Overall RS has the potential to lower the risk of many diet-related diseases and improve the human health [98, 155].

11.3 Hypoglycemic Effect of RS

FAO recommended increased intake of low GI foods with emphasis on diabetics and subjects with impaired glucose tolerance [156]. GI is a ranking of food/food products with respect to their influence on postprandial glycemia [1]. Hypoglycemia occurs as a side effect in diabetes mellitus patients and can occur in other diseases as well. RS-rich foods have slow digestion rate. This property of RS is of much importance to type II diabetic patients, due to its slow rate of glucose release as well as the time it takes to metabolize. The starch inaccessibility to digestive enzymes (such as α-amylase, isoamylase, and pullulanase) in starchy foods is responsible for reduced postprandial blood glucose and insulin response. Along with RS, diets rich in SDS are also good for health of diabetics as well as nondiabetic persons [13, 157]. Whole

grains containing high concentration of dietary fibers are a good example of low GI food, as they release glucose at slow rate [158]. Processing technique can lower the starch digestibility and can increase the SDS and RS content in cereal grains and can hence enhance the nutritional value of cereal grains. The outer layer and germ of wheat, barley, rye, and oat grains is rich in many bioactive compounds such as dietary fiber, antioxidants, phenolics, lignin, vitamin, and minerals [159]. Such compounds are effective in reduction of risk of cardiovascular diseases, cancer, diabetes, obesity, coronary heart disease, and other chronic diseases [160]. Food industry is focusing on producing functional foods with low GI using these cereal whole grains.

11.4 Hypocholesterolemic Effect of RS

Asp et al. [161] investigated the role of RS in relation to its hypocholesterolemic effect and protective effect against colorectal cancer. RS affects lipid metabolism, where total lipid, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL), triglycerides, and triglyceride-rich lipoprotein are all affected by RS [4]. Feeding of rat with RS diets containing up to 25% raw potato raises the cecal size, cecal pool of SCFA, absorption of SCFA in colon and lowers the plasma cholesterol and triglycerides [74]. Martinez-Flores et al. [162] observed hypocholesterolemic properties of cassava starch extruded with 9.7% RS. Such starches can be used in food to improve overall cardiovascular health. RS ingestion decreased the serum cholesterol level in rats fed with cholesterol-free diet [163]. Some contradictory reports on the role of RS in altering triglycerides and cholesterol levels are available, which emphasize the need of more research in this particular area to obtain a clear picture on effect of RS on lipid metabolism in humans.

11.5 RS Plays a Role in Energy and Weight Management

RS releases energy partially in small intestine as glucose and partially in large intestine as fermentation by-product such as acetate, which in turn provide balanced energy for long time after consumption. Energy value of RS is quite low and is calculated to be approximately 8 kJ g^{-1} (2 kcal g^{-1}) in comparison to energy provided by completely digestible starch, i.e., approximately 15 kJ g^{-1} [164]. RS has been reported to play a role in modification of fat oxidation, as satiety agent, and in weight management [4, 165, 166]. Mobilization and utilization of fat stored as an indirect result of reduction in insulin secretion is enhanced with RS-rich diets [167]. Compared to fully digestible carbohydrates, RS-enriched foods provide few calories along with lower glucose response. RS reduces the total and regional body fat accumulation. Diet enrichment with RS is a natural, endogenous way to increase

gut hormones which are effective in reducing energy intake [168]. RS fortification may be a very effective natural approach to treat obesity.

11.6 Reduction of Gallstone Formation

High secretion of insulin due to consumption of digestible starch-based food is leading to gallstone formation [74]. The occurrence of gallstone formation is quite less in Southern India where whole grains are preferred over flour, compared to North India, where majorly flour is used [169]. The lesser occurrence could be due to the high intake of dietary fibers and RS in South India. The dietary fiber intake in the USA, Europe, and Australia is two- to fourfold lower compared to India and China, where high starch diets are consumed. These results are correlated with the difference in the number of gall stone cases in these countries [74, 170].

11.7 Enhanced Absorption of Minerals

Studies on rat as well as human have shown that RS increase ileal absorption of a number of minerals. RS consumption enhances absorption of only calcium in humans, whereas in rats fed with RS-rich diets, increase in absorption of calcium, magnesium, zinc, iron, and copper was observed [151]. Consumption of diet containing 16.4% RS increases calcium and iron absorption in infant pigs [171]. Schulz et al. [172] reported increase in calcium and magnesium absorption by enhancing mineral solubility in the cecum and/or large intestine in rats by RS2 consumption [172]. Feeding raw potato starch to rat enhanced the fermentation in the distal part of digestive tract, resulting in increased absorption of calcium, magnesium, zinc, and copper owing to hypertrophy of the cecal wall and cecal acidification [173].

11.8 Other Health Benefits

RS reduces inflammatory bowel diseases such as ulcerative colitis and other large bowel problems such as diverticulitis and constipation [4]. As RS is involved in the production of SCFA, particularly, butyrate in vivo, it may prove a useful adjunct to traditional treatments of ulcerative colitis. Diets rich in RS2 as well as RS3 normalize the cell function-like activation of colonic cell proliferation, restoration of apoptotic response and uptake of SCFA, increased cecal level of butyrate, improved cecal and distal macroscopic and histological observations in rats with chemically induced colitis [174]. RS affects immune function through production of pro-inflammatory cytokines and expression of number of receptors on T- and B-lymphocytes that are required for initiation of immune response [175, 176].

12 Future Trends

The increased number of research papers that have appeared on RS is testimony to the importance of RS. RS in food is an active area of research. Research is being carried out on various aspects of RS, such as its increase in food through processing techniques, production of RS-fortified food, studies on health benefits of RS in vitro and in vivo, etc. Due to keen interest of researchers and nutritionists, several commercial preparations of RS are available which can be used to increase fiber content of food. More efforts are required for production of RS resistant to thermal processes during processing. Although several health benefits of consuming RS are reported, further studies are required for development of RS preparation with optional characteristics to claim specific health benefits such as improved blood health and lowering the GI. Although some figures have been proposed for specific health benefits, in general, it is very difficult to suggest a figure for RS consumption for general health benefit. Overall RS consumption has been decreased due to modern food processing practices. However, due to desirable functional and physiological properties of RS, there is an increasing trend to incorporate commercial preparation of RS in processed foods. In developed countries, where processed food daily intake is considerable, RS will provide dual benefit, one being dietary fiber and another being bioactive functional food component to increase gut hormones that reduce energy intake and hence treat obesity. As the demand for healthier food increases owing to its physiological benefits, RS can form essential ingredient of innovative foods to be developed in future.

Future may witness the more tailor made starch derivatives with multiple modifications. Buttriss and Stokes [140] reported development of insoluble resistant maltodextrins with similar functionality as that of RS. More such resistant components may appear in future. Nondigestible chemically modified starch derivatives may have increased application in food formulations [16]. As extrusion also enhances RS concentration of native starch, more extruded products with high RS can be expected in future [177].

13 Conclusions

Due to research on its different aspects (physical, chemical, and physiological) good understanding of RS has been achieved. Reports show that the consumption of RS has declined over the last few decades, possibly due to intake of low fiber foods and fast foods. Looking into this trend, food scientists have developed number of RS/dietary fiber-rich products easily available in the market. RS is ideal for fortification of ready to eat cereals, snacks, pasta, noodles, baked foods, and fried foods. These products can be labeled simply as "starch conferring additional nutraceutical benefits." Processing conditions can be altered to increase its content in food. Alterations in number of heating-cooling cycles, pH, temperature, time, freezing, and drying, etc., alter the RS content. Products prepared with RS incorporation have better crispness, mouthfeel, color, and flavor compared to those prepared with traditional

fibers. RS has assumed great importance due to its physiological properties that can reduce the risk of several diseases, including colon cancer and diabetes and also is useful in controlling obesity and diabetes. RS-fortified products have better consumer acceptability because of its unique physiochemical properties.

References

1. Bello-Perez LA, Paredes-Lopez O (2008) Starches of some food crops, changes during processing and their nutraceutical potential. Food Eng Rev 1:50–65
2. Asp NG (1992) Resistant starch. Proceedings from the second plenary meeting of EURESTA: European FLAIR concerted action, 11 on physiological implications of the consumption of resistant starch in man. Eur J Clin Nutr 46:SI
3. Birkett AM, Brown IL (2008) Resistant starch and health. In: Hamaker BR (ed) Technology of functional cereal products. CRC Press, West Palm Beach, pp 63–85
4. Nugent AP (2005) Health properties of resistant starch. British nutrition foundation. Nutr Bull 30:27–54
5. Srikaeo K, Sangkhiaw J (2014) Effects of amylose and resistant starch on glycaemic index of rice noodles. Food Sci Technol 59:1129–1135
6. British Nutrition Foundation (BNF) (1990) Complex carbohydrates in foods: the report of the British nutrition Foundation's task force. Chapman & Hall, London
7. Imberty A, Buleon A, Tran V, Perez S (1991) Recent advances in knowledge of starch structure. Starch 43:375–384
8. Topping DL, Clifton PM (2001) Short chain fatty acids and human colonic function: roles of resistant starch and non starch polysaccharides. Physiol Rev 81(3):1031–1064
9. Gerard C, Colonna P, Buleon A, Planchot V (2001) Amylolysis of maize mutant starches. J Sci Food Agric 81:1281–1287
10. Jane JL, Wong KS, McPherson AE (1997) Branch structure difference in starches of A- and B-type X-ray patterns revealed by their naegeli dextrin. Carbohydr Res 300:219–227
11. Oates CG (1997) Towards an understanding of starch granule structure and hydrolysis. Trends Food Sci Technol 8:375–382
12. Englyst HN, Wiggins HS, Cummings JH (1982) Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst 107:307–318
13. Englyst HN, Kingman SM, Cummings JH (1992) Classification and measurement of nutritionally important starch fractions. Eur J Clin Nutr 46:S33–S50
14. Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH (1999) Rapidly available glucose in foods: an *in vitro* measurement that reflects the glycemic response. Am J Clin Nutr 69:448–454
15. Englyst HN, Veenstra J, Hudson GJ (1996) Measurement of rapidly available glucose (RAG) in plant foods: a potential *in vitro* predictor of the glycaemic response. Brit J Nutr 75:327–337
16. Champ MMJ (2004) Physiological aspects of resistant starch and *in vivo* measurements. J AOAC Int 87(3):749–755
17. McCleary BV, Monaghan DA (2002) Measurement of resistant starch. J AOAC Int 85(3):665–675
18. Seal CJ, Daly ME, Thomas LC, Bal W, Birkett AM, Jeffcoat R, Mathers JC (2003) Postprandial carbohydrate metabolism in healthy subjects and those with type 2 diabetes fed starches with slow and rapid hydrolysis rates determined *in vitro*. Brit J Nutr 90:853–864
19. Zhang G, Ao Z, Hamaker BR (2006) Slow digestion property of native cereal starches. Biomacromolecules 7:3252–3258
20. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH (2003) Prevalence of a metabolic syndrome phenotype in adolescents. Arch Pediatr Adolesc Med 157:821–827

21. Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K, Pyorala K (2004) Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic european men and women. Arch Intern Med 164:1066–1076
22. Giugliano D, Ceriello A, Esposito K (2006) The effects of diet on inflammation. J Am Coll Cardiol 48:677–685
23. Shi YC, Cui X, Birkett AM, Thatcher MG (2003) Slowly digestible starch products. US Patent 20030219520, 20030215562
24. Shin SI, Choi HJ, Chung KM, Hamaker BR, Park KH, Moon TW (2004) Slowly digestible starch from debranched waxy sorghum starch: preparation and properties. Cereal Chem 81:404–408
25. Benmousaa M, Moldenhauer KAK, Hamaker BR (2007) Rice amylopectin fine structure variability affects digestion properties. J Agric Food Chem 55:1475–1479
26. Sang Y, Bean S, Seib PA, Pedersen J, Shi YC (2008) Structure and functional properties of sorghum starches differing in amylose content. J Agric Food Chem 56:6680–6685
27. Themeier H, Hollman J, Neese U, Lindhauer MG (2005) Structural and morphological factors influencing the quantification of resistant starch II in starches of different botanical origin. Carbohydr Polym 61:72–79
28. Richardson PH, Jeffcoat R, Shi YC (2002) High-amylose starches: from biosynthesis to their use as food ingredients. Mater Res Soc Bull 5:20–24
29. Schwall GP, Safford R, Westcott RJ, Jeffcoat R, Tayal A, Shi YCA (2000) Production of very-high-amylose potato starch by inhibition of SBE A and B. Nature. Australas Biotechnol 18(5):551–554
30. Morell MK, Kosar-Hashemi B, Cmiel M, Samuel MS, Chandler P, Rahman S (2003) Barley sex6 mutants lack starch synthase iia activity and contain a starch with novel properties. Plant J 34:173–185
31. Regina A, Bird A, Topping D, Bowden S, Freeman J, Barsby T (2006) High amylose wheat generated by RNA interference improves indices of large-bowel health in rats. Proc Nat Acad Sci USA 103:3546–3551
32. Zhua L, Liua Q, Wilson JD, Gu M, Shi Y (2011) Digestibility and physicochemical properties of rice (*Oryza sativa* L.) flours and starches differing in amylose content. Carbohydr Polym 86:1751–1759
33. Zhang G, Ao Z, Hamaker BR (2008) Nutritional property of endosperm starches from maize mutants: a parabolic relationship between slowly digestible starch and amylopectin fine structure. J Agric Food Chem 56:4686–4694
34. Zhang G, Hamaker BR (2009) Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. Crit Rev Food Sci Nutr 49:852–867
35. Englyst HN, Cummings JH (1987) Resistant starch a 'new' food component. A classification of starch for nutritional purposes. In: Morton ID (ed) Cereals in a European context. Ellis Horwood, Chichester, pp 221–233
36. Eerlingen RC, Delcour A (1995) Formation, analysis, structure and properties of type III enzyme resistant starch. J Cereal Sci 22:129–138
37. Brown I (1996) Complex carbohydrates and resistant starch. Nutr Rev 54:S115–S119
38. Fuentes-Zaragoza E, Sanchez-Zapata E, Sendra E, Sayas E, Navarro C, Fernandez-Lopez J, Pérez-Alvarez JA (2011) Resistant starch as prebiotic: a review. Starch-Starke 63:406–415
39. Leszczynski W (2004) Resistant starch – classification, structure, production. Pol J Food Nutr Sci 13(54):37–50
40. Hernandez O, Emaldi U, Tovar J (2008) *In vitro* digestibility of edible films from various starch sources. Carbohydr Polym 71:648–655
41. Nowotny E (1938) Effect of malt and barley amylase on raw non-gelatinised starch. Rocz Nauk Rol Leoen 45:1–38
42. Fuwa H, Nakajima M, Hamada A, Glover DV (1977) Comparative susceptibility to amylases of starches from different plant species and several single endosperm mutant and their double-mutant combinations with opaque-2 inbred oh43 maize. Cereal Chem 54:230–237

43. Sugimoto Y (1980) Scanning electron microscopic observation of starch granules attacked by enzyme. J Jap Soc Starch Sci 27:28–40
44. Kimura A, Robyt JF (1995) Reaction of enzymes with starch granules: kinetics and products of the reaction with glucoamylase. Carbohydr Res 277:87–107
45. Sarikaya E, Higasa T, Adachi M, Mikami B (2000) Comparison of degradation abilities of α- and β-amylases on raw starch granules. Process Biochem 35:711–715
46. Gallant DJ, Bouchet B, Baldwin PM (1997) Microscopy of starch: evidence of a new level of granule organization. Carbohydr Polym 32:177–191
47. Ridout MJ, Gunning AP, Parker ML, Wilson RH, Morris VJ (2002) Using AFM to image the internal structure of starch granules. Carbohydr Polym 50:123–132
48. Kossmann J, Lloyd J (2000) Understanding and influencing starch biochemistry. Crit Rev Plant Sci 19:171–226
49. Raigond P, Ezekiel R, Kaundal B (2014) Starch fractions of cooked potatoes at low temperature. Potato J 41(1):58–67
50. Sanz T, Salvador A, Fiszman SM (2008) Resistant starch (RS) in battered fried products: functionality and high-fiber benefit. Food Hydrocoll 22:543–549
51. Eerlingen RC, Crombez M, Delcour JA (1993) Enzyme-resistant starch. i. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation. Cereal Chem 70(3):339–344
52. Shamai K, Blanco-Peled H, Shimoni E (2003) Polymorphism of resistant starch type III. Carbohydr Polym 54:363–369
53. Colquhoun IJ, Parker R, Ring SG, Sun L, Tang HR (1995) An NMR spectroscopic characterization of the enzyme-resistant residue from α-amylolysis of an amylose gel. Carbohydr Polym 27:255–259
54. Eerlingen RC, Van den Broeck I, Delcour JA, Levine H (1994) Enzyme resistant starch. VI. Influence of sugars on resistant starch formation. Cereal Chem 71(5):472–476
55. Wepner B, Berghofer E, Miesenberger E, Tiefenbacher K (1999) Citrate starch: application as resistant starch in different food systems. Starch 51(10):354–361
56. Kim MJ, Choi SJ, Shin SI, Sohn MR, Lee CJ, Kim Y (2008) Resistant glutarate starch from adlay: preparation and properties. Carbohydr Polym 74:787–796
57. Hoover R, Zhou Y (2003) *In vitro* and *in vivo* hydrolysis of legume starches by α-amylase and resistant starch formation in legumes – a review. Carbohydr Polym 54:401–407
58. Ostergard K, Bjorck L, Gunnarsson A (1988) A study of native and chemically modified potato starch. Part I: analysis and enzyme availability *in vitro*. Starch 40:58–66
59. Raigond P, Ezekiel R, Raigond B (2014) Resistant starch in food: a review. J Sci Food Agric 95:1968–1978
60. Fausto FD, Kacchi AI, Mehta D (1997) Starch products in confectionery. Beverage & Food World 24(4):4–16
61. Tharanathan RN, Mahadevamma S (2003) Grain legumes: a boon to human nutrition. Trends Food Sci Technol 14:507–518
62. Aung KH, Surjani U, Udayasika P, Ingrid AMA, Amparo LR, Elliot PG (2010) The effect of acid dextrinisation on enzyme-resistant starch content in extruded maize starch. Food Chem 120:140–149
63. Raigond P, Ezekiel R, Singh B, Dutt S, Joshi A (2015) Resistant starch production technologies – a review. Potato J 42(2):81–94
64. Bello-Perez LA, Paredes-Lopez O (2009) Starches of some food crops, changes during processing and their nutraceutical potential. Food Eng Rev 1:50–65
65. Thompson DB (2000) On the non-random nature of amylopectin branching. Carbohydr Polym 43:223–239
66. Chou C, Wu M, Nurtama B, Lin J (2010) Effect of different heating treatment and storage time on formation of resistant starch from potato starch. Kasetsart J (Nat Sci) 44:935–942
67. Shin M, Woo K, Seib PA (2003) Hot-water solubilities and water sorptions of resistant starches at 25°C. Cereal Chem 80:564–566

68. Muir JG, O'Dea K (1992) Measurement of resistant starch. Factors affecting the amount of starch escaping digestion *in vitro*. Am J Clin Nutr 56:123–127
69. Garcia-Alonso A, Jimenez-Escrig A, Martin-Carron N, Bravo L, Saura-Calixto F (1999) Assessment of some parameters involved in the gelatinization and retrogradation of starch. Food Chem 66:181–187
70. Berry CS (1986) Resistant starch formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. J Cereal Sci 4:301–314
71. Bjorck I, Nyman M, Pedersen P, Siljestrom M, Asp NG, Eggum BO (1987) Formation of enzyme resistant starch during autoclaving of wheat starch: studies *in vitro* and *in vivo*. J Cereal Sci 6:159–172
72. Sievert D, Wursch P (1993) Thermal behaviour of potato amylase and enzyme-resistant starch from maize. Cereal Chem 70:333–338
73. Pomeranz Y, Sievert D (1990) Purified resistant starch products and their preparation. WO 9015147. University of Washington
74. Sajilata MG, Singhal RS, Kulkarni PR (2006) Resistant starch - a review. Compr Rev Food Sci F 5:1–17
75. Zeng F, Ma F, Kong F, Gao Q, Yu S (2015) Physiochemical properties and digestibility of hydrothermally treated waxy rice starch. Food Chem 172:92–98
76. Brumovsky JO, Thompson DB (2001) Production of boiling-stable granular resistant starch by partial acid hydrolysis and hydrothermal treatments of high-amylose maize starch. Cereal Chem 78(6):680–689
77. Hoover R, Vasanthan T (1994) The effect of annealing on the physicochemical properties of wheat, oat, potato and lentil starches. J Food Biochem 17:303–325
78. Lee CJ, Kim Y, Choi SJ, Moon TW (2012) Slowly digestible starch from heat moisture treated waxy potato starch: preparation, structural characteristics, and glucose response in mice. Food Chem 133(4):1222–1229
79. Hoover R, Manuel H (1996) The effect of heat-moisture treatment on the structure and physicochemical properties of normal maize, waxy maize, dull waxy maize and amylomaize v starches. J Cereal Sci 23:153–162
80. Chung H, Liu Q, Hoover R (2009) Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. Carbohydr Polym 75:436–447
81. Chung H, Liu Q, Hoover R (2010) Effect of single and dual hydrothermal treatments on the crystalline structure, thermal properties, and nutritional fractions of pea, lentil, and navy bean starches. Food Res Int 43:501–508
82. Gunaratne A, Hoover R (2002) Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches. Carbohydr Polym 49:425–437
83. Adebowale KO, Afolabi TA, Olu-Owolabi BI (2005) Hydrothermal treatments of finger millet (*Eleusine coracana*) starch. Food Hydrocoll 19:974–983
84. Adebowale KO, Olu-Owolabi BI, Olawumi EK, Lawal OS (2005) Functional properties of native, physically and chemically modified breadfruit (*Artocarpus Artilis*) starch. Ind Crop Prod 21:343–351
85. Watcharatewinkul Y, Puttanlek C, Rungsardthong V, Uttapap D (2009) Pasting properties of a heat-moisture treated canna starch in relation to its structural characteristics. Carbohydr Polym 75:505–511
86. Chung HJ, Jeong HY, Lima ST (2003) Effects of acid hydrolysis and defatting on crystallinity and pasting properties of freeze–thawed high amylose cornstarch. Carbohydr Polym 54:449–455
87. Dimantov A, Kesselman E, Shimoni E (2004) Surface characterization and dissolution properties of high amylose corn starch–pectin coatings. Food Hydrocoll 18:29–37
88. Jiranuntakul W, Puttanlek C, Rungsardthong V, Puncha-arnon S, Uttapap D (2011) Micro-structural and physicochemical properties of heat-moisture treated waxy and normal starches. J Food Eng 104:246–258

89. Arcila JA, Rose DJ (2015) Repeated cooking and freezing of whole wheat flour increases resistant starch with beneficial impacts on *in vitro* fecal fermentation properties. J Funct Foods 12:230–236
90. Agustiniano-Osornio JC, Gonzalez-Soto RA, Flores-Huicochea E, Manrique-Quevedo N, Sanchez-Harnandez L, Bello-Perez LA (2005) Resistant starch production from mango starch using a single-screw extruder. J Sci Food Agric 85:2105–2110
91. Hasjim J, Jane J (2009) Production of resistant starch by extrusion cooking of acid-modified normal-maize starch. J Food Sci 74(7):C556–C562
92. Dupuis JH, Liu Q, Yada RY (2014) Methodologies for increasing the resistant starch content of food starches: a review. Compr Rev Food Sci F 13:1219–1224
93. Chanvrier H, Thayakumaran S, Appelqvist IAM, Gidley MJ, Gilbert EP, Lopez-Rubio A (2007) Influence of storage conditions on the structure, thermal behaviour and formation of enyzme resistant starch in extruded starches. J Agric Food Chem 55:9883–9890
94. Huth M, Dongowski G, Gebhardt E, Flamme W (2000) Functional properties of dietary fibre enriched extrudates from barley. J Cereal Sci 32:115–128
95. Alsaffar AA (2011) Effect of food processing on the resistant starch content of cereals and cereal products-a review. Int J Food Sci Technol 46:455–462
96. Haralampu SG, Gross A (1998) Granular RS and method of making. U.S. Patent 58.49.090
97. Chiu CW, Henley M, Altieri P (1994) Process for making amylase resistant starch from high amylose starch. US Patent 5 281 276
98. Zhang H, Jin Z (2011) Preparation of resistant starch by hydrolysis of maize starch with pullulanase. Carbohydr Polym 83:865–867
99. Reddy CK, Suriya M, Haripriya S (2013) Physico-chemical and functional properties of resistant starch prepared from red kidney beans (*Phaseolus vulgaris*. L) Starch by enzymatic method. Carbohydr Polym 95:220–226
100. Gao Q, Suling L, Jian H, Liang S (2011) Preparation and properties of resistant starch from corn starch with enzymes. Afr J Biotechnol 10(7):1186–1193
101. McCleary BV (2000) Importance of enzyme purity and activity in the measurement of total dietary fibre and dietary fibre components. J AOAC Int 83(4):997–1005
102. Buttcher V, Welsh T, Mitzer LS, Kossmann J (1997) Cloning and characterization of the gene for amyloscurase from *Neisseria polysaccharea*: production of linear 1. 4-glucan. J Bacteriol 179:3324–3330
103. Hoover R (2000) Acid-treated starches. Food Rev Int 16(3):369–392
104. Shin S, Byun J, Park KH, Moon TW (2004) Effect of partial acid hydrolysis and heat-moisture treatment on formation of resistant tuber starch. Cereal Chem J 81(2):194–198
105. Koksel H, Masatcioglu T, Kahraman K, Ozturk S, Basman A (2008) Improving effect of lyophilization on functional properties of resistant starch preparations formed by acid hydrolysis and heat treatment. J Cereal Sci 47(2):275–282
106. Wurzburg OB (1995) Modified starches. In: Stephen AM (ed) Food polysaccharides and their applications. Marcel Dekker Inc, New York, pp 67–97
107. Tester RF, Karkalas J, Qi X (2004) Starch structure and digestibility. Enzyme-substrate relationship. World Poultry Sci J 60(2):186–195
108. Xie X, Liu Q (2004) Development and physicochemical characterization of new resistant citrate starch from different corn starches. Starch 56(8):364–370
109. Liu H, Liang R, Antoniou J, Liu F, Shoemaker CF, Li Y, Zhong F (2014) The effect of high moisture heat-acid treatment on the structure and digestion property of normal maize starch. Food Chem 159:222–229
110. Acquarone VM, Rao MA (2003) Influence of sucrose on the rheology and granule size of cross-linked waxy maize starch dispersions heated at two temperatures. Carbohydr Polym 51:451–458
111. Singh J, Kaur L, McCarthy OJ (2007) Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – a review. Food Hydrocoll 21:1–22

112. Seib PA, Woo K (1999) Food grade starch resistant to alpha-amylase and method of preparing the same. US Patent No. 5.855.946
113. Woo K, Seib PA (2002) Cross-linked resistant starch: preparation and properties. Cereal Chem 79:819–825
114. Lehmann U, Robin F (2007) Slowly digestible starch – its structure and health implications: a review. Trends Food Sci Technol 18:346–355
115. Heacock PM, Hertzler SR, Wolf B (2004) The glycemic, insulinemic, and breath hydrogen responses in humans to a food starch esterified by 1-octenylsuccinic anhydride. Nutr Res 24:581–692
116. Han JA, BeMiller JN (2007) Preparation and physical characteristics of slowly digesting modified food starches. Carbohydr Polym 67:366–374
117. He J, Liu J, Zhang G (2008) Slowly digestible waxy maize starch prepared by octenyl succinic anhydride esterification and heat-moisture treatement: glycemic response and mechanism. Biomacromolecules 9:175–184
118. Chung HJ, Shin DH, Lim ST (2008) *In vitro* starch digestibility and estimated glycemic index of chemically modified corn starches. Food Res Int 41:579–585
119. Sang Y, Seib PA (2006) Resistant starches from amylose mutants of corn by simultaneous heat-moisture treatment and phosphorylation. Carbohydr Polym 63:167–175
120. Wolf BW, Bauer LL, Fahey GC (1999) Effects of chemical modification on in vitro rate and extent of food starch digestion: an attempt to discover a slowly digested starch. J Agric Food Chem 47:4178–4183
121. Kahraman K, Koksel H, Ng PKW (2015) Optimisation of the reaction conditions for the production of cross-linked starch with high resistant starch content. Food Chem 174:173–179
122. Wang YJ, Wang L (2002) Characterization of acetylated waxy maize starches prepared under catalysis by different alkali and alkaline-earth hydroxides. Starch 54:25–30
123. Futch J (2009) Altering sweet potato starch functionality by amino acids and pH treatments. M. Sc. Thesis. B.S., Louisiana State University, USA
124. Carlos-Amaya F, Osorio-Diaz P, Agama-Acevedo E, Yee-Madeira H, Bello-Perez LA (2011) Physicochemical and digestibility properties of double modified banana (*Musa paradisiaca* L.) starches. J Agric Food Chem 59(4):1376–1382
125. Juansang J, Puttanlek C, Rungsardthong V, Puncha-arnon S, Uttapap D (2012) Effect of gelatinisation on slowly digestible starch and resistant starch of heat-moisture treated and chemically modified canna starches. Food Chem 131:500–507
126. Zeiba T, Kapelko M, Szumny A (2013) Effect of preparation method on the properties of potato starch acetates with an equal degree of substitution. Carbohydr Polym 94:193–198
127. Martin SM, Barbosa-Canovas G, Swanson B (2002) Food processing by high hydrostatic pressure. Crit Rev Food Sci Nutr 42:627–645
128. Katopo H, Song Y, Jane J (2002) Effect and mechanism of ultrahigh hydrostatic pressure on the structure and properties of starches. Carbohydr Polym 47:233–244
129. Vallons KJ, Arendt EK (2009) Effects of high pressure and temperature on the structural and rheological properties of sorghum starch. Innovative Food Sci Emerg Technol 10:449–456
130. Liu P, Hu X, Shen Q (2010) Effect of high hydrostatic pressure on starches: a review. Starch 62:615–628
131. Li W, Bai Y, Mousaa SAS, Zhang Q, Shen Q (2012) Effect of high hydrostatic pressure on physicochemical and structural properties of rice starch. Food Bioprocess Technol 5:2233–2241
132. Boluda-Aguilar M, Taboada-Rodríguez A, Lopez-Gomez A, Marín-Iniesta F, Barbosa-Canovas GV (2013) Quick cooking rice by high hydrostatic pressure processing. Food Sci Technol 51:196–204
133. Nasehi B, Javaheri S (2012) Application of high hydrostatic pressure in modifying functional properties of starches: a review. Middle-East J Sci Res 11:856–861

134. Deng Y, Jin Y, Luo Y, Zhong Y, Yune J, Song X, Zhao Y (2014) Impact of continuous or cycle high hydrostatic pressure on ultra structure and digestibility of rice starch granules. J Cereal Sci 60:302–310
135. Zhang B, Chen L, Zhao Y, Li X (2013) Structure and enzymatic resistivity of debranched high temperature pressure treated high-amylose corn starch. J Cereal Sci 57:348–355
136. Slade AJ, McGuire C, Loeffler D, Mullenberg J, Skinner W, Fazio G, Holm A, Brandt KM, Steine MN, Goodstal JF, Knauf VC (2012) Development of high amylose wheat through TILLING. BMC Plant Biol 14:12–69
137. Sparla F, Falini G, Botticella E, Pirone C, Talamè V, Bovina R, Salvi S, Tuberosa R, Sestili F, Trost P (2014) New starch phenotypes produced by TILLING in barley. PLoS One 9(10): e107779
138. Yue P, Waring S (1998) Resistant starch in food applications. Cereal Foods World 43(9):690–695
139. Aparicio-Saguilan A, Sayagoayerdi S, Vargastorres A, Tovar J, Ascencioatero T, Belloperez L (2007) Slowly digestible cookies prepared from resistant starch-rich lintnerized banana starch. J Food Compos Anal 20(3–4):175–181
140. Buttriss JL, Stokes CS (2008) Dietary fiber and health: an overview. British nutrition foundation. Nutr Bull 33:186–200
141. Baixauli R, Salvador A, Martinez-Cervera S, Fiszman SM (2008) Distinctive sensory features introduced by resistant starch in baked products. Food Sci Technol 41:1927–1933
142. Sajilata MG, Singhal RS (2005) Specialty starches for snack foods. Carbohydr Polym 59:131–151
143. Bednar GE, Patil AR, Murray SM, Grieshop CM, Merchen RR, Fahey GC (2001) Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability *in vitro* in a canine model. J Nutr 131:276–286
144. Report AACC (2001) The definition of dietary fiber[1]. Cereal Foods World 46(3):112–126
145. Champ M, Langkilde AM, Brovns F (2003) Advances in dietary fiber characterization 1. Definition of dietary fiber, physiological relevance, health benefits and analytical benefits. Nutr Res Rev 16:71–82
146. Wood PJ (2007) Cereal β-glucans in diet and health. J Cereal Sci 46:230–238
147. Topping DL, Fukushima M, Bird AR (2003) Resistant starch as a prebiotic and synbiotic: State of the art. Proc Nut Soc 62:171–176
148. Brown I, Warhurst M, Arcot J (1997) Fecal numbers of bifidobacteria are higher in pigs fed *Bifidobacterium longum* with a high amylose cornstarch that with a low amylose cornstarch. J Nutr 127:1822–1827
149. Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A (2012) Probiotic mechanisms of action. Ann Nutr Metab 61:160–174
150. Thompson DB (2007) Resistant starch. In: Biliaderis CG, Izydorczyk MS (eds) Functional food carbohydrates. CRC Press, Boca Raton, pp 73–95
151. Brown IL (2004) Applications and uses of resistant starch. J AOAC Int 87(3):727–732
152. Lopez HW, Levrat-Verny MA, Coudray C, Besson C, Krespine V, Messager A et al (2001) Class 2 resistant starches lower plasma and liver lipids and improve mineral retention in rats. J Nutr 131:1283–1289
153. Sharp R, Macfarlane GT (2000) Chemostat enrichments of human faeces with resistant starch are selective for adherent butyrate-producing clostridia at high dilution rates. Appl Environ Microbiol 66:4212–4221
154. Haralampu SG (2000) Resistant starch – a review of the physical properties and biological impact of RS3. Carbohydr Polym 41:285–292
155. Li S, Ward R, Gao Q (2011) Effect of heat-moisture treatment on the formation and physicochemical properties of resistant starch from mung bean (*Phaseolus radiatus*) starch. Food Hydrocoll 25:1702–1709

156. FAO/WHO (1998) Carbohydrates in human nutrition, Rome. Report of a joint FAO/WHO Expert Consultation, FAO/WHO, Rome
157. Sui Z, Shah A, BeMiller JN (2011) Cross-linked and stabilized in-kernel heat-moisture-treated and temperature-cycled normal maize starch and effects of reaction conditions on starch properties. Carbohydr Polym 86:1461–1467
158. Kavey R-E W, Daniels SR, Lauer RM, Atkins DL, Hayman LL, Taubert K (2003) Guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood. Circulation 107: 1562–1566.
159. Sidhu SJ, Kabir Y (2007) Functional foods from cereal grains. Int J Food Prop 10:231–244
160. Streppel MT, Ocke MC, Boshuizen HC, Kok FJ, Kromhout D (2008) Dietary fiber intake in relation to coronary heart disease and all-cause mortality over 40 y: the Zutphen study. Am J Clin Nutr 88:1119–1125
161. Asp NC, van Amelsvoort JMM, Hautvast JGAJ (1996) Nutritional implications of resistant starch. Nutr Res Rev 9:1–31
162. Martinez-Flores HE, Chang YK, Martinez-Bustos F, Sgarbierid V (2004) Effect of high fiber products on blood lipids and lipoproteins in hamsters. Nutr Res 24(1):85–93
163. Hashimoto N, Ito Y, Han KH, Shimada K, Sekikawa M, Topping DL (2006) Potato pulps lowered the serum cholesterol and triglyceride levels in rats. J Nutr Sci Vitaminol 52:445–450
164. Liversey G (1994) Energy value of resistant starch. In: Asp G, van Amelsvoort JMM, Hautvast JGAJ (eds) Proc concluding plenary meeting of EURESTA. Wageningen, The Netherlands, pp 56–62
165. Sharma A, Yadav BS, Ritika (2008) Resistant starch: physiological roles and food applications. Food Rev Int 24(2):193–234
166. Milkusova L, Sturdik E, Mosovska S, Brindzova L, Mikulajova A (2009) Development of new bakery products with high dietary fiber content and antioxidant activity for obesity prevention. In: Proc 4th International Dietary fiber conference. For Cereal Science and Technology. Vienna, Austria, Intl. Assoc. p. 185
167. Tapsell LC (2004) Diet and metabolic syndrome: where does resistant starch fit in? J AOAC Int 87(3):756–760
168. Keenan MJ, Zhou J, Mccutcheon KL, Raggio AM, Bateman HG, Todd E (2006) Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. Obesity 14:1523–1534
169. Fuentes-Zaragoza E, Riquelme-Navarrete MJ, Sanchez-Zapata E, Perez-Alvarez JA (2010) Resistant starch as functional ingredient: a review. Food Res Int 43:931–942
170. Dundar AN, Gocmen D (2013) Effect of autoclaving temperature and storing time on resistant starch formation and its functional and physicochemical properties. Carbohydr Polym 97 (2):767–771
171. Morais MB, Feste A, Miller RG, Lifichitz CH (1996) Effect of resistant starch and digestible starch on intestinal absorption of calcium, iron and zinc in infant pigs. Paediatr Res 39 (5):872–876
172. Schulz AGM, van Alemsvoort JMM, Beynen AC (1993) Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. J Nutr 123:1724–1731
173. Lopez HW, Coudray C, Bellanger J, Levrat-Verny MA, Demigne C, Rayssiguier Y, Remesy C (2000) Resistant starch improves mineral assimilation in rats adapted to a wheat bran diet. Nutr Res 20:141–155
174. Moreau NM, Martin LJ, Toquet CS (2003) Restoration of the integrity of rat caeco-colonic mucosa by resistant starch, but not by fructo-oligosaccharides, in dextran sulfate sodium induced experimental colitis. Brit J Nutr 90(1):75–85
175. Sotnikova EV, Martynova EA, Gorbacheva EV (2002) Resistant starches and immune system. Vopr Pitan 71(5):34–38
176. Segain JP, Raingeard de la Blétière D, Bourreille A, Leray V, Gervois N, Rosales C et al (2000) Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. Gut 47(3):397–403
177. Rudrapatnam N, Tharanathan RN (2005) Starch-value addition by modification. Crit Rev Food Sci Nutr 45:371–384

Gluten-Free Cereals and Pseudocereals: Nutrition and Health

29

Mario Fernández de Frutos, Bartosz Fotschki, Ricardo Fernández Musoles, and José Moisés Laparra Llopis

Contents

Abstract

Cereals constitute a staple food for large groups of population worldwide. However, the protein fraction of cereals continues receiving increasing attention from the clinical community because of its close involvement in both the development of immunological processes and intestinal disorders. Thus, together with constant technological innovations to the increasing demands of the consumer, makes necessary to optimize food formulations promoting health outcomes. In this context, beneficial health implications are being reported based on the advantageous nutritional profile of gluten-free cereals, but mostly pseudocereals. The latter represent a good source of proteins (albumins/globulins) reducing the

M. F. de Frutos · J. M. L. Llopis (✉)
Madrid Institute for Advanced Studies in Food, Madrid, Spain
e-mail: mfernandezdefrutos@gmail.com; moises.laparra@imdea.org

B. Fotschki
Department of Biological function of food, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland
e-mail: b.fotschki@pan.olsztyn.pl

R. F. Musoles
Valencian International University, Valencia, Spain
e-mail: rfernandez@universidadviu.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_60

intake of prolamins. Additionally, pseudocereals provide an optimal lipid profile (ratio of saturated versus unsaturated fatty acids) and bioactive compounds with a potential significant impact on the consumer's health. Currently, the underlying mechanisms by which these beneficial health effects occur still remain unsolved. Moreover, some recent data point to metabolic effects beyond their nutritional value. These could have an important impact on immunological processes, although studies on these aspects result inferential. Future research should approach epidemiologic studies and toward consolidating the mechanisms of action, especially in the human body.

Keywords
Cereals · Gluten-free · Health benefits · Metainflammation · Nutrition · Pseudocereals

1 Introduction

At present, continuous social development and commercial globalization are motivating the increase in food production, where constant technological innovations are needed to respond to the increasing demands of the consumer of high quality food, which is the closest thing to a fresh product with a processed minimum, healthy and nutritious. The important socioeconomic changes in most countries have led to a substantial change in dietary habits favoring an increase in the consumption of animal fats and proteins. This tendency is reflected in an increase in the incidence of diseases, directly or indirectly, associated with alterations of the hepatic homeostasis that give rise to a wide spectrum of pathologies related to the metabolic syndrome, obesity, and type 2 diabetes (T2D).

For the past two decades, the interest of researchers, the food industry, and consumers has focused on those foods that, in addition to containing essential nutrients, have bioactive components that promote health and/or prevent chronic diseases. Here, we can find cereals that have been cultivated worldwide from ancient times [1, 2]. In the case of cereals, in addition to playing an important nutritional function, they are responsible for multiple beneficial effects for health [3]. This has led to propose health claims for the consumption of whole grains in USA (1999) and, as of 2002, in England and Sweden [4]. The rich composition of whole grains or their fractions, along with their high dietary fiber content, have motivated many nutritional interventions that have focused on highlighting their potential for healthier, more nutritious foods [5, 6]. Thus, recent studies support the relationship between regular intakes of whole grains and the lower risk of diseases such as T2D, obesity, cardiovascular disorders, metabolic syndrome, and cancer [7–9].

The protein fraction of cereals has received much attention from the clinical community because of its close involvement in both the development of immunological processes and intestinal disorders. With the increasing development of immune function measuring systems and better understanding of the potential role of cereals in immunometabolic (i.e., obesity, T2D, nonalcoholic fatty liver disease)

[10–12] diseases, people became aware and more selective about food supply. Here, gluten-free cereals and pseudocereals emerged as advantageous alternatives to wheat in innovative healthier and nutritive goods [6, 13–15].

A healthy diet helps protect us from malnutrition as well as immune-metabolic disorders. Cereals are recognized as an essential food within a healthy diet and are recommended for frequent consumption. However, despite the recognized health benefits of cereal consumption, cereals are often considered as grains that provide a great deal of nutrients, forgetting to mention specific health benefits.

This chapter summarizes recent research on pseudocereals providing comprehensive information to better estimate their potential contribution to human health. Beyond its economic, nutritional, and environmental importance, cereals have played a key role in the history of mankind. Animal and human trials support the beneficial effects derived from whole grains consumption, mostly referring to wheat and its derivatives, rice, and maize. Otherwise, pseudocereals belong to a group of nongrasses, which can be consumed as seeds or flours; even kernels can be used to produce diverse food products. The consumption of these grains has experienced a significant increase in recent years worldwide. Thus, general features of those gluten-free cereals and their specific characteristics in comparison to most common cereals are presented.

2 Gluten-Free Cereals and Pseudocereals

According to recent statistics, world cereal trade in 2017–18 has been lifted by 8 million tons to a record 403 million tons, implying an 8.7 million ton (2.2%) expansion from 2016–17 [16]. The world's most important cereals in the diet are corn, wheat, and rice. Barley, oats, and rye are cereals whose consumption is not so widespread [17]. Other cereals such as sorghum and millet are mainly used for animal consumption, intended for human consumption in some regions [18]. Notably, pseudocereals (amaranth, quinoa, and buckwheat) receive increasing attention and are consumed with great acceptance due to their advantageous nutritional profile [3].

2.1 Food Uses

Good nutrition is our source of energy to live and be active, as well as the first defense against disease. Here, vegetal kingdom satisfies a considerable part of the nutritional needs for the human being; in this context, cereals have been part of the diet of most cultures and civilizations for millennia. Cereals constitute an agronomically important crop from the environmental point of view and an economic source of protein, both in human and animal feed. Pseudocereals show out to substitute wheat in different respects, but mainly due to their high protein concentration, minerals, vitamins, and fatty acids [3]. These grains have been part of human food for centuries, although as they have been neglected by food industries and nutrition,

Table 1 Several types of products prepared with most commonly used gluten-free cereals [15, 21, 24]

Gluten-free cereal	Amaranth		Quinoa		Buckwheat		
	Grain	Flour	Whole	Flour	Grain	Flour	Kernels
Product	Toasted, popped, extruded, milk	Bread, rolls, cakes, muffins, pancakes, cookies, noodles	Broths, soups, stews, rice-like products	Porridge, coarse bread, pasta, cookies	Unpeeled, semolina, omelets	Biscuits, cookies, bread, cakes	Tea

current knowledge is still limited. The advantageous nutritional features of these grains make them good candidates as part of strategies, both conventional and modern breeding approaches, in order to provide a public health benefit with the ultimate goal of overcoming deficiencies worldwide [6, 19]. Currently, it is assumed the high nutritional value of pseudocereals; however, the study to what extent these grains impact nutritional status resulted inferential.

Gluten-free cereals are incorporated into wheat-based products up to a certain proportion to improve the nutritional value of the resulting product [6, 20]; however, it still constitutes a great technological challenge. Traditional processing methods for these cereals include hand-operated wooden or stone pestle and mortar, which still are used today in certain regions. Today, processing occurs mainly based on applying the dry- and wet-milling processes. The latter are decided according to the objective to obtain flours at a high extent or fractions where there can be found other defined components. Gluten-free cereals can represent a good source of other ingredients of technofunctional importance (i.e., kernels, saponins, oils). There are numerous scientific publications that define the physicochemical and functional properties of the major components of pseudocereals [21–23].

Gluten-free cereals are being used in a wide variety of foods, devoted to human, pets, and animal feed. Commonly, they are used in several ways, either as seeds, whole or plain grain, and flour (Table 1). The transformations that occur in the distinct components of the flours derived from these cereals during the processing and cooking of foods improve their nutritional value, favor the reduction of "antinutritive" components, and, in some cases, the formation of bioactive compounds that can contribute significantly to the reduction of metainflammatory diseases [15, 25–28].

2.2 General Features of Most Commonly Consumed Pseudocereals

2.2.1 Quinoa

Chenopodium quinoa is a highly nutritious grain that has been cultivated from ancient years in South America. The origin of quinoa appears to be in the Andean

mountains. During recent times, there has been an increased interest worldwide for this grain in the United States, Europe, and Asia. Quinoa has been selected by the Food and Agriculture Organization of the United Nations (FAO) as one of the crops destined to significantly contribute to food security in the next century.

Quinoa seeds contain a higher percentage of bran than common cereals that makes feasible the carriage of higher levels of protein and fats in these [29]. One of the aspects that could limit the widespread utilization of quinoa is the relatively high amount of saponins that appear in the outer layer of the seeds and are considered as "antinutritional" factors. These are also rich in carbohydrates representing the most part of total fraction of nutrients. There can be found monosaccharides such as xylose, arabinose, fructose, and glucose, and disaccharides such as maltose and mainly sucrose [30]. Starch also represents an important source of carbohydrates in the grain. Dietary fiber in quinoa exhibits a composition where it composed mainly of cellulose, hemicellulose, beta-glucans, and lignin. Notably, quinoa carries a significant higher protein fraction compared to other cereals. Here, there can be found protease inhibitors affecting gastrointestinal proteolytic enzymes [25]. The genetic background is identified as a major cause for the variations in content and quality of these proteins. Additionally, environmental conditions and cultivation methods also contribute significantly to these variations [31, 32]. Lipid content in quinoa is composed mainly of unsaturated fatty acids (85%), particularly linoleic (~52%), oleic acid (~25%), as well as saturated palmitic acid (~10%) [33, 34]. In a global perspective, quinoa presents a favorable profile of polar lipids (phospholipids) (25.2 g/100 g total lipids) with potential benefits on inflammatory processes, cancer, cardiovascular diseases, neurological disorders, liver diseases, and antioxidant carrier [35]. This phospholipid profile is particularly important since quinoa constitutes a good source of bioactive compounds such as phytosterols, flavonoids, and tocopherols [36, 37].

2.2.2 Amaranth

There can be found up to 26 *Amaranthus* species, but the three grain species known as amaranths include *Amaranthus hypochondriacus* L., *Amaranthus cruentus* L., and *Amaranthus caudatus* L. These were domesticated in Central America (Tehuacan, Mexico) over 4000 years BC.

Amaranth seeds are of high nutritional value, the carbohydrates representing the major part of the total nutrient fraction in the seed. Starch is the most abundant component in the seeds accounting for up to 65% to 75%, while dietary fiber accounts for only 5% [38]. One of the most interesting advantages of amaranth starch is the production of very small granules (0.75–3 μm) that exhibit extremely high water-absorption capacity [39] providing unique functional properties to food. Numerous studies have shown that amaranth starch exerts important physiological functional properties due to its high digestibility [40]. The nutritional value of amaranth is closely connected to its high protein content and amino acid profile. They display a characteristic protein distribution of about 40% albumins, 20% globulins, 25–30% glutelins, and 2–3% prolamins [41, 42]. Amaranth is considered an essential candidate in special diets for patient with coeliac disease; however, the

Table 2 Fatty acid profile in gluten-free cereal and pseudocereals

Fatty acids (g/100 g)	Gluten-free cereal and pseudocereals[a]					
	Amaranth	Barley	Buckwheat	Quinoa	Rice bran	Wheat
Palmitic (16:0)	18.5–23.8	18.5–21.0	18.2–19.5	9.7–11.4	18.6	15.4
Stearic (18:0)	3.2–4	–	2.18	0.6–0.79	1.75	–
Oleic (18:1)	22–33.3	15.2–15.6	36.4–37.1	24.8–25.6	42.4	22.3
Linoleic (18:2)	38.2–44.8	24.0–52.4	34.8–35.5	52.3–52.8	34.8	54.2
Linolenic (18:3)	0.2–1.0	2.6–5.5	1.93	3.9–7.0	1.1	3.5
Other	7.08–11.3	5.4–6.8	3.8–10.6	2.44–6.8	1.23	4.6

[a]References: [47, 66–69]

recently identified participation of defined globulins in immunological processes [10] warrants further investigation. It has been reported that cooking and popping of seeds decreases the fraction of albumins and globulins [43]. But the immunomodulatory potential of the resulting peptides and other structures has not yet been clarified. For amaranth, the lipid fraction is mainly localized in the germ and seed coat. The oil content of amaranth is commonly estimated between 5% and 9% [44, 45], although a study reported values up to 19.3% [46]. These discrepancies could be caused by differences in the extraction and purification methods employed as well as by environmental factors such as temperature and latitude that influence the composition of the oil of amaranth seed [44]. Amaranth constitutes a good source of linoleic (C18:2), oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids (Table 2).

Amaranths are also of high nutraceutical value as they are important sources of phytochemicals and antioxidants [47–49]. Origin, accession, and location constitute key aspects determining the content of, for example, tocopherols [47, 48]. Otherwise, after different abiotic stresses, the expression of amaranthin-like genes results enhanced. Amaranthin is a homodimeric lectin that was first discovered in the seeds of *Amaranthus caudatus* and serves as a model for the family of amaranthin-like lectins. Molecular modeling studies have unraveled the structure of amaranthin-like proteins and their carbohydrate-binding sites. Their particular structure allows to interact with the proliferative region of normal human colonic epithelium and neoplastic lesions of the colon. However, a causal association of these lectins to physiological proliferation processes still results inferential.

2.2.3 Buckwheat

Despite the name, buckwheat (*Fagopyrum esculentum* Moench) is not related to wheat, but to sorrel, knotweed, and rhubarb. Native to the steppes of Central Asia and Siberia, it was spread to West being introduced to Turkey and Poland and later expanded to Central and South Europe. Today, the expansion of buckwheat reached Great Britain, Canada, and EE.UU.

Considerable pharmacological evidences both in vitro and in vivo support positive effects of *Fagopyrum* buckwheats such as antitumor, antioxidant, anti-

inflammatory, hepatoprotective, and antidiabetic [50]. These activities are mostly associated to their particular nutrient composition. Carbohydrates represent (~70%) the major proportion of buckwheat's endosperm, among these the main component is starch that comprises 55% of the total fraction [51, 52]. The starch present in buckwheat seeds has a different composition to the rest of cereals, with a higher concentration of amylose and lower of amylopectin and also a significant fraction resistant (35%) to digestion considered as a dietary fiber [53]. The buckwheat's starch capacity to retain water has been established as higher than that of wheat and corn starch [54]. Factors associated to low digestibility of buckwheat starch are small granule size, amylose content, starch-protein interactions, and lipid complexes. Buckwheat proteins exhibit an amino acid profile more nutritionally favorable than wheat. Notably, a more balanced concentration of amino acids is found, except for glutamine and proline [55]. The established higher composition of individual protein fractions such as albumins and globulins in buckwheat in comparison to wheat [55] supposes a significant difference in their potential immunomodulatory features. Meanwhile, globulins from wheat exert a clear inflammatory effect; the beneficial pharmacological effects described for buckwheat suggest different bioactive effects derived from buckwheat's globulins. Nowadays, the very low concentration of glutelins and prolamins in buckwheat makes it a good crop to be part of gluten-free diets [42]. In relation to other gluten-free cereals and pseudocereals, buckwheat provides lower oil content (~2–3 g/100 g) [44]. Otherwise, buckwheat shares with quinoa and amaranth a lipid profile rich in ϖ-3 (linoleic) and ϖ-6 (linolenic) acids as well as oleic acid. Notably, fatty acid content in buckwheat is influenced by seeding time in addition to environmental factors and variety [56]. For buckwheat, neutral lipids represent 85% of the total content, while polar lipids are found in proportions up to 15%. Similarly to other grains, buckwheat seeds are a good source of antioxidants such as tocopherols, phenols, and flavonoids, specially quercetin and rutin. This characteristic favored the interest and use of buckwheat hull by food industries. In addition, groats have also been used for porridges. Other bioactive compounds identified in buckwheat seeds are D-chiro-inositol, myo-inositol, and fagopyritols, which play an essential role in insulin sensitivity lowering glucose blood levels and blood pressure.

2.3 Health Implications

The nutrient content and profile alone does not describe the quality or physiological and health implications sufficiently (Fig. 1). Digestibility, available lysine, net nutrient utilization, and efficiency ratio are some other parameters that help to better estimate the nutritional value of the gluten-free cereals and pseudocereals. In general, the protein quality of pseudocereals is close to that of casein. Significant differences in starch digestibility for pseudocereals are found [25]. Rice and maize are low in protein, fiber, and folate that contrast those concentrations in pseudocereals [3, 57].

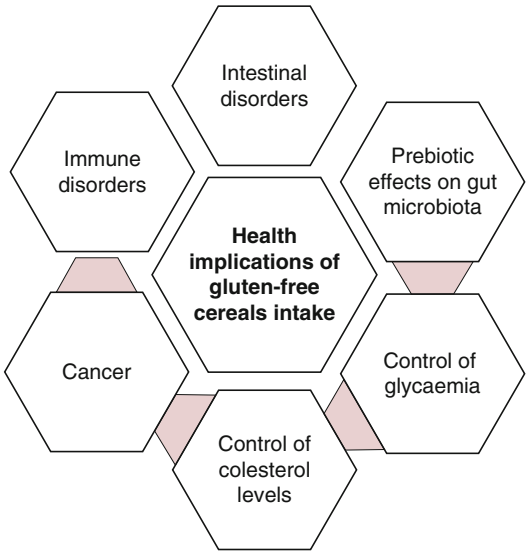


Fig. 1 Health-related outcomes associated to gluten-free cereals intake

2.3.1 Digestibility

The importance of proteins in gluten-free grains relies on their quality. The amino acid composition in pseudocereals provides to them a significant advantageous nutritional profile due to the high content of essential amino acids. Particularly, methionine, lysine, arginine, tryptophan, and several sulfur-containing amino acids are found at higher concentrations than in other gluten-free cereals. Because of the high proportion of lysine in globulins, pseudocereals provide about twofold higher amount of this amino acid than wheat or maize. Thus, they represent an excellent tool to fulfill the nutritional requirements for populations at risk such as children [58, 59]. There can be found several studies in relation to the impact of processing methods on protein quality. A literature search reveals that pseudocereals proteins remain stable, preserving their high quality and digestibility, after washing, cooking [60], and popping [43]. However, a recent study reported a decreased digestibility of quinoa proteins when using an extraction medium with pH values ranging from 8 to 11 [27]. These conditions could have an important impact during preliminary treatments to eliminate of saponins from quinoa by using a "washing" step.

2.3.2 Immunity

Pseudocereals, contrary to common grains such as wheat, contain low or no storage prolamin proteins [61], which are the main storage proteins in cereals and the toxic proteins in celiac disease (CD). Thus, these grains received increasing attention as safe and suitable for CD patients as part of the gluten-free diet. Otherwise, high proportions of pseudocereals proteins are constituted by albumins (40%) and globulins (20%) [59]. Two main classes of globulins can be differentiated in amaranth: 7S (conamaranthin) and 11S (amaranthin) storage globulins [62]. The 11S globulins (salt-soluble proteins) are one of the major storage protein fractions in amaranth.

Amaranth 11S globulin has a molecular mass around 398 kDa, and under reduced conditions, it shows characteristic bands of the acidic (36−32 kDa) and basic (24−22 kDa) subunits of 11S proteins. Additionally, a 59−55 kDa band corresponds to the nonprocessed proglobulin with structural characteristics similar to other 11S globulins [63]. Similarly, in quinoa, the 11S (chenopodin) and the 2S globulin account for 37% and 35% of the total protein content, respectively [64].

Among the different buckwheat proteins, there has been identified a 13S globulin seed storage protein with significant allergenic potential (Fagag1, 61.2 kDa, accession number AF152003, Genbank) usually present as a hexamer linked by disulfide bond [65].

The broad umbrella of heterogeneous conditions identified for reactions to gluten have revealed the need to establish an international consensus and new nomenclature and classification for these; including CD, nonceliac gluten sensitivity, and allergy affecting up to 10% of the general population [70]. Gluten or the epitopes triggering these disorders are found in all *Triticum* species; wheat, spelt wheat, einkorn wheat, emmer, durum wheat as well as rye, barley, triticale, and oat. Nowadays, cereal grains that are considered safe to CD patients are rice, maize, millet, and sorghum. However, recent immunological studies have identified several immunogenic components of the globulin fraction, α-amylase/trypsin inhibitors [10, 11]. The different downstream signaling pathways triggered by these globulins engage the chemokine receptor CXCR3 and either MyD88-dependent or independent signals associated to the Toll-like receptor (TLR)-4. The potential physiological benefit derived from an inhibited amylase activity favored the use of these globulins for type 2 diabetes and obese patients. Notably, these pathologies constitute important risk factors for nonalcoholic fatty liver disease (NAFLD) that today has become the most common liver pathology worldwide affecting an estimated 15–30% of most populations. Thus, 10–20% of subjects with NAFLD develop the severe variant of nonalcoholic steatohepatitis (NASH), hepatic inflammation, and the development of liver fibrosis with high liver-related morbidity and mortality, part of which is due to the development of hepatocellular carcinoma. However, current evidence for a causal association as well as definition of the potential role of globulins from pseudocereals within these disorders has largely been inferential. Buckwheat leaf and flowers are source of α and β amylase inhibitor activity [71, 72]; however, extracts from buckwheat inhibit proinflammatory signals in macrophages (264.7 cells) stimulated with the prototypical TLR4 agonist; lipopolysaccharide (LPS) from Gram negative bacteria [73]. Dietary buckwheat has been associated to prevention of lymphocyte immunosenescence in aged mice [74]. Taken together these studies, there could be hypothesized the more preserved prevention of reactive oxygen species derived from TLR4 stimulation. In this sense, inclusion of flours from pseudocereals into bread formulations as substitute of wheat improved the nutritional iron status [6]. Systemic iron homeostasis is tightly regulated by the liver through synthesis of hepcidin, which associates to inflammatory stimuli regulated by immunological transcription factors such as the proliferator-activated receptor-γ coactivator-1α (PGC1α) that participates in the regulation of metabolic, but also immune activation. Thus, scarce existing studies suggest innate immune-mediated alleviating effects on iron homeostasis dysregulation.

This effect is important not only from a nutritional point of view, but also because nutritional iron is an effective modulator of the TLR4 signaling. For example, it has to be kept in mind that activation of aryl hydrocarbon receptor mediates suppression of hypoxia-inducible factor-dependent erythropoietin expression. The flavonoids content of buckwheat has been indicated to interact with the aryl hydrocarbon receptor at intestinal level increasing its expression [75], thus also influencing TLR4-mediated response(s).

2.3.3 Metainflammation

Inflammation constitutes a physiological process necessary to keep functional appropriate cellular response(s). The innate branch of immune system has a crucial role in determining proper inflammatory homeostasis. Notably, a low-grade inflammation represents a common feature to many metainflammatory diseases such as NAFLD and associated comorbidities such as T2D and obesity. The scarce studies existing show the positive effects reducing inflammatory conditions within the gut-liver axis [6]. Notably, pseudocereals were used with lower proportions (5–25%) in relation to wheat flour. It was demonstrated that amaranth albumin proteins are capable to form disulfide bonds with gluten proteins [22]. The data do not allow drawing out definitive conclusions about the modulatory effect of proteins bioactivity. Otherwise, open a new way to further research in order to produce innovative food formulations from which there could be expected more controlled physiological effects. The literature search reveals continuous and intense research efforts in the baking industry to incorporate gluten-free cereals and pseudocereals [22, 63, 76].

2.3.4 Prebiotic Effects

Currently, the close association between the beneficial effects attributed to prebiotics and prevention of gastrointestinal disorders is well accepted. Prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating the growth and/or activity in specific intestinal bacteria. The latter, through the production of short chain fatty acids, are able to modulate the intestinal inflammatory environment [77]. Fermentation of prebiotics exert important metabolic consequences in liver-related disorders contributes to lipid and cholesterol synthesis in the liver as well as regulating the expression of different transcription factors.

Pseudocereals provide significantly higher amounts of dietary fiber than wheat and related cereals [3]. Despite nutritional benefits, pseudocereals have been shown favorable to the adaptability of yeast and most lactic acid bacteria of technological interest [78, 79]. Preclinical studies demonstrated that enterobacteria and other potential commensal harmful bacteria do not benefit from this prebiotic effect of buckwheat [80]. In addition to metabolic effects, it has been reported the participation of defined receptors (i.e., aryl hydrocarbon receptor) in the buckwheat-mediated controlled of the proliferation of *Bacteroides* spp. [75]. This association between the modulatory potential of buckwheat on microbiota composition and receptors with an important role in cancer progression could open the way to nutraceutical uses of defined components from pseudocereals.

2.3.5 Control of Glycemia

Typical Western diet is characterized by fast digestion and absorption of carbohydrates determining a high postprandial glucose and insulin responses, additionally influencing the risk of liver and biliary tract cancers, although convincing evidence is currently lacking [81]. Current data demonstrate higher glycemic values for gluten-free breads as well as pasta than for their respective counterparts [82, 83]. To date, scarce data support the positive effects of amaranth oil and seeds at controlling insulin responses [84]. The effects of buckwheat on glycemic response(s) remain controversial [85, 86]. Studies on the digestibility of pseudocereals starch predict a poor glycemic value of those [25, 87]. In line with these studies, there have been reported delayed fluxes of glucose release that are reflected in delayed AUC curves of postprandial glucose in rats [88]. These effects were accompanied of an upregulated hepatic expression of the PPARγ receptor that is a key regulator of whole body glucose homeostasis and substrate distribution for energy expenditure. Taking into consideration the influence of other nutrients than starch in the modulation of glycemic values (i.e., protein, fiber and fat), pseudocereals constitute promising "ingredients" to optimize food for populations at risk [45, 89]. Technological features of pseudocereals as well as their behavior during household processes can also greatly impact its glycemic value.

2.3.6 Control of Cholesterolemia

Information from human trials supporting the cholesterol-lowering activity of pseudocereals is scarce [90–92]. The main hypotheses to explain the potential contribution of pseudocereals to the hypocholesterolemic effect are based on their nutritional profile. Otherwise, distinct experimental approaches have been performed to support the cholesterol-lowering activity of quinoa [92, 93], amaranth [14, 94], and buckwheat [90, 91]. Some of those have also focused on the impact of processing treatments (i.e., heat-expanded amaranth) [14] or to what extent can alterations on lipid homeostasis [94] be controlled through pseudocereals intake. Overall, the underlying mechanisms to explain the hypocholesterolemic effects of pseudocereals remain unclear. For example, it has been proposed that buckwheat cholesterol-lowering activity could be derived from its poor digestibility [95, 96] in contrast to the commonly assumed high quality of its proteins. An interesting hypothesis is also the assumption of metabolic effects beyond the changes in lipid homeostasis derived from its nutritional profile [97]. Probably, at least in part, these effects could occur through an increased energy expenditure promoting oxidative metabolism. The existing uncertainties warrant further research in this sense.

3 Conclusion and Future Perspectives

Cereals have been associated to human diet from ancient times. Domestication of cereals was facilitated by polyploidization events that led to genetic determinants, which excluded potentially adaptive alleles. At present, an intense and continuous social development, as well as commercial globalization, motivates the increase in

food production, where constant technological innovations are needed to respond to the increasing demands of the consumer of high quality food. Thus, important socioeconomic changes in most countries leading to a substantial change in dietary habits favor the appearance of metainflammatory diseases (i.e., NAFLD, T2D, obesity). Here, the strong social demand of gluten-free products increased worldwide and even gluten-free foods started being consumed by many other groups of population than those with immunological imbalances such as celiac patients. Several distinct reasons are considered for this switch on "food choice" and consumer's behavior; nutritional quality and health benefits beyond this nutrition are mostly considered. Pseudocereals represent a good option both from a nutritional and technological point of view to improve the nutritional profile of food, while reducing the intake of defined food ingredients associated to intestinal and immunological disorders. Three distinct grains are majorly known as pseudocereals; quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus cruentus*), and buckwheat (*Fagopyrum esculentum* and *Fagopyrum tartaricum*). These grains offer good technofunctional options to use them as substitutes of wheat flour, thus producing gluten-free foods. Moreover, ascription to the gluten-free diet is commonly associated to nutritional deficiencies. Here, the inclusion of pseudocereals improve the nutritional profile and reduces the risk of deleterious effects on the nutritional status.

Pseudocereals consumption has been associated to a wide spectrum of beneficial effects beyond its mere nutritional value in relation to immune regulation including prevention of intestinal imbalances in gut microbiota composition and inflammatory disorders. Many of these effects could derive from their potential to stimulate innate branch of the immune system because of their particular composition on globulins, but also to its prebiotic effect and potential capacity of other naturally occurring biologically active compounds. Although the scarce data existing encourage the use of pseudocereals as adjuvant in nutritional intervention strategies, a complete description of the underlying molecular mechanisms and processes implied is still unknown. This manuscript has been limited to those existing studies where key aspects such as bioavailability of the different compounds as well as concentrations used could result close to the effective physiological ones. The retrieval from literature revision highlights the need to clarify these aspects in order to pave the way for a full translational and transferable usage of pseudocereals into the clinical practice and society to improve self-management strategies of diseases.

Acknowledgments JML thanks Spanish MINECO for his "Ramón y Cajal" contract. BF thanks KNOW Consortium "Healthy Animal – Safe Food," MS&HE Decision No. 05-1/KNOW2/2015.

References

1. Ulbricht C, Abrams T, Conquer J, Costa D, Grims-Serrano JM, Taylor S, Varguese M (2009) An evidence-based systematic review of amaranth (*Amaranthus* spp.) by the natural standard research collaboration. J Diet Suppl 6:390–417. https://doi.org/10.3109/19390210903280348
2. Charmet G (2011) Wheat domestication: lessons for the future. C R Biol 334:212–220. https://doi.org/10.1016/j.crvi.2010.12.013

3. Alvarez-Jubete L, Arendt EK, Gallagher E (2009) Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. Trends Food Sci Tech 21:106–113. https://doi.org/10.1016/j.tifs.2009.10.014
4. Marquart L, Wiemer KL, Jones JM, Jacob B (2003) Whole grains health claims in the USA and other efforts to increase whole-grain consumption. Proc Nutr Soc 62(1):151–160. https://doi.org/10.1079/PNS2003242
5. Liu RH (2007) Whole grain phytochemicals and health. J Cereal Sci 46:207–219. https://doi.org/10.1016/j.jcs.2007.06.010
6. Laparra JM, Haros M (2016) Inclusion of ancient Latin-American crops in bread formulation improves intestinal iron absorption and modulates inflammatory markers. Food Funct 7(2):1096–1102. https://doi.org/10.1039/c5fo01197c
7. Marventano S, Vetrani C, Vitale M, Godos J, Riccardi G, Grosso G (2017) Whole grain intake and glycaemic control in healthy subjects: a systematic review and meta-analysis of randomized controlled trials. Forum Nutr 9:769. https://doi.org/10.3390/nu9070769
8. Li Y, Li S, Meng X, Gan RY, Zhang JJ, Li HB (2017) Dietary natural products for prevention and treatment of breast cancer. Forum Nutr 9:728. https://doi.org/10.3390/nu9070728
9. Jenkins DJA, Boucher BA, Ashbury FD et al (2017) Effect of current dietary recommendations on weight loss and cardiovascular risk factors. J Am Coll Cardiol 69:1103–1112. https://doi.org/10.1016/j.jacc.2016.10.089
10. Junker Y, Zeissig S, Kim SJ, Barisani D, Wieser H, Leffler DA, Zevallos V, Libermann TA, Dillon S, Freitag TL, Kelly CP, Schuppan D (2012) Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. J Exp Med 209:2395–2408. https://doi.org/10.1084/jem.20102660
11. Kaliszewska A, Martinez V, Laparra JM (2016) Proinflammatory responses driven by non-gluten factors are masked when they appear associated to gliadins. Food Chem Toxicol 95:89–95. https://doi.org/10.1016/j.fct.2016.06.030
12. Ojo B, Simenson AJ, O'Hara C, Wu L, Gou X, Peterson SK, Lin D, Smith BJ, Lucas EA (2017) Wheat germ supplementation alleviates insulin resistance and cardiac mitochondrial dysfunction in an animal model of diet-induced obesity. Br J Nutr 118:241–249. https://doi.org/10.1017/S0007114517002082
13. Bergamo P, Maurano F, Mazzarella G, Iaquinto G, Vocca I, Rivelli AR, De Falco E, Gianfrani C, Rossi M (2011) Immunological evaluation of the alcohol-soluble protein fraction from gluten-free grains in relation to celiac disease. Mol Nutr Food Res 55:1266–1270. https://doi.org/10.1002/mnfr.201100132
14. Caselato-Sousa VM, Ozaki MR, de Almeida EA, Amaya-Farfan J (2014) Intake of heat-expanded amaranth grain reverses endothelial dysfucntion in hypercholesterolemic rabbits. Food Funct 5:3281–3286. https://doi.org/10.1039/c4fo00468j
15. Sanz-Penella JM, Laparra JM, Sanz Y, Haros M (2012) Bread supplemented with amaranth (*Amaranthus cruentus*): effect of phytates on in vitro iron absorption. Plant Foods Hum Nutr 67:50–56. https://doi.org/10.1007/s11130-011-0269-6
16. Food and Agriculture Organization of the United Nations (2017) http://www.fao.org/worldfoodsituation/csdb/en/
17. Food and Agriculture Organization of the United Nations (2011) http://www.fao.org/worldfoodsituation/csdb/en/
18. US Department of Agriculture (USDA) (2007) National Nutrient Database for Standard References. https://ndb.nal.usda.gov/ndb/
19. Hurrell RF, Reddy MB, Burri J, Cook JD (2000) An evaluation of EDTA compounds for iron fortification of cerealbased foods. Br J Nutr 84:903–910. https://doi.org/10.1017/S0007114500002531
20. Sanz-Penella JM, Wronkowska M, Soral-Śmietana M, Haros M (2013) Effect of whole amaranth flour on bread properties and nutritive value. LWT-Food Sci Tech 50:679–685. https://doi.org/10.1016/j.lwt.2012.07.031
21. Chauhan GS, Eskin NAM, Tkachuck R (1992) Nutrients and antinutrients in quinoa seed. Cereal Chem 69:85–88

22. Oszvald M, Tamás C, Rakszegi M, Tömösközi S, Békés F, Tamás L (2009) Effects of incorporated amaranth albumins on the functional properties of wheat dough. J Sci Food Agric 89:882–889. https://doi.org/10.1002/jsfa.3528
23. Schönlechner R, Drausinger J, Ottenschlaeger V, Jurackova K, Berghofer E (2010) Functional properties of gluten-free pasta produced from amaranth, quinoa and buckwheat. Plant Foods Hum Nutr 65:339–349. https://doi.org/10.1007/s11130-010-0194-0
24. Jacobsen S-E (2003) The worldwide potential for quinoa (*Chenopodium quinoa Willd*). Food Rev Int 19:167–177. https://doi.org/10.1081/FRI-120018883
25. Wolter A, Hager AS, Zannini E, Arendt EK (2014) Influence of sourdough on in vitro starch digestibility and predicted glycemic indices of gluten-free breads. Food Funct 5:564–572. https://doi.org/10.1039/c3fo60505a
26. Mithila MV (2015) Khanum F (2015) effectual comparison of quinoa and amaranth supplemented diets in controlling appetite; a biochemical study in rats. J Food Sci Technol 52:6735–6741. https://doi.org/10.1007/s13197-014-1691-1
27. Ruiz GA, Opazo-Navarrete M, Meurs M, Minor M, Sala G, van Boekel M, Stieger M, Janssen AE (2016) Denaturation and in vitro gastric digestion of heat-treated quinoa protein isolates obtained at various extraction pH. Food Biophys 11:184–197. https://doi.org/10.1007/s11483-016-9429-4
28. Tang Y, Tsao R (2017) Phytochemicals in quinoa and amaranth grains and their antioxidant, anti-inflammatory, and potential health beneficial effects: a review. Mol Nutr Food Res 2017:61(7). https://doi.org/10.1002/mnfr.201600767
29. Bressani R (2003) Amaranth. In: Caballero B (ed) Encyclopedia of food science and nutrition. Academic Press, Oxford, pp 166–173
30. Berghofer E, Scoenlenchner R (2007) Pseodocereals – An Overview, Department of Food Science and Technology, University of Natural Resources and Applied Life Sciences, Vienna, Austria. http://projekt.sik.se/traditionalgrains/review/Oral%20presentation%20PDF%20files/Berghofer%20.pdf. Accessed 10 Oct 2017
31. Wood SG, Lawson LD, Fairbanks DJ, Robison LR, Andersen WR (1993) Seed lipid content and fatty acid composition of three quinoa cultivars. J Food Compos Anal 6:41–44. https://doi.org/10.1006/jfca.1993.1005
32. Vega-Galvez A, Miranda M, Vergara J, Uribe E, Puente L, Martínez EA (2010) Nutrition facts and functional potential of quinoa (*Chenopodium quinoa willd*), an ancient Andean grain: a review. J Sci Food Agric 90:2541–2547. https://doi.org/10.1002/jsfa.4158
33. Wijngaard HH, Arent EK (2006) Buckwheat. Cereal Chem 83:391–401. https://doi.org/10.1094/CC-83-0391
34. Valcárcel-Yamani B, Caetano S, Lannes S (2012) Applications of quinoa (*Chenopodium Quinoa Willd.*) and qmaranth (*Amaranthus Spp.*) and their influence in the nutritional value of cereal based foods. Food and. Public Health 2:265–275. https://doi.org/10.5923/j.fph.20120206.12
35. Torres García J, Durán Agüero S (2014) Phospholipids: properties and health effects. Nutr Hosp 31:76–83. https://doi.org/10.3305/nh.2015.31.1.7961
36. Repo-Carrasco R, Espinoza C, Jacobsen S-E (2003) Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and Kañiwa (*Chenopodium pallidicaule*). Food Rev Int 19:179–189. https://doi.org/10.1081/FRI-120018884
37. Carrion R, Murphy K, Ganjyal G, Kowalski R, Noratto G (2014) Quinoa as source of bioactive compounds with potential for intestinal health. FASEB J 28:647.18
38. Venskutonis PR, Kraujalis P (2013) Nutritional components of amaranth seeds and vegetables: a review on composition, properties, and uses. Compr Rev Food Sci Food Saf 12:381–412. https://doi.org/10.1111/1541-4337.12021
39. Uriyapongson J, Rayas-duarte P (1994) Comparison of yield and properties of amaranth starches using wet and dry-wet milling processes. Cereal Chem 71:571–577
40. Caselato-Sousa VM, Amaya-Farfán J (2012) State of knowledge on amaranth grain: a comprehensive review. J Food Sci 77:93–104. https://doi.org/10.1111/j.1750-3841.2012.02645.x

41. Segura-Nieto M, Shewry PR, Paredes-Lopez O (1994) Globulins of the pseudocereals: Amaranth, quinoa and buckwheat. In: Shewry PR, Casey R (eds) Seed Proteins. Kluwer Academic Publishers, Dordrecht, pp 453–475
42. Gorinstein S, Pawelzik E, Delgado-Licon E, Haruenkit R, Weisz M, Trakhtenberg S (2002) Characterisation of pseudocereal and cereal proteins by protein and amino acid analyses. J Sci Food Agric 82:886–891. https://doi.org/10.1111/j.1750-3841.2012.02645.x
43. Gamel TH, Linssen JP, Alink GM, Mossallem AS, Shekib LA (2004) Nutritional study of raw and popped seed proteins of *Amaranthus caudatus* L and *Amaranthus cruentus L*. J Sci Food 84:1153–1158. https://doi.org/10.1002/jsfa.1781
44. Berganza BE, Moran AW, Rodriguez GM, Coto NM, Santamaría M, Bressani R (2003) Effect of variety and location on the total fat, fatty acids and squalene content of amaranth. Plant Foods Hum Nutr 58:1–6. https://doi.org/10.1023/B:QUAL.0000041143.24454.0a
45. Pina-Rodriguez AM, Akoh CC (2010) Composition and oxidative stability of a structured lipid from amaranth oil in a milk-based infant formula. J Food Sci 75:140–146. https://doi.org/10.1111/j.1750-3841.2009.01460.x
46. Singhal RS, Kulkarni PR (1998) Amaranths as underutilized resource. Int J Food Sci Tech 23:125–139. https://doi.org/10.1111/j.1365-2621.1988.tb00559.x
47. Budin JT, Breene WM, Putnam DH (1996) Some compositional properties of seeds and oils of eight Amaranthus species. J Am Oil Chem Soc 73:475–481. https://doi.org/10.1007/BF02523922
48. Bruni R, Medici A, Guerrini A, Scalia S, Poli F, Muzzoli M, Sacchetti G (2001) Wild Amaranthus Caudatus seed oil, a nutraceutical resource from Ecuadorian flora. J Agric Food Chem 49:5455–5460. https://doi.org/10.1021/jf010385k
49. Rocchetti G, Chiodelli G, Giuberti G, Masoero F, Trevisan M, Lucini L (2017) Evaluation of phenolic profile and antioxidant capacity in gluten-free flours. Food Chem 228:367–373. https://doi.org/10.1016/j.foodchem.2017.01.142
50. Jing R, Li HQ, Hu CL, Jiang YP, Qin LP, Zheng CJ (2016) Phytochemical and pharmacological profiles of three fagopyrum buckwheats. Int J Mol Sci 17(4). https://doi.org/10.3390/ijms17040589
51. Steadman K, Burgoon M, Lewis B, Edwardson SE, Obendorf RL (2001) Buckwheat seed milling fractions: description, macronutrient composition and dietary fibre. J Cereal Sci 33:271–278. https://doi.org/10.1006/jcrs.2001.0366
52. Steadman K, Burgoon M, Schuster R et al (2000) Fagopyritols, D-chiro-inositol, and other soluble carbohydrates in buckwheat seed milling fractions. J Agric Food Chem 48:2843–2847. https://doi.org/10.1021/jf990709t
53. Skrabanja V, Kreft I (1998) Resistant starch formation following autoclaving of buckwheat (*Fagopyrum esculentum* Moench) groats. An in vitro study. J Agric Food Chem 46:2020–2023. https://doi.org/10.1021/jf970756q
54. Qian J, Rayas-Duarte P, Grant L (1998) Partial characterization of buckwheat (*Fagopyrum esculentum*) starch. Cereal Chem 75:365–373. https://doi.org/10.1094/CCHEM.1998.75.3.365
55. Aubrecht E, Biacs PÁ (2001) Characterization of buckwheat grain proteins and its products. Acta Aliment 30:71–80. https://doi.org/10.1556/AAlim.30.2001.1.8
56. Cai YZ, Corke H, Whum HX (2004) Amaranth. In: Corke H, Walker CE, Wrigley C (eds) Encyclopedia of grain science. Elsevier, Oxford, pp 1–10
57. Hager AS, Wolter A, Jacob F, Zannini E, Arendt EK (2012) Nutritional properties and ultrastructure of commercial gluten free flours from different botanical sources compared to wheat flours. J Cereal Sci 56:239–247. https://doi.org/10.1016/j.jcs.2012.06.005
58. Grobelnik MS, Turinek M, Jakop M, Bavec M, Bavec F (2009) Nutrition value and use of grain amaranth: potential future application in bread making. Agricultura 6:43–53
59. Schoenlechner R, Siebenhandl S, Berghofer E (2008) Pseudocereals, Chapter 7. In: Arendt EK, Bello FD (eds) Gluten-free cereal products and beverages. Academic Press, San Diego, pp 149–190
60. Gross R, Koch F, Malaga I, Miranda AF, Schoeneberger H, Trugo LC (1989) Chemical composition and protein quality of some local Andean food sources. Food Chem 34:25–34. https://doi.org/10.1016/0308-8146(89)90030-7

61. Aubrecht E, Horacsek M, Gelencser E, Dworschak E (1998) Investigation of prolamin content of cereals and different plant seeds. Acta Aliment 27:119–125
62. Marcone MF, Rickey YY (1997) Evidence for the phosophorylation and glycosylation of the amaranth 11S globulin (Amaranthin). J Food Biochem 21:341–369. https://doi.org/10.1111/j.1745-4514.1997.tb00203.x
63. Carrazco-Peña L, Osuna-Castro JA, De León-Rodríguez A, Maruyama N, Toro-Vazquez JF, Morales-Rueda JA, Barba de la Rosa AP (2013) Modification of solubility and heat-induced gelation of Amaranth 11S globulin by protein engineering. J Agric Food Chem 61:3509–3516. https://doi.org/10.1021/jf3050999
64. Brinegar C, Sine B, Nwokocha L (1996) High-cy steine 2S seed storage proteins from quinoa (*Chenopodium quinoa*). J Agric Food Chem 44:1621–1623. https://doi.org/10.1021/jf950830+
65. Shigemori S, Yonekura S, Sato T, Otani H, Shimosato T (2013) Expression of the immunoreactive buckwheat major allergenic storage protein in Lactococcus lactis. Appl Microbiol Biotechnol 97:3603–3611. https://doi.org/10.1007/s00253-012-4608-9
66. Jahaniaval F, Kakuda Y, Marcone MF (2000) Fatty acid and triacylglycerol compositions of seed oils of five Amaranthus accessions and their comparison to other oils. J Am Oil Chem Soc 77:847–852. https://doi.org/10.1007/s11746-000-0135-0
67. Ruales J, Nair BM (1993) Content of fat, vitamins and minerals in quinoa (*Chenopodium quinoa, Willd*.) seeds. Food Chem 48:131–136. https://doi.org/10.1016/0308-8146(93)90047-J
68. León-Camacho M, García-González DL, Aparicio R (2001) A detailed and comprehensive study of amaranth (*Amaranthus cruentus L*.) oil fatty profile. Eur Food Res Technol 213:349–355. https://doi.org/10.1007/s002170100340
69. Vidueiros SM, Curti RN, Dyner LM, Binaghi MJ, Peterson G, Bertero HD, Pallaro AN (2015) Diversity and interrelationships in nutritional traits in cultivated quinoa (*Chenopodiumquinoa Willd*.) from Northwest Argentina. J Cereal Sci 62:87–93. https://doi.org/10.1016/j.jcs.2015.01.001
70. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M et al (2012) Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med 10:13. https://doi.org/10.1186/1741-7015-10-13
71. Lee MH, Lee JS, Yang HC (2008) α-Amylase inhibitory activity of flower and leaf extracts from buckwheat (*Fagopyrum esculentum*). J Kor Soc Food Sci Nutrition 37:42–47
72. Prakash S, Deshwal S (2013) α/β-amylase activity of *Fagopyrum esculentum* (buckwheat): a medicinal plant. Janaki Med Coll J Med Sci 1:53–58
73. Karki R, Kim DW (2013) Extract of buckwheat sprouts scavenges oxidation and inhibits pro-inflammatory mediators in lipopolysaccharide-stimulated macrophages (RAW264.7). J Integr Med 11:246–252. https://doi.org/10.3736/jintegrmed2013036
74. Alvarez P, Alvarado C, Puerto M, Schlumberger A, Jiménez L, De la Fuente M (2006) Improvement of leukocyte function in prematurely aging mice after five weeks of diet supplementation with polyphenol-rich cereals. Nutrition 22:913–921. https://doi.org/10.1016/j.nut.2005.12.012
75. Li Y, Innocentin S, Withers DR, Roberts NA, Gallagher AR, Grigorieva EF, Wilhelm C, Veldhoen M (2011) Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell 147:629–640. https://doi.org/10.1016/j.cell.2011.09.025
76. Schoenlechner R, Drausinger J, Ottenschlaeger V, Jurackova K, Berghofer E (2010) Functional properties of gluten-free pasta produced from amaranth, quinoa and buckwheat. Plant Foods Hum Nutr 65:339–349. https://doi.org/10.1007/s11130-010-0194-0
77. Cavaglieri CR, Nishiyama A, Fernandes LC, Curi R, Miles EA, Calder PC (2003) Differential effects of short-chain fatty acids on proliferation and production of pro-and anti-inflammatory cytokines by cultured lymphocytes. Life Sci 73:1683–1690. https://doi.org/10.1016/S0024-3205(03)00490-9
78. Vogelmann SA, Seitter M, Singer U, Brandt MJ, Hertel C (2009) Adaptability of lactic acid bacteria and yeasts to sourdoughs prepared from cereals, pseudocereals and cassava and use of

competitive strains as starters. Int J Food Microbiol 130:205–212. https://doi.org/10.1016/j.ijfoodmicro.2009.01.020
79. Bianchi F, Rossi EA, Gomes RG, Sivieri K (2014) Potentially synbiotic fermented beverage with aqueous extracts of quinoa (*Chenopodium quinoa Willd*) and soy. Food Sci Tech Int 21:403–415. https://doi.org/10.1177/1082013214540672
80. Préstamo G, Pedrazuela A, Peñas E, Lasunción MA, Arroyo G (2003) Role of buckwheat diet on rats as prebiotic and healthy food. Nutr Res 23:803–814. https://doi.org/10.1016/S0271-5317(03)00074-5
81. Fedirko V, Lukanova A, Bamia C, Trichopolou A, Trepo E, Nöthlings U (2013) Glycemic index, glycemic load, dietary carbohydrate, and dietary fiber intake and risk of liver and biliary tract cancers in western Europeans. Ann Oncol 24:543–553. https://doi.org/10.1093/annonc/mds434
82. Atkinson FS, Foster-Powell K, Brand-Miller JC (2008) International tables of glycemic index and glycemic load values: 2008. Diabetes Care 31:2281–2283. https://doi.org/10.2337/dc08-1239
83. Bacchetti T, Saturni L, Turco I, Ferretti G (2014) The postprandial glucose response to some varieties of commercially available gluten-free pasta: a comparison between healthy and celiac subjects. Food Funct 5:3014–3019. https://doi.org/10.1039/c4fo00745j
84. Kim HK, Kim MJ, Cho HY, Kim EK, Shin DH (2006) Antioxidative and anti-diabetic effects of amaranth (*Amaranthus esculantus*) in streptozotocin-induced diabetic rats. Cell Biochem Funct 24:195–199. https://doi.org/10.1002/cbf.1210
85. Stringer DM, Taylor CG, Appah P, Blewett H, Zahradka P (2013) Consumption of buckwheat modulates the post-prandial response of selected gastrointestinal satiety hormones in individuals with type 2 diabetes mellitus. Metabolism 62:1021–1031. https://doi.org/10.1016/j.metabol.2013.01.021
86. Su-Que L, Ya-Ning M, Xing-Pu L, Ye-Lun Z, Guang-Yao S, Hui-Juan M (2013) Effect of consumption of micronutrient enriched wheat steamed bread on postprandial plasma glucose in healthy and type 2 diabetic subjects. Nutr J 17:64–71. https://doi.org/10.1186/1475-2891-12-64
87. Cavallero A, Empilli S, Brighenti F, Stanca AM (2002) High (1→3,1→4)-β-glucan barley fractions in bread making and their effects on human glycemic response. J Cereal Sci 36:59–66. https://doi.org/10.1006/jcrs.2002.0454
88. Laparra JM, Haros M (2017) Inclusion of whole flour from Latin-American crops into bread formulations as substitute of wheat delays glucose release and uptake (*Personal communication*)
89. Pina-Rodriguez AM (2009) Akoh CC (2009) synthesis and characterization of a structured lipid from amaranth oil as a partial fat substitute in milk-based infant formula. J Agric Food Chem 57:6748–6756. https://doi.org/10.1021/jf901048x
90. He J, Klag MJ, Whelton PK, Mo JP, Chen JY, Qian MC, Mo PS (1995) He GQ (1995) oats and buckwheat intakes and cardiovascular disease risk factors in an ethnic minority of China. Am J Clin Nutr 61:366–372
91. Zhang HW, Zhang YH, MJ L, Tong WJ, Cao GW (2007) Comparison of hypertension, dyslipidaemia and hyperglycaemia between buckwheat seed-consuming and non-consuming Mongolia-Chinese population in Inner Mongolia, China. Clin Exp Pharmacol Physiol 34:838–844. https://doi.org/10.1111/j.1440-1681.2007.04614.x
92. De Carvalho FG, Ovidio PP, Padovan GJ, Jordao Junior AA, MArchini JS, Navarro AM (2014) Metabolic parameters of postmenopausal women after quinoa or corn flakes intake–a prospective and double-blind study. Int J Food Sci Nutr 65:380–385. https://doi.org/10.3109/09637486.2013.866637
93. Paśko P, Barton H, Zagrodzki P, Izewska A, Krosniak M, Gawlik M, Gawlik M, Gorinstein S (2010) Effect of diet supplemented with quinoa seeds on oxidative status in plasma and selected tissues of high fructose-fed rats. Plant Foods Hum Nutr 65:146–151. https://doi.org/10.1007/s11130-010-0164-6

94. Lucero López VR, Razzeto GS, Escudero NL, Gimenez MS (2013) Biochemical and molecular study of the influence of Amaranthus Hypochondriacus flour on serum and liver lipids in rats treated with ethanol. Plant Foods Hum Nutr 68:396–402. https://doi.org/10.1007/s11130-013-0388-3
95. Kayashita J, Shimaoka I, Nakajoh M, Yamazaki M, Kato N (1997) Consumption of buckwheat protein lowers plasma cholesterol and raises fecal neutral sterols in cholesterol-fed rats because of its low digestibility. J Nutr 127:1395–1400
96. Tomotake H, Shimaoka I, Kayashita J, Yokoyama F, Nakajoh M, Kato N (2000) A buckwheat protein product suppresses gallstone formation and plasma cholesterol more strongly than soy protein isolate in hamsters. J Nutr 130:1670–1674
97. Foucault AS, Even P, Lafont R, Dioh W, Veillet S, Tomé D, Huneau JF, Hermier D, Quignard-Boulangé A (2014) Quinoa extract enriched in 20-hydroxyecdysone affects energy homeostasis and intestinal fat absorption in mice fed a high-fat diet. Physiol Behav 128:226–231. https://doi.org/10.1016/j.physbeh.2014.02.002

Part V

Food Carotenoids

Natural Food Pigments and Colorants

30

Delia B. Rodriguez-Amaya

Contents

Abstract

Extensive structure elucidation resulted in detailed information about anthocyanins, betacyanins, carotenoids, and chlorophylls, the major natural pigments in plant-derived foods. Modifications of the basic skeleton form a broad diversity of structures for anthocyanins and carotenoids. The chromophores responsible for the pleasant colors and the factors affecting them have been delineated. Identification of sources and determination of the composition in foods have also been widely pursued. Stability and influencing factors, alterations during

D. B. Rodriguez-Amaya (✉)
Faculty of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil

Universidade Federal da Fonteira Sul, Laranjeiras do Sul, Parana, Brazil
e-mail: dramaya2012@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_12

processing and storage of foods, and stabilization methods have been studied as part of the effort to retain the natural color of foods and to substitute artificial food dyes with natural colorants, this substitution being justified by concern about the safety of artificial colorants and by the potential health benefits of the natural colorants. Carotenoids have been the most investigated in terms of health effects, involving epidemiological, in vitro, animal, and human intervention studies. A wide range of biological activities have been attributed to anthocyanins, based mainly on cell culture and animal studies; human clinical studies are lacking. Investigations of the potential health benefits of betacyanin and chlorophyll are in their initial stages.

Keywords
Natural pigments · Natural colorants · Anthocyanin · Betacyanin · Carotenoid · Chlorophyll · Bioactive compounds · Health benefits

1 Introduction

The natural color of foods of plant origin is due primarily to four groups of pigments: the green chlorophylls, the yellow-orange-red carotenoids, the red-blue-purple anthocyanins, and the red betacyanin. A few animal-derived foods are colored by carotenoids. These pigments are also incorporated into food products by direct addition or indirectly through animals' feed.

Although earlier investigations were motivated by the color, recent studies have been stimulated by potential health effects. These possible health benefits, along with consumers' concern about safety, have led to efforts to replace artificial food dyes with natural colorants. This is not an easy task, however. Natural colorants are usually less stable, more costly, and not as easily utilized as synthetic dyes, besides having weaker tinctoral strength, interaction with food components, and limited range of hues [1, 2].

2 Anthocyanins

2.1 Chemical Structures, Properties, and Occurrence

Anthocyanins are water-soluble pigments belonging to the family of compounds called flavonoids. They are glycosides or acylglycosides of six commonly found aglycone anthocyanidins: pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Fig. 1). Cyanidin is the most commonly occurring anthocyanidin in nature. Anthocyanidins are flavylium (2-phenylbenzopyrylium) structures with varying hydroxyl and methoxyl substituents. They consist of two aromatic rings (rings A and B) linked by a three carbon heterocyclic ring (ring C) that contains oxygen. Eight conjugated double bonds carrying a positive charge on the

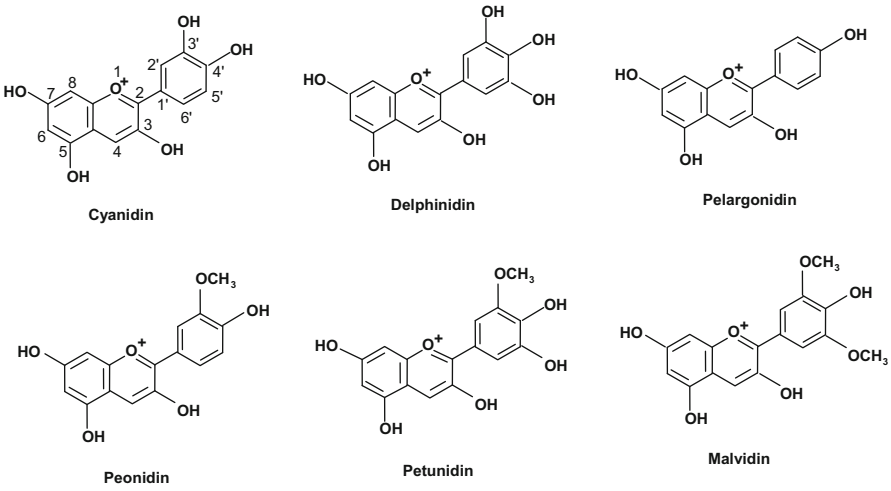


Fig. 1 Six anthocyanins commonly found in foods

heterocyclic oxygen constitutes the chromophore responsible for the intense color of anthocyanins. The color of anthocyanin-rich fruits and vegetables vary from vivid red as in strawberry, purple as in eggplant, and dark blue as in blueberry.

Anthocyanins are known to vary in the number and position of hydroxyl groups; the degree of methylation of these hydroxyl groups; the identity and number of sugar moieties and the positions at which they are attached; and the extent of sugar acylation and the identity of the acylating agent [3–5]. The most commonly encountered sugar is D-glucose, but anthocyanidins can also be conjugated to L-rhamnose, D-xylose, D-galactose, arabinose, and fructose [6], as well as rutinose (6-*O*-α-L-rhamnosyl-D-glucose), sophorose (2-*O*-β-D-glucosyl-D-glucose), gentiobiose (6-*O*-β-D-glucosyl-D-glucose), sambubiose (2-*O*-β-D-xylosyl-D-glucose), xylosylrutinose, and glycosylrutinose [3, 7]. Sugar is typically attached at C-3 on the C-ring (3-glycoside) or at both C-3 and C-5 on the A-ring (3,5-diglycoside). Glycosylation at C-7 on the A-ring and C-3′, C-5′, and C-4′ on the B-ring (not at both C-3′ and C-4′) have also been demonstrated.

The sugar moieties may be acylated with aromatic acids such as *p*-coumaric, caffeic, sinapic, ferulic, gallic, and *p*-hydroxybenzoic acids and/or aliphatic acids such as malonic, acetic, malic, succinic, or oxalic acids [8, 9]. The acyl substituents are usually bound to the C-3 sugar, esterified to the 6-OH, or less frequently to the 4-OH of the sugars. Anthocyanins with complicated acylation patterns on different sugar moieties had also been reported [10, 11].

In aqueous medium such as foods, anthocyanins undergo reversible structural transformations with pH (Scheme 1), with concomitant change in color [1, 12, 13]. The red favylium cation predominates at pH below 2. At pH 3–6, rapid hydration of the flavylium cation occurs at C-2 to form the colorless carbinol pseudobase. The carbinol by ring-opening tautomerization gives rise to the (*Z*)-chalcone, which can

Scheme 1 Structural transformations of anthocyanins with pH

isomerize to the (*E*)-chalcone. At slightly acidic to neutral conditions, deprotonation of the flavylium cation generates the blue quinoidal base.

Anthocyanins are found in fruits (especially berries), vegetables, nuts, grains, roots, and flowers [14–18]. Major sources are purple grapes, cherry, plum, raspberry, strawberry, blackberry, blueberry, black currant, cranberry, chokeberry, red cabbage, and red wine [17–20].

The anthocyanin composition of foods is affected by several factors, such as cultivar/variety [21–29], maturity [23, 24, 28], cultivation practices [27, 30], growing area or season/climate [28, 31, 32], and storage conditions [33]. Amarowicz et al. [34] reviewed extensively the influence of postharvest processing and storage on the contents of flavonoids (including anthocyanins). They concluded that for most of the subclasses in question, the effect of storage and processing on the polyphenol content is negligible in comparison with the differences between different varieties of plants.

2.2 Stability and Alterations During Processing and Storage of Food

Various factors affect anthocyanin color and stability, such as the chemical structure and concentration of the anthocyanin, temperature, pH, light, oxygen, presence of enzymes, proteins, metallic ions, other flavonoids and phenolics, ascorbic acid, and sugar [5, 6, 35–40].

Generally, hydroxylation induces a bathochromic shift and reduces stability, whereas methylation has the opposite effects [35, 41]. Glycosylation of the anthocyanin, especially in C-3, increases stability and solubility in water. The superior color stability of red raspberry products compared to that of processed strawberry and blackberry has been attributed to glycosylation with the disaccharide sophorose [42]. Acylation of sugar moieties also improves stability. Diacylation in the red radish anthocyanin as compared to the monoacylated anthocyanins in red-fleshed potato might be responsible for its greater stability [9]. Diacylated anthocyanins may be stabilized by a sandwich type stacking caused by hydrophobic interactions between the planar aromatic residues of the acyl groups and the positively charged pyrylium nucleus, thus diminishing the formation of the pseudobase [43]. In monoacylated anthocyanins, only one side of the pyrylium ring can be protected against the attack of water [44].

Utilization of anthocyanins as food colorants and functional ingredients has been limited by their low stability and interaction with other compounds in the food matrix. Grape-skin extract has been used as a colorant for a long time. Research has been directed toward the search for better sources and enhancement of extraction efficiency and stability. Examples of possible sources of anthocyanins as colorants are red radish [9, 45–48], red cabbage [23, 36, 49], black carrots [50, 51], red [48, 52], and purple sweet potatoes [29, 52–54].

Because anthocyanins with complex patterns of glycosylation and acylation exhibit remarkable stability to pH changes, heat treatment, and light exposure [55, 56], attributed to copigmentation, self-association, and metal complexing [10, 44, 57],

stabilization efforts have been directed along this line. In nature, anthocyanins are protected by these stabilizing mechanisms [43, 58, 59].

Copigmentation can occur through two mechanisms: (a) intramolecular interaction, which is based on the stacking of the hydrophobic acyl moiety covalently bound to sugar and the flavylium nucleus [56, 60]; and (b) intermolecular interactions in which anthocyanins interact via van der Waals interactions (vertical π-π stacking) between the planar polarizable nuclei of the anthocyanin with colorless copigment (e.g., phenolics) [61, 62]. The anthocyanin-copigment complexes adopt a sandwich-type stacking that stabilizes the favylium cation chromophore and partially protect it from the nucleophilic attack of water, thereby preventing color loss [10, 58]. This molecular association usually produces an increase in absorbance (hyperchromic effect) and a shift to longer wavelength of the visible absorption maximum (bathocromic effect) [43, 65]. A recent review revisited copigmentation, providing a comprehensive description of the nature of binding (the dispersion and electrostatic components of π-π stacking, the hydrophobic effect, and possible hydrogen-bonding between pigment and copigment), and of spectral modifications occurring in copigmentation complexes [7].

Many factors influence the magnitude of copigmentation, including the structures and concentrations of the anthocyanin and copigment, their molar ratio, the pH, temperature, and ionic strength [40, 61, 63, 64]. The effects of these different factors were demonstrated in commercial anthocyanin extracts [57]. At a given pH, color stability was mainly dependent on the structures of anthocyanins and of colorless phenolic compounds. Colorants rich in acylated anthocyanins (purple carrot, red radish, and red cabbage) exhibited great stability due to intramolecular copigmentation. Protection of the red chromophore was greater for diacylated anthocyanins in red radish and red cabbage. For colorants without acylated anthocyanins (grape-marc, elderberry, black currant, and chokeberry), intermolecular copigmentation played a key role; colorants rich in flavonols and with the highest copigment/pigment ratio showed remarkable stability.

Anthocyanins with ortho-dihydroxy system in the B-ring form complexes with certain metal cations, such as AL, Fe, Sn, and Cu [65–67]. Glycosides of cyanidin, delphinidin, and petunidin can form such complexes, but those of malvidin, pelargonidin, and peonidin cannot.

Ascorbic acid accelerates anthocyanin degradation [68, 69]. Initially thought to be due to a direct interaction between the two molecules, it is now believed that it is hydrogen peroxide formed from the oxidation of ascorbic acid that attacks the C-2 position of the anthocyanin, provoking the cleavage of the pyrylium ring and producing colorless esters and coumarin derivatives [37].

Monomeric anthocyanin combines with bisulfite at the pH of most foods and beverages to form a colorless sulfonic acid addition adduct. Addition of the sulfonate adduct was shown to take place at the C_4 position [70], interrupting the conjugated double bond system at the center of the molecule. Polymeric anthocyanins will not undergo this reaction since the 4-position is unavailable, being covalently linked to another phenolic compound [71].

Thermal processing reduces the anthocyanin content of foods [e.g., 26, 27, 29, 38, 72–78]. Monoacylated anthocyanins showed higher resistance against heat treatment (steaming, pressure cooking, microwaving, and frying) than di- and nonacylated anthocyanins [29]. In black currant, cyanidin-3-rutinoside was the most stable to heat treatment at 95 °C; cyanidin and delphinidin rutinosides were the most stable during storage for 12 months at 8 °C [79]. Cyanidin-3-rutinoside was also best preserved in processing purees and low-sugar jams prepared from strawberry cultivars [27]. Pelargonidin-3-glucoside had the most losses.

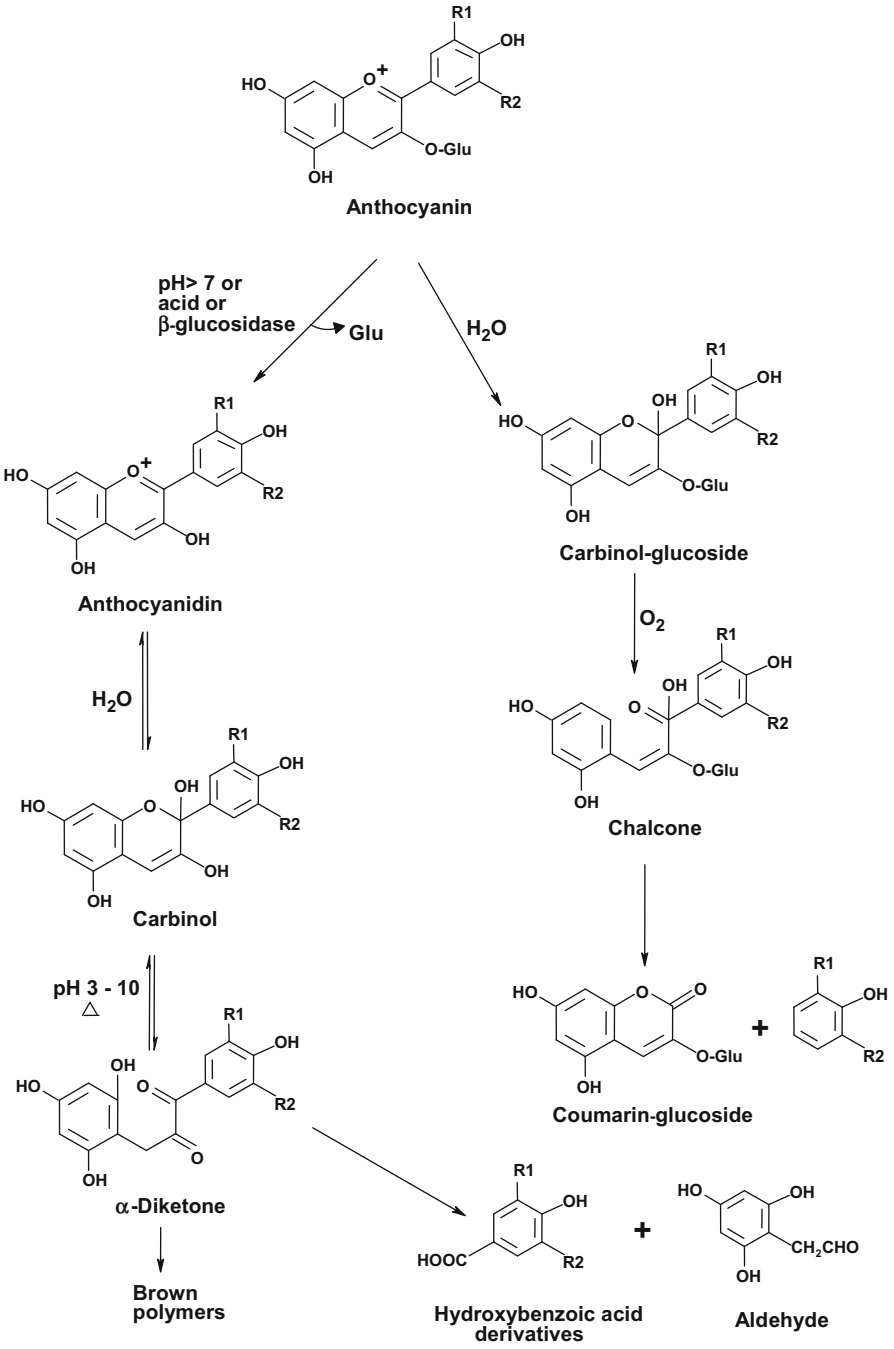
Suggested pathways for anthocyanin alterations during processing involve polymerization (browning) or cleavage (loss of color) [37]. In alkaline or acid medium, or in the presence of the enzyme β-glucosidase, anthocyanin is hydrolyzed, releasing the anthocyanidin, which can be transformed first into the carbinol base and subsequently into α-diketone (Scheme 2). The latter can polymerize, forming brown products, or fragment into an aldehyde and a derivative of hydroxybenzoic acid, both of which are colorless. On the other hand, anthocyanin can also be converted to carbinol and later into chalcone, and finally cleaved into a coumarin derivative and a compound corresponding to the B ring. Coumarin 3,5-diglycoside is a common product of the degradation of anthocyanins (3,5-diglycosides). The degradation depends on the anthocyanin present and the temperature.

In heat-treated (over 6 h at 95 °C, pH 3.5) elderberry and strawberry pigment isolates, acylated anthocyanins initially underwent hydrolysis, splitting the acylglycoside moieties from the flavylium backbone, forming anthocyanidin (aglycone) [80, 81]. Pentoses were more readily split off than hexoses. Opening of the pyrylium ring then followed. Finally, phenolic acids (4-hydroxybenzoic acid from pelargonidin and protocatechuic acid from cyanidin) and phloroglucinaldehyde (from both cyanidin and pelargonidin) were formed as terminal degradation products, residues of the B- and A-ring.

Stabilization methods for anthocyanins include the addition of copigment compounds, such as polymers, phenolic compounds, and metals; exclusion of O_2 during processing and storage; encapsulation techniques [39, 82]. In model beverages, the addition of polyphenols delayed color fading, the most notable improvement being observed with green tea extract [83]. In blackberry juice, the addition of sugars, especially trehalose, improved anthocyanin stability during thermal processing [77].

Encapsulation has been considered of great potential for protecting natural pigments [84], such as anthocyanins [85]. Microencapsulation/encapsulation of anthocyanins has been investigated to develop natural colorants with improved stability, solubility, dispersibility, and bioavailability [51, 86–89]. In black soybean anthocyanin, a combination of copigmentation and nanoencapsulation can be an effective technique for improving the color and antioxidant stability of anthocyanin.

Anthocyanins and their interactions have a decisive role in the color intensity and the stability of wine. Copigmentation appears to account for between 30% and 50% of the color in young wines [58]. Evolution of the red wine color during aging is a complex process that is attributed to copigmentation and to the progressive conversion of the original athocyanins into new, more stable pigments [90, 91].



Scheme 2 Structural alterations of anthocyanins during processing and storage of foods based on [37] and [41]

3 Betacyanins

3.1 Chemical Structures, Properties, and Occurrence

Betalains are water-soluble nitrogen-containing pigments, consisting of the red to red-violet betacyanins and yellow-orange betaxanthins. They are immonium conjugates of betalamic acid with *cyclo*-dopa[cyclo-3-(3,4-dihydroxyphenylalanine)] and amino compounds, respectively. The bathochromic shift of 50–70 nm of betacyanins compared to betaxanthins is due to the aromatic ring of cyclo-dopa. Glycosylation of betanidin results in a hypsochromic shift of the resulting betacyanin with glucose attached at C-6 being less effective than that linked to C-5 [92]. Esterification with aliphatic acyl moieties had little impact on the maximum absorption of betacyanins [93, 94].

Khan and Giridhar [95] presented a list of betalain-accumulating plants reported so far, highlighting pigment occurrence and accumulation pattern, and reviewed betalain biosynthesis. Stintzing and Carle [41] focused on the technological aspects and human health effects.

For a long time, beetroot was considered the sole source of betacyanin for use as food colorant, betanin (betanidin 5-*O*-β-glucoside) being the most abundant pigment. Recent years have seen an increased search for other sources, such as *Ullucus tuberosus*, one of the most widely grown and economically important root crops in the Andean region of South America [96]; *Basella rubra*, commonly known as Malabar spinach, a leafy vegetable that accumulates pigments in its fruits [97]; cactus pear (*Opuntia ficus-indica* and *O. stricta*) [98–102]; red-purple pitaya *Hylocereus polyrhizus* [93, 94, 99, 100, 103–106]; and *Amaranthus* species [92, 107–110].

3.2 Stability and Alterations During Processing and Storage of Food

Betalain stability is increased by high pigment content, high degree of glucosylation, and acylation, low a_w, low pH, antioxidants, chelating agents, low temperature, darkness, and nitrogen atmosphere [104]. Degrading enzymes (peroxidase, polyphenol oxidase, glucosidase), low degree of glucosylation and acylation, high a_w, metal cations, pH <3 or >7, high temperature, light, O_2, and H_2O_2 lowers stability. Glycosylation increased the half-life of betanidin and isobetanidin by about 17 times in O_2-saturated solutions, a behavior attributed to the lower oxidation-reduction potential of betanidin as compared to betanin [111]. Betacyanin stability may also be heightened by substitution with aromatic acids, the 6-*O*-substitution being more effective than 5-*O*-substitution, due to intramolecular stacking, since the U-shape folding of the molecule may protect the aldimine bond from hydrolytic attack [112].

Betalains have been reported to be stable over a broad pH range, from 3 to 7; the pH optimum for betanin is between 4 and 6. Elevated temperature shifted this optimum towards 6 [113]. In the presence of oxygen, betanin was most stable

between pH 5.5 and 5.8; under anaerobic conditions, lower pH values (4.0–5.0) were more favorable [114]. Betanin stability decreased linearly with increasing oxygen concentration [115]. Light and oxygen were observed to have additive effects, the betanin degradation being 15.6 and 14.6%, individually, rising to 28.6% with the simultaneous presence of both [116]. Since betanin undergoes water-dependent hydrolysis, a_w is a crucial factor for betanin susceptibility to aldimine bond cleavage [107–109, 117]. Improved betanin stability was found at lower a_w, the most effective being below 0.63 [118]. In a stability study of encapsulated beetroot pigments, betanin degradation was greatest at $a_w = 0.64$ [119].Greater betanin stability was explained by decreasing mobility of the reactants at lower a_w and by dilution effects at higher a_w.

Metal cations (Fe^{2+}, Fe^{3+}, Sn^{2+}, Al^{3+}, Cr^{3+}, Cu^{2+}) have been shown to accelerate betanin degradation [120–122]. EDTA can prevent metal-catalyzed betanin degradation by pigment stabilization and complex formation with metal ions [120].

Considerable decomposition may be catalyzed by betalain degrading enzymes. Several polyphenoloxidases were isolated from red beet [123, 124]. A betalain oxidase in red beet was reported to catalyze betanin degradation to cyclo-Dopa-5-*O*-β-glucoside, betalamic acid, and 2-hydroxy-2-hydro-betalamic acid [125].

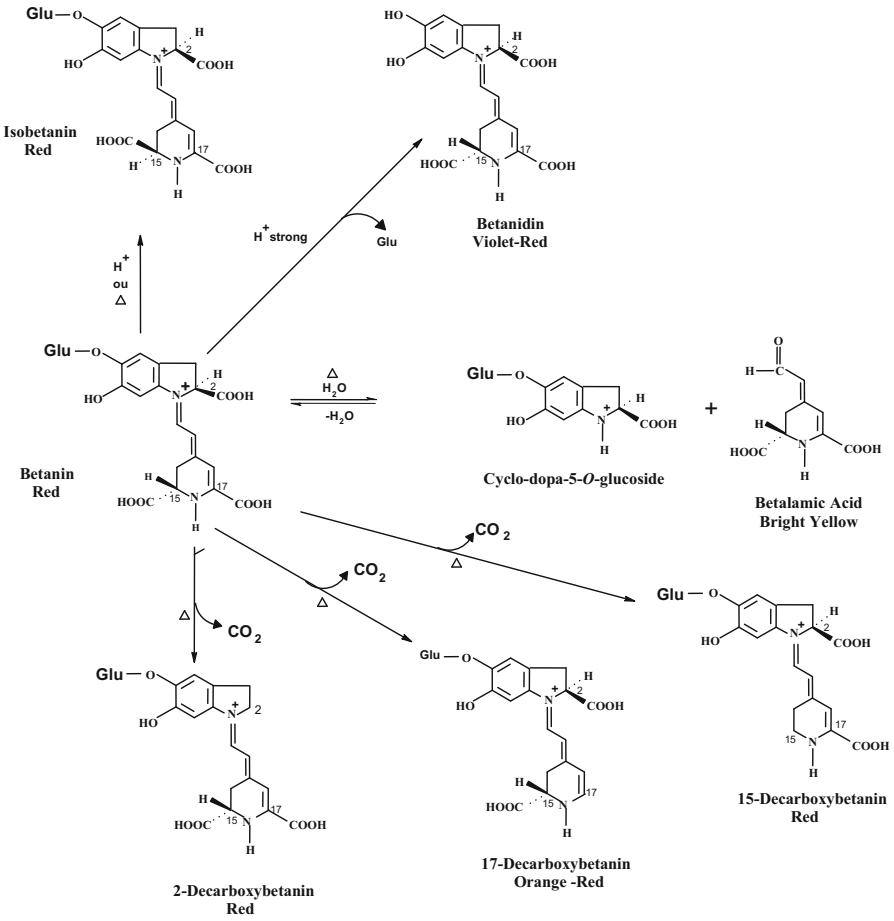
Betalain degradation on thermal processing has been reported in many papers [e.g., 103–105, 113, 115, 121, 126, 127]. The color shift to orange of red beet juice on thermal treatment was explained by the formation of yellow and orange-red degradation products [127].

Betacyanin undergoes isomerization, deglosylation, hydrolysis, decarboxylation, and dehydrogenation, with or without concomitant chromatic changes [117]. Betacyanins are generally accompanied by their respective isobetacyanins, the ratio depending on the food source. However, isomerization can be induced by both acidic [128–130] and alkaline conditions [128]. It has also been observed during thermal treatment of red beet juice [127, 131], the betanin/isobetanin ratio decreasing from 25:1 to 9:1 and 2.5:1 in raw, blanched, and sterilized red beet juice, respectively [131]. On the other hand, thermal treatment of purple pitaya juice elevated the betanin/isobetanin ratio [104]. Betanin and isobetanin exhibit the same chromatic properties.

Under strongly acidic conditions [128], at high temperature [132], or in the presence of β-glucosidase, the glucose moiety of betanin may be cleaved. This deglycosylation results in a bathochromic shift of about 4 nm [117] and an increased susceptibility towards oxidation [41].

At pH above 6 or during thermal processing, betanin undergoes cleavage to the bright yellow betalamic acid and the colorless *cyclo*-Dopa-5-*O*-β-glucoside [129]. This was found to be more pronounced during heat treatment in purified solution than in red beet and purple pitaya juice [133]. Acylation with aromatic or aliphatic acids may protect the aldimine bond from cleavage [112, 133].

Recondensation of betalamic acid and *cyclo*-Dopa derivatives occurs after short-term heating [115, 134]. This regeneration is partial [131, 134] and is greater at pH 6 in the absence of oxygen [114]. No recondensation was observed at pH 7. Ascorbic, isoascorbic, metaphosphoric, and gluconic acids improved the regeneration of red beet juice pigments after heating [135].



Scheme 3 Structural alterations of betanin during processing and storage of foods based on [127]

Betanin may be decarboxylated at the C-2, C-15, and/or C-17 positions, the carboxyl groups differing in their susceptibility toward decarboxylation [133]. Decarboxylation at either C-2 or C-15 do not alter the betanidin chromophore, thereby maintaining the chromatic characteristics of the original betacyanin. On the other hand, 17-decarboxy betanin exhibit an orange hue [117]. Monocarboxylated betanin, phyllocactin (malonyl-betanin, and hylocerenin (3′-hydroxy-3′-methyl-glutaryl-betanin) were shown to be considerably more stable toward degradation than nondecarboxylated betacyanins [114, 136]. Isobetanin undergoes decarboxylations similar to those of betanin (not shown in Scheme 3).

Yellow neobetanin (14,15,-dehydrobetanin) was shown to be a natural constituent of red beet [137, 138] and prickly pear [92, 139]. However, neobetanin formation upon thermal treatment of red beet juice under aerobic conditions has been confirmed [127]. Moreover, dehydrogenation of phyllocactin and hylocerenin on

thermal treatment of purple pitaya juice resulted in the formation of the corresponding yellow neo-derivatives [103]. Thus, dehydrogenation had been held responsible for the noticeable color (hypsochromic) shift during heat treatment of red beet juice and purple pitaya juice [103, 104, 117, 127].

On prolonged thermal treatment, a diversity of betacyanin degradation products may ensue by multiple decarboxylation or by combined decarboxylation and dehydrogenation of both betanin and neobetanin [117]. Mixtures of mono-, di-, and tridecarboxylated betacyanins, together with their corresponding neobetacyanins, were identified in purified extracts of red beet and purple pitaya [106, 140]. Main products were 17-decarboxy-betacyanin, 17-decarboxy-isobetanin, 2-decarboxy-betanin, 2,17-bidecarboxybetanin, and 2,17-decarboxyisobetanin, and 14,15-dehydrogenated-neobetanin. Heating of betanin, phyllocactin, and hylocerenin resulted in decarboxylated neoderivatives together with the corresponding decarboxylated betacyanins [133]. Thus, neobetanin is also decarboxylated in a manner similar to that of betanin and isobetanin (not shown in Scheme 3). Generation of neobetanin, neophyllocactin, and neohylocerenin was observed during heat treatment of red beet and purple pitaya juice [103, 127].

4 Carotenoids

4.1 Chemical Structures, Properties, and Occurrence

The lipophilic carotenoids are generally C_{40} tetraterpenes/tetraterpenoids formed from eight C_5 isoprenoid units joined head to tail, except at the center where a tail-to-tail linkage reverses the order. The most distinctive structural feature is a centrally located, conjugated double bond system. The basic linear and symmetrical skeleton is modified in many ways, including cyclization, hydrogenation, dehydrogenation, introduction of oxygen containing groups, migration of the double bonds, rearrangement, chain shortening or extension, or combinations thereof, resulting in a wide array of structures.

Carotenoids may be acyclic (e.g., lycopene, ζ-carotene) or may have a six-membered ring at one (e.g., γ-carotene, δ-carotene) or both ends (e.g., β-carotene, α-carotene) of the molecule, except capsanthin and capsorubin which have five-membered rings. Hydrocarbon carotenoids (e.g., β-carotene, lycopene) are known as carotenes, and the oxygenated derivatives are called xanthophylls. Common oxygen-containing substituents are hydroxyl (as in β-cryptoxanthin), keto (as in cantha-xanthin), epoxy (as in violaxanthin), and aldehyde (as in β-citraurin) groups.

Plants are able to synthesize carotenoids de novo. Thus, along with the principal carotenoids, low levels of their biosynthetic precursors and derivatives are found in plant-derived foods, making the carotenoid composition variable and usually complex [141]. The carotenoids are located in subcellular organelles (plastids), mainly associated with proteins in the chloroplasts and deposited in crystalline form or as oily droplets in chromoplasts [142].

Carotenoids are not as widely distributed in foods of animal origin and the composition is simpler. Unable to carry out carotenogenesis, animals are limited to absorbing dietary carotenoids, which are accumulated unchanged or slightly altered to form carotenoids typical of animal species.

Hydroxycarotenoids in ripe fruits are mostly esterified with fatty acids [143–147]. In a few fruits, particularly those that remain green when ripe (e.g., kiwi) [148], limited or no esterification of carotenols occur. In green leaves [149], carotenols are unesterified; those of corn [150, 151] are mostly unesterified. Lutein, the principal carotenoid, occurs free or esterified in one (monoester) or both hydroxyl groups (diester) in nasturtium [152] and marigold [153] flowers, with the esters predominating. Esterification occurs progressively during maturation and appears to be important physiologically. Acylation increases the lipophilic character of the xanthophylls, facilitating their accumulation in the chromoplasts [148]. Moreover, in red and hot chili peppers, mono- and diester carotenoids showed greater processing stability than the nonesterified counterparts [154].

Astaxanthin is the main carotenoid of some fish, such as salmon and trout, as well as crustaceans. It may be found free, esterified in one or both hydroxyl groups with fatty acids, or as a complex with proteins (carotenoproteins) or lipoproteins (carotenolipoproteins) [155]. Crustacean astaxanthin is a mixture of the three ester forms.

Carotenoids in which the carbon skeleton has been shortened by removal of fragments from one or both ends of the usual C_{40} structure are called apocarotenoids. Natural examples are bixin, the major pigment of annatto, and crocetin, the main coloring component of saffron. As with epoxy carotenoids, apocarotenoids are initial products of the oxidative degradation of carotenoids. Thus, traces of these compounds are often found in foods. For example, β-Apo-8′-carotenal, β-Apo-10′-carotenal, and β-citraurin are common minor carotenoids of citrus fruits.

The conjugated double bond system comprises the light-absorbing chromophore that confers the carotenoid's attractive color and is mainly responsible for their special properties and many functions [141]. As the light yellow ζ-carotene, a carotenoid should have at least seven conjugated double bonds in order to have perceptible color, phytoene (three conjugated double bonds) and phytofluene (five conjugated double bonds) are colorless, whereas lycopene (11 conjugated double bonds in an acyclic structure) is red. The monocyclic γ-carotene and the bicyclic β-carotene, although having the same number of conjugated double bonds as lycopene, are red-orange and yellow-orange, respectively. This is because cyclization takes the π electrons of the ring double bond out of plane with those of the chain due to steric hindrance between the ring methyl group at C-5 and the hydrogen at C-8 of the polyene chain. Hydroxyl substituents do not affect the chromophore; thus, both α-carotene and its dihydroxy derivative lutein are pale yellow. Similarly, the monohydroxy and dihydroxy derivatives of β-carotene, β-cryptoxanthin and zeaxanthin, have the same color as β-carotene. Capsanthin with a conjugated double bond system consisting of nine double bonds in the polyene chain, one double bond in the β-ring, and the carbonyl group double bond, and capsorubin with its nine conjugated double bonds in the polyene chain extended by the double bonds of two

carbonyl groups, give the intense color of red pepper. Astaxanthin, which has nine conjugated double bond in the polyene chain extended by two double bonds in β-rings and two carbonyl group double bonds, is responsible for the vivid red color of cooked shrimp, lobster, and crabs. In the raw crustaceans, astaxanthin is complexed with protein, transforming the color to blue, black, or grey. Heating denatures the protein and the red color of free astaxanthin ensues. Astaxanthin also gives the reddish color of salmon and trout flesh.

In nature, carotenoids exist primarily in the all-*E* configuration. An exception is bixin, which occurs naturally in the *Z*-form. Theoretically, each carbon-carbon double bond in the polyene chain of carotenoids may exhibit *E*-*Z* isomerization. However, some double bonds are prevented from undergoing this isomerization because the *Z*-configuration is sterically hindered [156, 157]. The *Z*-isomers of β-carotene [158–160] and zeaxanthin [160–163] commonly found in foods are the 9-*Z*-, 13-*Z*-, and the 15-*Z*-isomers. Carbon-carbon double bonds located in the cyclic part of the carotenoid structure, as the C-5,6 double bond in β-carotene, are also sterically hindered and are not isomerized. However, this double bond in the acyclic lycopene is unhindered and 5-*Z*-lycopene is found in tomato and tomato products, along with the 9-*Z*-, 13-*Z*-, and the 15-*Z*-isomers [164–167]. Being unsymmetrical, all-*E*-α-carotene [158], β-cryptoxanthin [158], and all-*E*-lutein [160–163, 168] give rise to 13′-*Z*- and 9′-*Z*-isomers in addition to 13-*Z*-, 9-*Z*-, and 15-*Z*-isomers in foods.

The carotenoids most commonly encountered in foods are β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin (Fig. 2) [169–174]. Rich sources of β-carotene are some palm fruits, some squash cultivars, green leafy and non-leafy vegetables, carrot, orange-fleshed sweet potato, cantaloupe, mango, and apricot. β-Carotene is sometimes accompanied by α-carotene, as in carrot, some varieties of squashes, pumpkins, and palm fruits. β-Cryptoxanthin is the main carotenoid of orange-fleshed fruits, such as papaya, tangerine, orange, loquat, persimmon, and tree tomato. Lycopene-rich foods are tomato and tomato products, pitanga, pink-fleshed guava, red-fleshed papaya, and watermelon. The richest sources of lycopene, however, are the Asian gac (*Momordica cochinchinensis*) fruit [175, 176] and the Spanish sarsaparilla [147]. Corn and corn products and some varieties of squash are the major dietary sources of zeaxanthin/lutein. Leafy and other green vegetables are the main sources of lutein.

The different factors affecting the carotenoid composition of the fresh produce have been extensively investigated, such as cultivar/variety, maturity, climate or season, geographic site of production, agronomic practices, part of the plant utilized, harvesting, and postharvest handling [141, 148, 173, 174, 177–179].

4.2 Stability and Alterations During Processing and Storage of Food

Carotenoid stability is affected by the nature of the carotenoid (carotene or xanthophyll, *E*- or *Z*-isomer, esterified or unesterified) and the food (fruit, root, leaf, juice, puree), disruption (peeled, sliced, shredded) of the food matrix, oxygen, light,

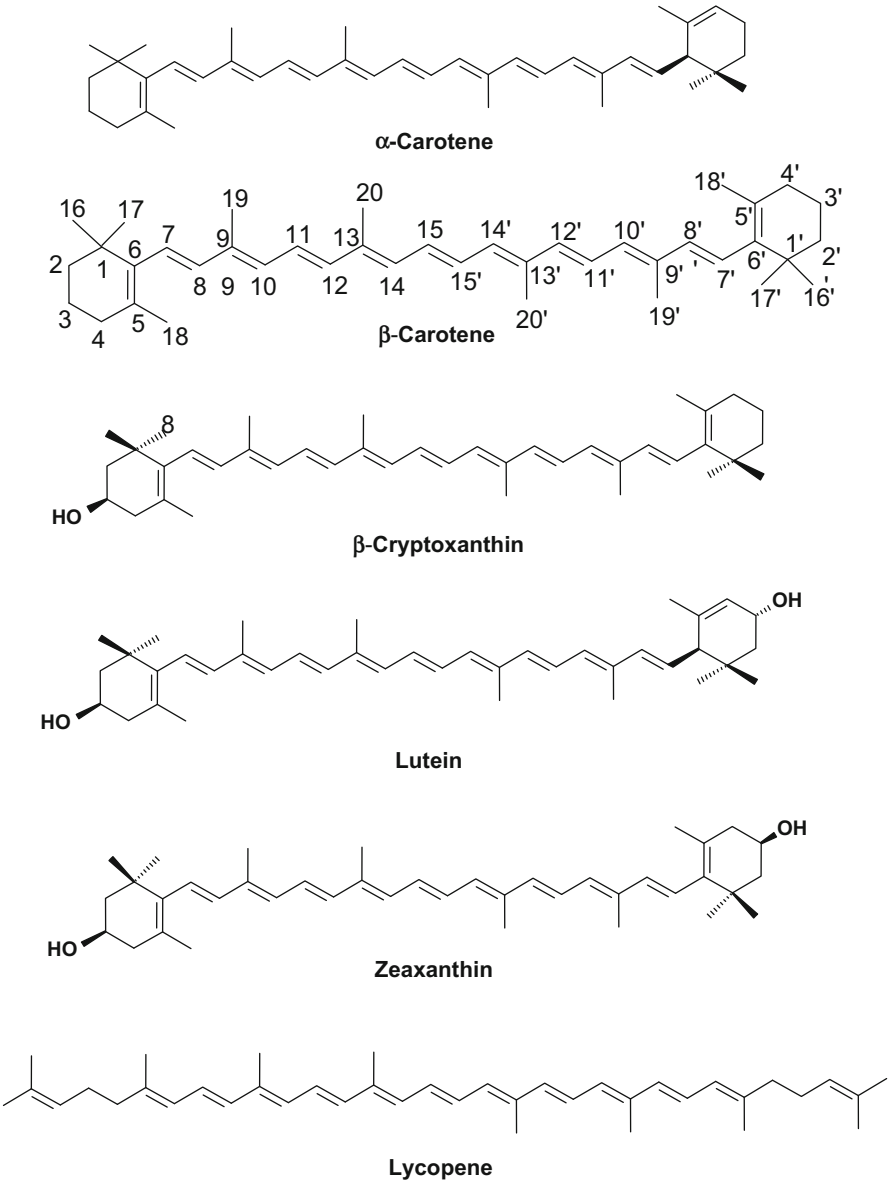


Fig. 2 Carotenoids commonly found in foods

processing method and condition (especially temperature and duration), and storage condition and duration, water content/activity, atmosphere, oxidizing enzymes, antioxidants, metal catalysts and prooxidants, and free radical initiators and inhibitors [174, 180, 181, 182, 183, 184, 185].

Considering the individual effects along with the interplay of many influencing factors, results of the numerous studies on the effects of home and industrial processing may sometimes appear conflicting. Moreover, in spite of the great progress in carotenoid analytical capability, existence of some errors in the analysis and in the calculation of retention or loss cannot be ruled out. Isomerization and oxidation of carotenoids during analysis and/or during storage of samples may also be attributed erroneously to the processing and storage of foods.

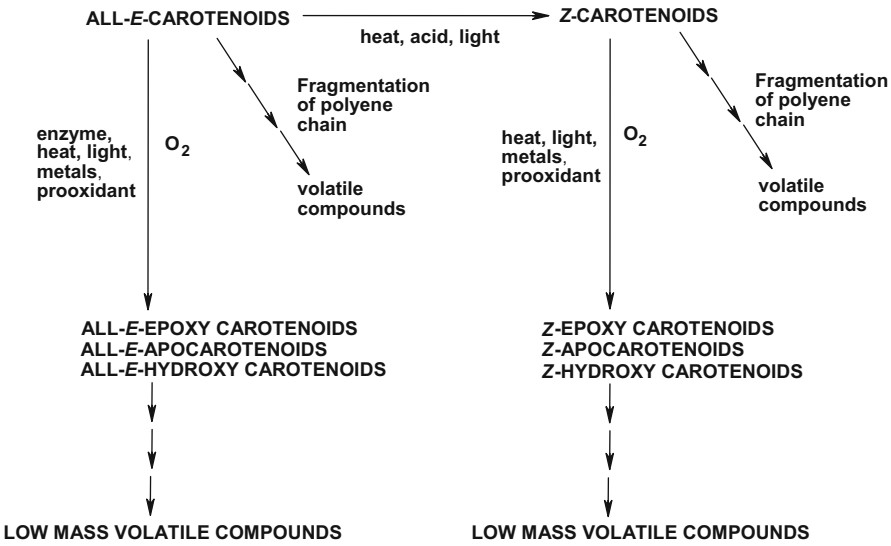
Carotenoid degradation is known to increase with the destruction of the food cellular structure, greater surface area or porosity, duration and severity of processing conditions, duration and inadequate conditions of storage, permeability of packaging material to O_2, and exposure to light. Processing and storage effects on food carotenoids had been reviewed [179–187], with tomato, carrot, and pepper as the most investigated foods.

In paprika and chili powder, mono- and diesters showed greater processing stability than their nonesterified counterparts [154]. This was probably due to the more lipophilic nature of the esterified carotenoids, resulting in their better integration into membrane structures, thereby protecting them from thermal degradation. Capsanthin, capsorubin, zeaxanthin, and β-cryptoxanthin mono- and diesters exhibited similar stabilities, whereas the susceptibility to degradation of the nonesterified carotenoids varied considerably.

A review of earlier studies [180] drew some conclusions, which continue to be supported by recent studies [174]. Carotenoids differ in their susceptibility to degradation. Moreover, their stability differs in different foods even when the same processing and storage conditions are used. The main cause of carotenoid loss during processing and storage of foods is enzymatic or nonenzymatic oxidation. Enzymatic oxidation of carotenoids can occur to a greater extent than thermal decomposition in many foods. Whatever the processing method chosen, retention of carotenoids decreases with longer processing time, higher processing temperatures, and cutting or maceration of the food. The heat treatment in blanching may provoke some losses of carotenoids, but the inactivation of oxidative enzymes will prevent further and greater losses during processing and storage. Because the carotenoids are more concentrated in the peel than in the pulp, peeling and juicing result in substantial losses of carotenoids, often surpassing those of heat treatment. Exclusion of oxygen (through vacuum or hot filling, oxygen-impermeable packaging, or inert atmosphere), protection from light, and storage at low temperatures all protect carotenoids from decomposition.

Alteration or loss of carotenoids during processing and storage of foods occurs through physical removal (e.g., peeling), geometric isomerization, and enzymatic or nonenzymatic oxidation [174, 181].

Isomerization of all-E-carotenoids to the Z-isomers carotenoids is promoted by acids, heat, and light. The release of organic acids during slicing, pulping, or juicing of fruits can be sufficient to provoke this isomerization, but it occurs to a greater extent during cooking or thermal processing, as shown for various carotenoids in many foods [158, 162, 186, 188–195]. During typical cooking of tomatoes, β-carotene and lutein isomerized to a greater extent than δ-carotene, γ-carotene, and



Scheme 4 General scheme for the oxidative degradation of carotenoids reproduced from [174]

lycopene [196]. Individual carotenoids and their isomeric forms also behaved differently during production and storage of canned tomato juice [197]. Notably, tetra-*Z*-lycopene was drastically reduced and isomerized to other geometric forms, including all-*E*-lycopene.

The technological, analytical, and nutritional implications of the occurrence of carotenoid Z-isomers in food were reviewed by Schieber and Carle [198]. Pronounced *E*-*Z* isomerization leads to a decrease in color intensity and the ability to quench singlet oxygen [199, 200]. It also results in loss of provitamin A activity and alteration of bioavailability and metabolism.

Enzyme-catalyzed oxidation takes place prior to heat treatment, during peeling, slicing, and pulping. It can also occur in minimally processed food and in unblanched frozen food during thawing [141, 174].

Nonenzymatic oxidation (also called autoxidation) is accompanied by isomerization, and both the *Z*- and *E*-isomers are oxidized [201, 202]. Oxidation initially involves epoxidation, cleavage to apocarotenals, and hydroxylation [201, 203, 204]. Subsequent fragmentations result in a series of compounds of low molecular masses, similar to those produced in fatty acid oxidation. Cleavages at different sites of the polyene chain can also directly produce short volatile fragments [174]. An overall carotenoid degradation scheme is shown in Scheme 4.

Epoxidation of β-carotene commences with oxygen attack in the terminal double bond of the conjugated double bond system on one side of the molecule, and then on the other side, forming β-carotene-5,6-epoxide and β-carotene-5,6,5′,6′-diepoxide, respectively. Rearrangement of the 5,6- to the 5,8-epoxide yields β-carotene-5,8-epoxide and β-carotene-5,8,5′,8′-diepoxide [201, 203–207].

Epoxidation of lycopene occurs both at the terminal conjugated double bonds and at the isolated double bonds with the formation of lycopene-1,2-epoxide and lycopene-1,2,1′, 2′-diepoxide, along with lycopene-5,6-epoxide and lycopene-5,6,5′,6′-diepoxide [202, 208, 209]. Combinations of these epoxides and cyclization result in a greater number of products and a more complicated epoxidation scheme.

Introduction of the 5,6-epoxide moiety and transformation of this group to the 5,8-furanoid is a common reaction in foods. A good example is the conversion of violaxanthin to auroxanthin, as observed in bottled mango juice [210], mango juice and canned mango slices [211], and orange xanthophylls [212–214].

Cleavage of β-carotene results in β-apo-carotenals (β-apo-15-carotenal, β-apo-14′-carotenal, β-apo-12′-carotenal, β-apo-10′-carotenal, and β-apo-8′-carotenal). A similar scheme takes place with lycopene, forming apo-15-lycopenal, apo-14′-lycopenal, apo-12′-lycopenal, apo-10′-lycopenal, apo-8′-lycopenal, and apo-6′-lycopenal [202, 215].

Both Z- and epoxy-β-carotenes were detected in corn oil containing β-carotene, oxidized in the Rancimat at 110 °C from 1 to 14 h [216]. Likewise, some Z- and epoxycarotenoids were found in cashew apple juice heated at 60°C and 90 °C [217].

Volatile compounds are generated from carotenoids by direct cleavage of the carotenoid polyene chain, sequential cleavage, and transformation of the initial volatile [174]. These are mostly aldehydes, ketones, alcohols, hydrocarbons, furan, and pyran [218–221]. Now devoid of color, they contribute to the desirable flavor of foods and beverages, as in wine and tea, but also to off-flavor, as in dehydrated carrot.

A prominent change during ripening of fruits is the enhanced biosynthesis of the vividly colored carotenoids and their oxidative cleavage to volatile compounds that contribute to the typical aroma/flavor of ripe fruits. There is similarity between the volatile compounds resulting from autoxidation and the enzymatic oxidation of carotenoids during ripening [174].

The utilization of carotenoids as colorant additives and functional ingredients in foods and beverages can be problematic because of their insolubility in water, instability, and low bioavailability. The first two problems have been addressed by the formulation of water-dispersable market products, as colloidal suspensions, emulsions, or dispersions in suitable colloids. In recent years, attention has centered on encapsulation and nanoencapsulation [174].

5 Chlorophylls

5.1 Structures, Properties, and Occurrence

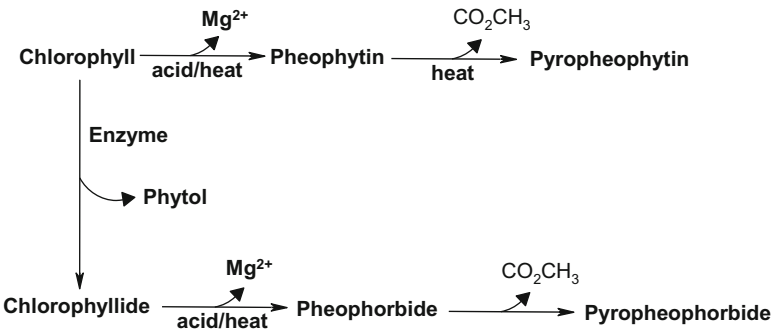
Chlorophyll a and b are the typical green pigments of higher plants, occurring in an approximate ratio of 3:1 in lettuce leaves [222] and in five commonly consumed Mediterranean leafy vegetables [223], but with a ratio of 1:2 to 1:4 in different varieties of the tree tomato fruit [224]. Chlorophylls are porphyrins, which are macrocyclic tetrapyrrole pigments in which the pyrrole rings are joined by methyne

bridges and the double bond system forms a closed, conjugated loop [37]. A centrally located magnesium atom is coordinated with the nitrogen of the four pyrroles. Chlorophyll a has a methyl group and chlorophyll b has a formyl group at C-3. Both have a vinyl and an ethyl group at the C-2 and C-4 position, respectively; a carbomethoxy group at the C-10 position of an isocyclic ring; and a phytol group esterified to propionate at the C-7 position. Phytol is a 20-carbon monounsaturated isoprenoid alcohol. Chloropyll a appears blue-green and chlorophyll b yellow-green [225]. The chlorophyll molecule has a hydrophilic part, the macrocycle, and a hydrophobic segment, the phytol. The closed circuit of conjugated double bonds is the chromophore that allows them to absorb light.

5.2 Stability and Alterations During Processing and Storage of Food

The green color of vegetables and fruits, due to the presence of chlorophyll, is affected by aging, enzymes, weak acids, oxygen, heat, and light. Degradation of chlorophyll occurs during ripening of fruits, senescence of green vegetables, and thermal processing of foods [226]. Epimerization is the first alteration observed when chlorphyll is exposed to heat [37, 227, 228]. Mild heating is sufficient to induce the inversion of the C-10 carbomethoxy group located in the isocyclic ring, forming isomers designated as chlorophylls a′ and b′.

The chlorophyll degradation route is shown in Scheme 5. It has long been known that the olive brown color of cooked and canned vegetables is due to the formation of pheophytin, the most common alteration of chlorophyll reported in thermally treated green vegetables, such as spinach [229], broccoli [230], pepper [194], squash, green beans, peas, leek, broccoli, and spinach [231]. Industrial processing resulted in 55–75% loss of chlorophyll a and 50–89% of chlorophyll b, and an increase in pheophytin a and b in both broccoli florets and stems [230]. Disrupting and breaking the cell walls, heat induces the release of acids, decreasing the pH. Under acidic condition, the magnesium atom is replaced by hydrogen to form pheophytin.



Scheme 5 Alterations of chlorophyll during processing and storage of foods

The green chlorophyllide is formed by the removal of phytol, catalyzed by the naturally occurring enzyme chlorophyllase. Prolonged heating causes elimination of the carbomethoxy group at C-10, giving rise to pyropheophytins, as observed in heat processed green vegetables [232].

Chlorophyll b was reported to be thermally more stable than chlorophyll a [37, 233, 234], the higher thermal stability of the former being attributed to the electron-withdrawing effect of its C-3 formyl group [235]. However, in a study on the effect of microwave and conventional cooking methods on chlorophyll pigments of six green vegetables, chlorophyll a was found more heat resistant compared with chlorophyll b, except in peas [231].

A similar early stage degradation occurs in degreening senescent leaves, consisting of the transformation of chlorophyll to chlorophyllide, pheophorbide, and pyropheophorbide, catalyzed respectively by chlorophyllase, Mg-dechelatase, and pheophorbide a oxygenase [226, 236, 237]. Pheophorbide a undergoes oxygenolytic opening of the porphyrin macrocycle, catalyzed by pheophorbide a oxygenase; subsequent reactions produce flourescent chlorophyll catabolites. The latter undergo catabolism to nonflourescent chlorophyll catabolites. This general catabolic pathway in senescent leaves was found to be also active in olive fruits during maturation [238].

Efforts to retain the green color in processed foods include: acid neutralization, high temperature short-time processing, enzymatic conversion of chlorophyll to chlorophyllide, and commercial application of metallo complex [37]. Pheophytin and pyropheophytin will complex with copper and zinc ions to form complexes with more attractive green color and are more stable to light. While pheophytin and pyropheophytin were found in conventionally processed green beans, in Veri-green canned beans, the green color was maintained due to the formation of the more stable zinc complexes, Zn-pheophytin a and Zn-pyropheophytin b [239]. The Veri-green process is a patented procedure in which blanching of green vegetables is undertaken in the presence of zinc salts [240].

6 Health Benefits

Of the natural pigments, the most studied in terms of human health are the carotenoids. Their best established function is the provitamin A activity, but they have been credited with other health-promoting effects, such as immunoenhancement and reduction of the risk of developing chronic degenerative diseases, such as cancer, cardiovascular diseases, cataract, and macular degeneration [241–245].

Carotenoids' action against diseases has been widely attributed to their antioxidant activity [246–248]. However, nonantioxidant mechanisms have been increasingly reported for these bioactive compounds such as retinoid-dependent signaling, modulation of carcinogen metabolism, regulation of cell growth, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of intercellular gap junction communication, gene regulation, modulation of DNA repair mechanisms, induction of detoxifying enzymes, hormonal and immune system modulation, and filtering of blue light [241, 242, 244, 249, 250].

Lycopene's possible role in human health has drawn considerable attention [251–256], especially in relation to prostate cancer [257–260]. Lycopene has also been associated with reduced risk for lung [261], pancreatic [262], colorectal [263], and digestive tract cancer [264].

Lutein and zeaxanthin selectively accumulate in the macula of the human retina [265, 266], and most epidemiological studies have shown that dietary intake or plasma or retina levels of lutein and/or zeaxanthin are associated with reduced risk of age-related macular degeneration, the major cause of irreversible blindness in the elderly [e.g., 265, 267–272]. These two carotenoids have also been consistently linked to the reduction of the risk for cataract [267, 270, 273, 274]. A meta-analysis of six studies showed, however, that dietary lutein and zeaxanthin were significantly related with reduced risk of late macular degeneration, but not with decreased risk of early macular degeneration [275]. Supplementation with lutein could improve visual function in patients suffering from macular degeneration and cataract [276, 277].

For the protective role against macular degeneration and cataract, lutein and zeaxanthin may act in two ways: (1) as filters of damaging blue light, and (2) as antioxidants quenching excited triplet-state sensitizers or singlet oxygen and scavenging harmful reactive oxygen species like lipid peroxides or the superoxide radical anion [278].

Dietary intake or serum levels of lutein and/or zeaxanthin have also been inversely associated with cardiovascular diseases [279–281]. Based on their survey of coronary mortality in 16 countries, Connor et al. [282] concluded that a diet low in foods containing folate and lutein/zeaxanthin might be the major contributing factor to increased coronary risk observed in the countries of Central and Eastern Europe.

As enumerated in review articles, a wide range of biological activities have been attributed to anthocyanins, such as antioxidant, antiallergic, anti-inflammatory, antiviral, antiproliferative, antimicrobial, antimutagenic, antitumor activities; microcirculation improvement; and peripheral capillary fragility prevention [4, 13, 20, 283, 284]. Anthocyanins are associated with low prevalence or alleviation of some diseases/disorders such as cancer, cardiovascular diseases, diabetes, obesity, and cognitive decline.

Antidiabetic properties include lowering of blood glucose levels by protecting β-cells, improving insulin resistance, increasing insulin secretion, improving liver function, inhibiting carbohydrate hydrolyzing enzymes, antioxidant capacity, and regulation of adipocyte function [283, 285].

Anticancer activity has been attributed to the additive effect of multiple mechanisms, such as antimutagenic activity, anti-inflammatory activity, antiproliferative effect, inhibition of DNA damage, inhibition of carcinogen activation, induction of phase II enzymes for detoxification, cell cycle arrest, inhibition of COX-2 enzymes, induction of apoptosis and antiangiogensis [13, 283, 286, 287].

Prevention of cardiovascular diseases encompasses increasing serum antioxidant capacity, strong inhibition of lipid peroxidation, active oxygen radical scavenging, capillary permeability, vasorelaxation, reduced plasmatic total cholesterol and hepatic triglyceride levels, inhibition of atherosclerotic plaque progression, improving endothelial dysfunction, inhibiting oxidized LDL formation [288, 289].Neuroprotection and vision improvement have also been cited for anthocyanins [283, 284].

The numerous reported biological activities and health-promoting effects of anthocyanins have been based mostly on in vitro cell studies and animal studies. Properly designed human clinical trials need to be carried out to provide definitive evidence.

Potential health-promoting activities (antioxidant, anti-inflammatory, anticancer, antilipidemic, antimicrobial activities) have also been attributed to betalains [290–292], but investigations are very much at the initial stage. Studies on the possible health effects of chlorophyll (anticancer, antimutagenic, and anti-proliferative activities; inhibition of heme-induced cytotoxic and hyperproliferative effects) are even more limited [293–296].

7 Concluding Remarks

The impressive work that has been accomplished on the chemical and technological aspects of natural pigments and colorants of foods, particularly anthocyanins, betacyanins, carotenoids, and chlorophylls, has provided a wealth of information on a wide range of important topics, including structures, chemical properties, stability, alterations during processing and storage, and stabilization methods. The potential health-promoting effects of carotenoids have been investigated for some time, but because of some inconsistent or inconclusive results, more human clinical studies are needed. The wide range of biological activities indicated by cell culture and animal model research for anthocyanins require confirmation by more human studies. Currently, at an initial stage, investigation of the health benefits of betacyanin and chlorophyll should be continued.

References

1. Wrolstad RE, Culver CA (2012) Alternatives to those artificial FD&C food colorants. Annu Rev Food Sci Technol 3:59–77
2. Sigurdson GT, Tang P, Giusti MM (2017) Natural colorants: food colorants from natural sources. Annu Rev Food Sci Technol 8:261–280
3. Clifford MN (2000) Anthocyanins – nature, occurrence and dietary burden. J Sci Food Agric 80:1063–1072
4. Kong J-M, Chia L-S, Goh N-K, Chia T-F, Brouillard R (2003) Analysis and biological activities of anthocyanins. Phytochemistry 64:923–933
5. Castañeda-Ovando A, Pacheco-Hernández ML, Páez-Hernández ME, Rodríguez JA, Galán-Vidal CA (2009) Chemical studies of anthocyanins. Food Chem 113:859–871
6. Francis FJ, Markakis PC (1989) Food colorants: anthocyanins. Crit Rev Food Sci Nutr 28:273–314
7. Trouillas P, Sancho-Garcia JC, de Freitas V, Gierschner J, Otyepka M, Dangles O (2016) Stabilizing and modulating color by copigmentation: insights from theory and experiment. Chem Rev 116:4937–4982
8. Giusti MM, Rodríguez-Saona LE, Wrolstad RE (1999) Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. J Agric Food Chem 47:4631–4637

9. Giusti MM, Wrolstad RE (2003) Acylated anthocyanins from edible sources and their applications in food systems. Biochem Eng J 14:217–225
10. Goto T (1987) Structure, stability and color variation of natural anthocyanins. Prog Chem Org Nat Prod 52:113–158
11. Odake K, Terahara N, Saito N, Toki K, Honda T (1992) Chemical structures of two anthocyanins from purple sweet potato, *Ipomoea batatas*. Phytochemistry 31:2127–2130
12. Pina F, Oliveira J, de Freitas V (2015) Anthocyanins and derivatives are more than flavylium cations. Tetrahedron 71:3107–3114
13. He J, Giusti MM (2010) Anthocyanins: natural colorants with health-promoting properties. Annu Rev Food Sci Technol 1:163–187
14. De Brito ES, de Araújo MCP, Alves RE, Carkeet C, Clevidence BA, Novotny JA (2007) Anthocyanins present in selected tropical fruits: acerola, jambolão, jussara, and guajiru. J Agric Food Chem 55:9389–9394
15. Einbond LS, Reynertson KA, Luo X-D, Basile MJ, Kennelly EJ (2004) Anthocyanin antioxidants from edible fruits. Food Chem 84:23–28
16. Harborne JB, Williams CA (2001) Anthocyanins and other flavonoids. Nat Prod Rep 18:310–333
17. Wu X, Prior RL (2005) Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. J Agric Food Chem 53:2589–2599
18. Wu X, Prior RL (2005) Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: vegetables, nuts, and grains. J Agric Food Chem 53:3101–3113
19. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL (2006) Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem 54:4069–4075
20. Mazza GJ (2007) Anthocyanins and heart health. Ann Ist Super Sanita 43:369–374
21. Lee J, Finn CE (2007) Anthocyanin and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. J Sci Food Agric 87:2665–2675
22. Dossett M, Lee J, Finn CE (2010) Variation in anthocyanins and total phenolics of black raspberry populations. J Funct Foods 2:292–297
23. Ahmadiani N, Robbins RJ, Collins TM, Giusti MM (2014) Anthocyanins contents, profiles, and color characteristics of red cabbage extracts from different cultivars and maturity stages. J Agric Food Chem 62:7524–7531
24. Jin AL, Ozga JA, Kennedy JA, Koerner-Smith JL, Botar G, Reinecke DM (2015) Developmental profile of anthocyanin, flavonol, and proanthocyanidin type, content, and localization in Saskatoon fruits (*Amelanchier alnifolia* Nutt.) J Agric Food Chem 63:1601–1614
25. Jorjong S, Butkhup L, Samappito S (2015) Phytochemicals and antioxidant capacities of Mao-Luang (*Antidesma bunius* L.) cultivars from Northeastern Thailand. Food Chem 181:248–255
26. Kim HJ, Park WS, Bae J-Y, Kang SY, Yang MH, Lee S, Lee H-S, Kwak S-S, Ahn M-J (2015) Variations in the carotenoid and anthocyanin contents of Korean cultural varieties and home-processed sweet potatoes. J Food Compos Anal 41:188–193
27. Kovacevic DB, Putnik P, Dragovic-Uzelac V, Vahcic N, Babojelic MS, Levaj B (2015) Influences of organically and conventionally grown strawberry cultivars on anthocyanin content and color in purees and low-sugar jams. Food Chem 181:94–100
28. Szalóki-Dorkó L, Stéger-Máté M, Abrankó L (2015) Evaluation of colouring ability of main European elderberry (*Sambucus nigra* L.) varieties as potential resources of natural food colourants. Int J Food Sci Technol 50:1317–1323
29. Xu J, Su X, Lim S, Griffin J, Carey E, Katz B, Tomich J, Smith JC, Wang W (2015) Characterization and stability of anthocyanins in purple-fleshed sweet potato P40. Food Chem 186:90–96
30. Rotray W, Orsat V (2011) Blackberries and their anthocyanins: factors affecting biosynthesis and properties. Compr Rev Food Sci Food Saf 10:303–320

31. Olsen H, Aaby K, Borge GIA (2010) Characterization, quantification, and yearly variation of the naturally occurring polyphenols in a common red variety of curly kale (*Brassica oleracea* L. convar. *acephala* var. *sabellica* cv. "Redbor"). J Agric Food Chem 58:11346–11354
32. Pervaiz T, Songtao J, Faghihi F, Haider MS, Fang J (2017) Naturally occurring anthocyanin, structure, functions and biosynthetic pathway in fruit plants. J Plant Biochem Physiol 5:187. https://doi.org/10.4172/2329-9029.1000187
33. Kovacevic DB, Putnik P, Dragovic-Uzelac V, Pedisíc S, Jambrak AR, Herceg Z (2016) Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegrate juice. Food Chem 190:317–323
34. Amarowicz R, Carle R, Dongowski G, Durazzo A, Galensa R, Kammerer D, Maiani G, Piskula MK (2009) Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods. Mol Nutr Food Res 53:S151–S183
35. Mazza G, Brouillard R (1987) Recent developments in the stabilization of anthocyanins in food products. Food Chem 25:201–225
36. Bridle P, Timberlake CF (1997) Anthocyanins as natural food colours – selected aspects. Food Chem 58:103–109
37. Schwartz SJ, von Elbe JH, Giusti MM (2008) Colorants. In: Damodaran S, Parkin KL, Fennema OR (eds) Fennema's food chemistry. CRC Press Taylor & Francis Group, Boca Raton
38. Patras A, Brunton NP, O'Donnell C, Tiwar BK (2010) Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends Food Sci Technol 21:3–11
39. Cavalcanti RN, Santos DT, Meireles MAA (2011) Non-thermal stabilization mechanisms of anthocyanins in model and food systems – an overview. Food Res Int 44:499–509
40. Malaj N, de Simone BC, Quartarolo AD, Russo N (2013) Spectrophotometric study of copigmentation of malvidin-3-*O*-glucoside with *p*-coumaric, vanillic and syringic acids. Food Chem 141:3614–3620
41. Stintzing FC, Carle R (2004) Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends Food Sci Technol 15:19–38
42. Boyles MJ, Wrolstad RE (1993) Anthocyanin composition of red raspberry juice: influences of cultivar, processing, and environmental factors. J Food Sci 58:1135–1141
43. Goto T, Kondo T (1991) Structure and molecular stacking of anthocyanins – flower color variation. Angew Chem Int Ed 30:17–33
44. Brouillard R (1983) The in vivo expression of anthocyanin colour in plants. Phytochemistry 22:1311–1323
45. Giusti MM, Wrolstad RE (1996) Radish anthocyanin extract as a natural red colorant for maraschino cherries. J Food Sci 61:688–694
46. Giusti MM, Ghanadan H, Wrolstad RE (1998) Elucidation of the structure and conformation of red radish (*Raphanus sativus*) anthocyanins using one- and two-dimensional nuclear magnetic resonance techniques. J Agric Food Chem 46:4858–4863
47. Giusti MM, Rodríguez-Saona LE, Bagett JR, Reed GL, Durst RW, Wrolstad RE (1998) Anthocyanin pigment composition of red radish cultivars as potential food colorants. J Food Sci 63:219–224
48. Rodríguez-Saona LE, Giusti MM, Wrolstad RE (1999) Color and pigment stability of red radish and red-fleshed potato anthocyanins in juice model systems. J Food Sci 64:451–456
49. Baublis A, Spomer A, Berber-Jiménez MD (1994) Anthocyanin pigments: comparison of extract stability. J Food Sci 59:1219–1221, 1233
50. Stintzing FC, Stintzing AS, Carle R, Frei B, Wrolstad RE (2002) Color and antioxidant properties of cyanidin-based anthocyanin pigments. J Agric Food Chem 50:6172–6181
51. Ersus S, Yurdagel U (2007) Microencapsulation of anthocyanin pigments of black carrot (*Daucus carota* L.) by spray drier. J Food Eng 80:805–812
52. Reyes LF, Cisneros-Zevallos L (2007) Degradation kinetics and colour of anthocyanins in aqueous extracts of purple- and red-flesh potatoes (*Solanum tuberosum* L.) Food Chem 100:885–894

53. Cai Z, Qu Z, Lan Y, Zhao S, Ma X, Wan Q, Jing P, Li P (2016) Conventional, ultrasound-assisted, and accelerated-solvent extractions of anthocyanins from purple sweet potatoes. Food Chem 197:266–272
54. Heinonen J, Farahmandazad H, Vuorinen A, Kallio H, Yang B, Sainio T (2016) Extraction and purification of anthocyanins from purple-fleshed potato. Food Bioprod Process 99: 136–146
55. Francis FJ (1992) A new group of food colorants. Trends Food Sci Technol 3:27–30
56. Dangles O, Saito N, Brouillard R (1993) Anthocyanin intramolecular copigment effect. Phytochemistry 34:119–124
57. Malien-Aubert C, Dangles O, Amiot MJ (2001) Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra- and intermolecular copigmentation. J Agric Food Chem 49:170–176
58. Boulton R (2001) The copigmentation of anthocyanins and its role in the color of red wine: a critical review. Am J Enol Vitic 52:67–87
59. Yoshida K, Kondo T, Goto T (1991) Unusually stable monoacylated anthocyanin from purple yam *Dioscorea alata*. Tetrahedron Lett 32:5579–5580
60. George F, Figueiredo P, Toki K, Tatsuzawa F, Saito N, Brouillard R (2001) Influence of trans-cis isomerisation of coumaric acid substituents on colour variance and stabilisation in anthocyanins. Phytochemistry 57:791–795
61. Mazza G, Brouillard R (1990) The mechanism of co-pigmentation of anthocyanins in aqueous solutions. Phytochemistry 29:1097–1102
62. Saito N, Tatsuzawa F, Yoda K, Yokoi M, Kasahara K, Iida S, Shigihara A, Honda T (1995) Acylated cyanidin glycosides in the violet-blue flowers of *Ipomoea purpurea*. Phytochemistry 40:1283–1289
63. Davies AJ, Mazza G (1993) Copigmentation of simple and acylated anthocyanins with colorless phenolic compounds. J Agric Food Chem 41:716–720
64. Gordillo B, Rodríguez-Pulido FJ, Escudero-Gilete ML, González-Miret ML, Heredia FJ (2012) Comprehensive colorimetric study of anthocyanic copigmentation in model solutions. Effects of pH and molar ratio. J Agric Food Chem 60:2896–2905
65. Goto T, Tamura H, Kawai T, Hoshino T, Harada N, Kondo T (1986) Chemistry of metalloanthocyanins. Ann N Y Acad Sci 471:155–173
66. Dangles O, Elhabiri M, Brouillard R (1994) Kinetic and thermodynamic investigation of the aluminum–anthocyanin complexation in aqueous solution. J Chem Soc Perkin Trans 2:2587–2596
67. Elhabiri M, Figueiredo P, Toki K, Saito N, Brouillard R (1997) Anthocyanin–aluminium and –gallium complexes in aqueous solution. J Chem Soc Perkin Trans 2:355–362
68. Skrede G, Wrolstad RE, Lea P, Enersen G (1992) Color stability of strawberry and blackcurrant syrups. J Food Sci 57:172–177
69. Martí N, Pérez-Vicente A, García-Viguera C (2001) Influence of storage temperature and ascorbic acid addition on pomegranate juice. J Sci Food Agric 82:217–221
70. Berké B, Chèze C, Vercauteren J, Deffieux G (1998) Bisulfite addition to anthocyanins: revisited structures of colourless adducts. Tetrahedron Lett 39:5771–5774
71. Wrolstad RE, Durst RW, Lee J (2005) Tracking color and pigment changes in anthocyanin products. Trends Food Sci Technol 16:423–428
72. Skrede G, Wrolstad RE, Durst RW (2000) Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.) J Food Sci 65:357–364
73. Franke AA, Custer LJ, Arakaki C, Murphy SP (2004) Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. J Food Compos Anal 17:1–35
74. Kırca A, Özkan M, Cemeroğlu B (2007) Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. Food Chem 101:212–218
75. Brownmiller C, Howard LR, Prior RI (2008) Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blueberry products. J Food Sci 73:H72–H79

76. Xu B, Chang SKC (2009) Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (*Phaseolus vulgaris* L.) as affected by thermal processing. J Agric Food Chem 57:4754–4764
77. Kopjar M, Pilizota V (2011) Prevention of thermal degradation of anthocyanins in blackberry juice with the addition of different sugars. CyTA-J Food 9:237–242
78. Brauch JE, Buchweitz M, Schweiggert RM, Carle R (2016) Detailed analyses of fresh and dried maqui (*Aristotelia chilensis* (Mol.) Stuntz) berries and juice. Food Chem 190:308–316
79. Rubinskiene M, Viskelis P, Jasutiene I, Viskeliene R, Bobinas C (2005) Impact of various factor on the composition and stability of black currant anthocyanins. Food Res Int 38:867–871
80. Sadilova E, Carle R, Stintzing FC (2007) Thermal degradation of anthocyanins and its impact on color and in vitro antioxidant capacity. Mol Nutr Food Res 51:1461–1471
81. Sadilova E, Stintzing FC, Carle R (2006) Thermal degradation of acylated and nonacylated anthocyanins. J Food Sci 71:C504–C512
82. Cortez R, Luna-Vital DA, Margulis D, de Mejia EG (2017) Natural pigments: stabilization methods of anthocyanins for food applications. Compr Rev Food Sci Food Saf 16:180–198
83. Chung C, Rojanasasithara T, Mutilangi W, McClements DJ (2016) Stabilization of natural colors and nutraceuticals: inhibition of anthocyanin degradation in model beverages using polyphenols. Food Chem 212:596–603
84. Sajilata MG, Singhal RS (2006) Isolation and stabilisation of natural pigments for food application. Stewart Postharvest Rev 5:11
85. Yousuf B, Gul K, Wani AA, Singh P (2016) Health benefits of anthocyanins and their encapsulation for potential use in food systems: a review. Crit Rev Food Sci Nutr 56:2223–2230
86. Bakowska-Barczak AM, Kolodziejczyk PP (2011) Black currant polyphenols: their storage stability and microencapsulation. Ind Crop Prod 34:1301–1309
87. Idham Z, Muhamad II, Setapar SHM, Sarmidi MR (2012) Effect of thermal processes on roselle anthocyanins encapsulated in different polymer matrices. J Food Process Preserv 36:176–184
88. Mahdavi AS, Jafari SM, Ghorbani M, Assadpoor E (2014) Spray-drying microencapsulation of anthocyanins by natural biopolymers: a review. Dry Technol 32:509–518
89. Robert P, Freedes C (2015) The encapsulation of anthocyanins from berry-type fruits. Molecules 20:5875–5888
90. Liao H, Cai Y, Haslam E (1992) Polyphenol interactions. Anthocyanins: co-pigmentation and colour changes in red wines. J Sci Food Agric 59:299–305
91. Brouillard R, Dangles O (1994) Anthocyanin molecular interactions: the first step in the formation of new pigments during wine aging? Food Chem 51:365–371
92. Stintzing FC, Kammerer D, Schieber A, Adama H, Nacoulma OG, Carle R (2004) Betacyanins and phenolic compounds from *Amaranthus spinosus* L. and *Boerhavia erecta* L. Z. Naturforsch 59c:1–8
93. Stintzing FC, Schieber A, Carle R (2002) Betacyanins in fruits from red-purple pitaya *Hylocereus polyrhizus* (Weber) Britton & Rose. Food Chem 77:101–106, 517
94. Wybraniec S, Platzner I, Geresh S, Gottlieb HE, Haimberg M, Mogilnitzki M, Mizrahi Y (2001) Betacyanins from vine cactus *Hylocereus polyrhizus*. Phytochemistry 58:1209–1212
95. Khan MI, Giridhar P (2015) Plant betalains: chemistry and biochemistry. Phytochemistry 117:267–295
96. Cejudo-Bastante MJ, Hurtado N, Mosquera N, Heredia FJ (2014) Potential use of new Colombian sources of betalains. Color stability of ulluco (*Ullucus tuberosus*) extracts under different pH and thermal conditions. Food Res Int 64:465–471
97. Kumar SS, Manoj P, Shetty NP, Prakash M, Giridhar P (2015) Characterization of major betalain pigments – gomphrenin, betanin and isobetanin from *Basella rubra* L. fruit and evaluation of efficacy as a natural colourant in product (ice cream) development. J Food Sci Technol 52:4994–5002

98. Castellar R, Obón JM, Alacid M, Fernández-López JA (2003) Color properties and stability of betacyanins from *Opuntia* fruits. J Agric Food Chem 51:2772–2776
99. Stintzing FC, Schieber A, Carle R (2003) Evaluation of colour properties and chemical quality parameters of cactus juices. Eur Food Res Technol 216:303–311
100. Moβhammer MR, Stintzing FC, Carle R (2005) Colour studies on fruit juice blends from *Opuntia* and *Hylocereus* cacti and betalain-containing model solutions derived therefrom. Food Res Int 38:975–981
101. Moβhammer MR, Stintzing FC, Carle R (2005) Development of a process for the production of a betalain-based colouring foodstuff from cactus pear. Innov Food Sci Emerg Technol 6:221–231
102. Mosshammer MR, Stintzing FC, Carle R (2006) Evaluation of different methods for the production of juice concentrates and fruit powders from cactus pear. Innov Food Sci Emerg Technol 7:275–287
103. Herbach KM, Stintzing FC, Carle R (2004) Thermal degradation of betacyanins in juices from purple pitaya [*Hylocereus polyrhisus* (Weber) Britton & Rose] monitored by high-performance liquid chromatography-tandem mass spectrometric analyses. Eur Food Res Technol 219: 377–385
104. Herbach KM, Rohe M, Stintzing FC, Carle R (2006) Structural and chromatic stability of purple pitaya (*Hylocereus polyrhisus* [Weber] Britton & Rose) betacyanins as affected by the juice matrix and selected additives. Food Res Int 39:667–677
105. Herbach KM, Maier C, Stintzing FC, Carle R (2007) Effects of processing and storage on juice color and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice. Eur Food Res Technol 224:649–658
106. Wybraniec S, Mizrahi Y (2005) Generation of decarboxylated and dehydrogenated betacyanins in thermally treated purified fruit extract from purple pitaya (*Hylocereus polyrhizus*) monitored by LC-MS/MS. J Agric Food Chem 53:6704–6712
107. Cai YZ, Corke H (2000) Production and properties of spray-dried *Amaranthus* betacyanin pigments. J Food Sci 65:1248–1252
108. Cai YZ, Corke H (2001) Effect of postharvest treatments on *Amaranthus* betacyanin degradation evaluated by visible/near-infrared spectroscopy. J Food Sci 66:1112–1118
109. Cai Y, Sun M, Corke H (1998) Colorant properties and stability of *Amaranthus* betacyanin pigments. J Agric Food Chem 46:4491–4495
110. Cai Y-Z, Sun M, Corke H (2005) Characterization and application of betalain pigments from plants of the *Amaranthaceae*. Trends Food Sci Technol 16:370–376
111. Von Elbe JH, Attoe EL (1985) Oxygen involvement in betanine degradation – measurement of active oxygen species and oxidation reduction potentials. Food Chem 16:49–67
112. Schliemann W, Strack D (1998) Intramolecular stabilization of acylated betacyanins. Phytochemistry 49:585–588
113. Havliková I, Miková K, Kyzlink V (1983) Heat stability of betacyanins. Z Lebensm-Unters - Forsch 177:247–250
114. Huang AS, von Elbe JH (1987) Effect of pH on the degradation and regeneration of betanine. J Food Sci 52:1689–1693
115. Czapski J (1985) The effect of heating conditions on losses and regeneration of betacyanins. Z Lebensm-Unters -Forsch 180:21–25
116. von Elbe JH, Maing I-Y, Asmundson CH (1974) Color stability of betanin. J Food Sci 39:334–337
117. Herbach KM, Stintzing FC, Carle R (2006) Betalain stability and degradation – structural and chromatic aspects. J Food Sci 71:R41–R50
118. Kearsley MW, Katsaboxakis KZ (1980) Stability and use of natural colours in foods. Red beet powder, copper chlorophyll powder and cochineal. Int J Food Sci Technol 15: 501–514
119. Serris GS, Biliaderis CG (2001) Degradation kinetics of beetroot pigment encapsulated in polymeric matrices. J Sci Food Agric 81:691–700

120. Attoe EL, von Elbe JH (1984) Oxygen involvement in betanin degradation – oxygen uptake and influence of metal ions. Z Lebensm-Unters -Forsch 179:232–236
121. Czapski J (1990) Heat stability of betacyanins in red beet juice and in betanine solutions. Z Lebensm-Unters -Forsch 191:275–278
122. Sobkowska E, Czapski J, Kaczmarek R (1991) Red table beet pigment as food colorant. Int Food Ingredient 3:24–28
123. Escribano J, Cabanes J, Chazarra S, Garcia-Carmona F (1997) Characterization of monophenolase activity of table beet polyphenol oxidase. Determination of kinetic parameters on the tyramine/dopamine pair. J Agric Food Chem 45:4209–4214
124. Escribano J, Gandía-Herrero F, Cabellero N, Pedreño MA (2002) Subcellular localization and isoenzyme pattern of peroxidase and polyphenol oxidase in beet root (*Beta vulgaris* L.) J Agric Food Chem 50:6123–6129
125. Zakharova NS, Petrova TA, Bokuchava MA (1987) Betanin enzymatic conversion. Appl Biochem Microbiol 25:768–774
126. Merin U, Gagel S, Popel G, Bernstein S, Rosenthal I (1987) Thermal degradation kinetics of prickly pear fruit red pigment. J Food Sci 52:485–486
127. Herbach KM, Stintzing FC, Carle R (2004) Impact of thermal treatment on color and pigment pattern of red beet (*Beta vulgaris* L.) preparations. J Food Sci 69:C491–C498
128. Wilcox ME, Wyler H, Dreiding AS (1965) Stereochemistry of betanidin and isobetanidin VIII. Structure of the bark pigment betanin. Helv Chim Acta 48:1134–1147
129. Schwartz SJ, von Elbe JH (1983) Identification of betanin degradation products. Z Lebensm-Unters-Forsch 176:448–453
130. Hilpert H, Siegfried MA, Dreiding AS (1985) Total synthese von decarboxybetalainen durch photochemische ringöffnung von 3-(4-pyridyl)alanin. Helv Chim Acta 68:1670–1678
131. Von Elbe JH, Schwartz SJ, Hildenbrand BE (1981) Loss and regeneration of betacyanin pigments during processing of red beets. J Food Sci 46:1713–1715
132. Jackman RL, Smith JL (1996) Anthocyanins and betalains. In: Hendry GAF, Houghton JD (eds) Natural food colorants, 2nd edn. Blackie Academic and Professional, Glasgow
133. Herbach KM, Stintzing FC, Carle R (2005) Identification of heat-induced degradation products from purified betanin, phyllocactin and hylocerenin by high-performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 19:2603–2616
134. Huang AS, von Elbe JH (1985) Kinetics of the degradation and regeneration of betanine. J Food Sci 50:1115–1120, 1129
135. Han D, Kim SJ, Kim SH, Kim DM (1998) Repeated regeneration of degraded red beet juice pigments in the presence of antioxidants. J Food Sci 63:69–72
136. Herbach KM, Stintzing FC, Carle R (2006) Stability and color changes of thermally treated betanin, phyllocactin and hylocerenin solutions. J Agric Food Chem 54:390–398
137. Alard D, Wray V, Grotjahn L, Reznik H, Strack D (1985) Neobetanin: isolation and identification from *Beta vulgaris*. Phytochemistry 24:2383–2385
138. Kujala T, Loponen J, Pihlaja K (2001) Betalains and phenolics in red beetroot (*Beta vulgaris*) peel extracts: extraction and characterization. Z Naturforsch C 56:343–348
139. Strack D, Engel U, Wray V (1987) Neobetanin: a new natural plant constituent. Phytochemistry 26:2399–2400
140. Wybraniec S (2005) Formation of decarboxylated betacyanins in heated purified betacyanin fractions from red beet root (*Beta vulgaris* L.) monitored by LC-MS/MS. J Agric Food Chem 53:3483–3487
141. Rodriguez-Amaya DB (1999) A guide to carotenoid analysis in foods. International Life Sciences Institute (ILSI) Press, Washington, DC
142. Bartley GE, Scolnik PA (1995) Plant carotenoids: pigments for photoprotection, visual attraction, and human health. Plant Cell 7:1027–1038
143. Breithaupt DE, Bamedi A (2001) Carotenoid esters in vegetables and fruits: a screening with emphasis on β-cryptoxanthin esters. J Agric Food Chem 49:2064–2070

144. Weller P, Breithaupt DE (2003) Identification and quantification of zeaxanthin esters in plants using liquid chromatrography-mass spectrometry. J Agric Food Chem 51:7044–7049
145. Inbaraj BS, Lu H, Hung CF, Wu WB, Lin CL, Chen BH (2008) Determination of carotenoids and their esters in fruits of *Lycium barbarum* Linnaeus by HPLC-DAD-APCI-MS. J Pharm Biomed Anal 47:812–818
146. Mertz C, Gancel A-L, Gunata Z, Alter P, Dhuique-Mayer C, Vaillant F, Perez AM, Ruales J, Brat P (2009) Phenolic compounds, carotenoids and antioxidant capacity of three tropical fruits. J Food Compos Anal 22:381–387
147. Delgado-Pelayo R, Hornero-Méndez D (2012) Identification and quantitative analysis of carotenoids and their esters from sarsaparilla (*Smilax aspera* L.) berries. J Agric Food Chem 60:8225–8232
148. Gross J (1987) Pigments in fruits. Academic, London
149. Kobori CN, Rodriguez-Amaya DB (2008) Uncultivated Brazilian green leaves are richer sources of carotenoids than commercially produced leafy vegetables. Food Nutr Bull 29:333–341
150. De Oliveira GPR, Rodriguez-Amaya DB (2007) Processed and prepared products of corn as sources of lutein and zeaxanthin. Compositional variation in the food chain. J Food Sci 72: S79–S85
151. Rodriguez-Amaya DB, Kimura M (2004) HarvestPlus handbook for carotenoid analysis. International Food Policy Research Institute, Washington, DC
152. Niizu PY, Rodriguez-Amaya DB (2005) The flowers and leaves of *Tropaeolum majus* as rich sources of lutein. J Food Sci 70:S605–S609
153. Breithaupt D, Wirt U, Bamedi A (2002) Differentiation between lutein monoester regioisomers and detection of lutein diesters from marigold flowers (*Tagetes erecta* L.) and several fruits by liquid chromatography-mass spectrometry. J Agric Food Chem 50:66–70
154. Schweiggert U, Kurz C, Schieber A, Carle R (2007) Effects of processing and storage on the stability of free and esterified carotenoids of red peppers (*Capsicum annuum* L) and hot chili peppers (*Capsicum frutescens* L.) Eur Food Res Technol 225:261–270
155. Shahidi F, Metusalach, Brown JA (1998) Carotenoid pigments in seafoods and aquaculture. Crit Rev Food Sci Nutr 38:1–67
156. Liaaen-Jensen S (2004) Basic carotenoid chemistry. In: Krinsky NI, Mayne ST, Sies H (eds) Carotenoids in health and disease. Marcel Dekker, New York
157. Weedon BCL, Moss GP (1995) Structure and nomenclature. In: Britton G, Liaaen-Jensen S, Pfander H (eds) Carotenoids vol. 1A, isolation and analysis. Birkhaüser Verlag, Basel
158. Lessin WJ, Catigani GL, Schwartz SJ (1997) Quantification of cis-trans isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. J Agric Food Chem 45:3728–3732
159. Marx M, Schieber A, Carle R (2000) Quantitative determination of carotene stereoisomer in carrot juices and vitamin supplemented (ATBC) drinks. Food Chem 70:403–408
160. Dachtler M, Glaser T, Kohler K, Albert K (2001) Combined HPLC-MS and HPLC-NMR on-line coupling for the separation and determination of lutein and zeaxanthin stereoisomers in spinach and in retina. Anal Chem 73:667–674
161. Humphries JM, Khachick F (2003) Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. J Agric Food Chem 51:1322–1327
162. Updike AA, Schwartz SJ (2003) Thermal processing of vegetables increases cis isomers of lutein and zeaxanthin. J Agric Food Chem 51:6184–6190
163. Aman R, Biehl J, Carle R, Conrad J, Beifuss U, Schieber A (2005) Application of HPLC coupled with DAD, APcI-MS and NMR to the analysis of lutein and zeaxanthin stereoisomers in thermally processed vegetables. Food Chem 92:753–763
164. Schierle J, Bretzel W, Bühler I, Faccin N, Hess D, Steiner K, Schüep W (1997) Content and isomeric ratio of lycopene in food and human blood plasma. Food Chem 59:459–465
165. Tiziani S, Schwartz SJ, Vodovotz Y (2006) Profiling of carotenoids in tomato juice by one- and two-dimensional NMR. J Agric Food Chem 54:6094–6100

166. Li H, Deng Z, Liu R, Loewen S, Tsao R (2012) Ultra-performance liquid chromatographic separation of geometric isomers of carotenoids and antioxidant activities of 20 tomato cultivars and breeding lines. Food Chem 132:508–517
167. Stinco CM, Rodríguez-Pulido FJ, Escudero-Gilete ML, Gordillo B, Vicario IM, Meléndez-Martínez AJ (2013) Lycopene isomers in fresh and processed tomato products: correlations with instrumental color measurements by digital image analysis and spectroradiometry. Food Res Int 50:111–120
168. Achir N, Randrianatoandro VA, Bohuon P, Laffargue A, Avallone S (2010) Kinetic study of β-carotene and lutein degradation in oils during heat treatment. Eur J Lipid Sci Technol 112:349–361
169. Holden JM, Eldridge AL, Beecher GR, Buzzard IM, Bhagwat S, Davis CS, Douglass LW, Gebhardt S, Haytowitz D, Schakel S (1999) Carotenoid content of US foods: an update of the database. J Food Compos Anal 12:169–196
170. Murkovic M, Gams K, Draxl S, Pfannhauser W (2000) Development of an Austrian carotenoid database. J Food Compos Anal 13:435–440
171. Furtado JD, Siles X, Campos H (2004) Carotenoid concentrations in vegetables and fruits common to the Costa Rican diet. Int J Food Sci Nutr 55:101–113
172. Reif C, Arrigoni E, Schärer H, Nyström L, Hurrell RF (2013) Carotenoid database of commonly eaten Swiss vegetables and their estimated contribution to carotenoid intake. J Food Compos Anal 29:64–72
173. Rodriguez-Amaya DB, Kimura M, Godoy HT, Amaya-Farfan J (2008) Updated Brazilian database on food carotenoids: factors affecting carotenoid composition. J Food Compos Anal 21:445–463
174. Rodriguez-Amaya DB (2016) Food carotenoids: chemistry, biology and technology. IFT Press/Wiley, Oxford
175. Ishida BK, Turner C, Chapman MH, McKeon TA (2004) Fatty acid and carotenoid composition of Gac (*Momordica cochinchinensis* Spreng) fruit. J Agric Food Chem 52:274–279
176. Vuong LT, Franke AA, Custer LJ, Murphy SP (2006) *Momordica chochinchinensis* Spreng. (gac) fruit carotenoids reevaluated. J Food Compos Anal 19:664–668
177. Gross J (1991) Pigments in vegetables. Chlorophylls and carotenoids. Avi Van Nostrand Reinhold, New York
178. Rodriguez-Amaya DB, Amaya-Farfan J, Rodriguez EB (2008a) Carotenoids in fruits: biology, chemistry, technology and health benefits. In: Francesco E (ed) Trends in phytochemistry. Research Signpost, Kerala
179. Maiani G, Castón MJP, Catasta G, Toti E, Cambrodón IG, Bysted A, Granado-Lorencio F, Olmedilla-Alonso B, Knuthsen P, Valoti M, Böhm V, Mayer-Miebach E, Behsnilian D, Schlemmer U (2009) Carotenoids: actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Mol Nutr Food Res 53:S194–S218
180. Rodriguez-Amaya DB (1997) Carotenoids and food preparation: the retention of provitamin A carotenoids in prepared, processed, and stored foods. Opportunities for Micronutrient Intervention (OMNI), Arlington
181. Rodriguez-Amaya DB (1999b) Changes in carotenoids during processing and storage of foods. Arch Latinoam Nutr 49:38S–47S
182. Xianquan S, Shi J, Kakuda Y, Yueming J (2005) Stability of lycopene during food processing and storage. J Med Food 8:413–422
183. Hager TJ, Howard LR (2006) Processing effects on carrot phytonutrients. HortSci 41:74–79
184. Shi I, le Maguer M (2000) Lycopene in tomatoes: chemical and physical properties affected by food processing. Crit Rev Food Sci Nutr 40:1–42
185. Pénicaud C, Archir N, Dhuique-Mayer C, Dornier M, Bohuon P (2011) Degradation of β-carotene during fruit and vegetable processing or storage: reaction mechanisms and kinetic aspects: a review. Fruits 66:417–440
186. Nguyen ML, Schwartz SJ (1998) Lycopene stability during food processing. Exp Biol Med 218:101–105

187. Namitha KK, Negi PS (2010) Chemistry and biotechnology of carotenoids. Crit Rev Food Sci Nutr 50:728–760
188. Marx M, Stuparic M, Schieber A, Carle R (2003) Effects of thermal processing on trans-cis-isomerization of β-carotene in carrot juice and carotene-containing preparations. Food Chem 83:609–617
189. Shi J, le Maguer M, Bryan M, Kakuda Y (2003) Kinetics of lycopene degradation in tomato puree by heat and light irradiation. J Food Process Eng 25:485–498
190. Seybold C, Fröhlich K, Bitsch R, Otto K, Böhm V (2004) Changes in contents of carotenoids and vitamin E during tomato processing. J Agric Food Chem 52:7005–7010
191. Mayer-Miebach E, Behsnilian D, Regier M, Schuchmann HP (2005) Thermal processing of carrots: lycopene stability and isomerization with regard to antioxidant potential. Food Res Int 38:1103–1108
192. Vásquez-Caicedo AL, Schilling S, Carle R, Neidhart S (2007) Effects of thermal processing and fruit matrix on β-carotene stability and enzyme inactivation during transformation of mangoes into purée and nectar. Food Chem 102:1172–1186
193. Imsic M, Winkler S, Tomkins B, Jones R (2010) Effect of storage and cooking on β-carotene isomers in carrots (*Daucus carota* L. cv. 'Stefano'). J Agric Food Chem 58: 5109–5113
194. Cervantes-Paz B, Yahia EM, Ornelas-Paz JJ, Victoria-Campos CI, Ibarra-Junquera V, Pérez-Martínez JD, Escalante-Minakata P (2014) Antioxidant activity and content of chlorophylls and carotenoids in raw and heat-processed Jalapeño peppers at intermediate stages of ripening. Food Chem 146:188–196
195. Knockaert G, Pulissery SK, Colle I, van Buggenhout S, Hendrickx M, van Loey A (2012) Lycopene degradation, isomerization and in vitro bioaccessibility in high pressure homogenized tomato puree containing oil: effect of additional thermal and high pressure processing. Food Chem 135:1290–1297
196. Nguyen M, Francis D, Schwartz S (2001) Thermal isomerization susceptibility of carotenoids in different tomato varieties. J Sci Food Agric 81:910–917
197. Rubio-Diaz DE, Santos A, Francis DM, Rodriguez-Saona LE (2010) Carotenoid stability during production and storage of tomato juice made from tomatoes with diverse pigment profiles measured by infrared spectroscopy. J Agric Food Chem 58:8692–8698
198. Schieber A, Carle R (2005) Occurrence of carotenoid cis-isomers in food: technological, analytical, and nutritional implications. Trends Food Sci Technol 16:416–422
199. Conn PF, Schalch W, Truscott TG (1991) The singlet oxygen and carotenoid interaction. J Photochem Photobiol B 11:41–47
200. Stahl W, Sies H (1993) Physical quenching of singlet oxygen and cis-trans isomerization of carotenids. Ann N Y Acad Sci 691:10–19
201. Rodriguez EB, Rodriguez-Amaya DB (2007) Formation of apocarotenals and epoxycarotenoids from β-carotene by chemical reactions and by autoxidation in model systems and processed foods. Food Chem 101:563–572
202. Rodriguez EB, Rodríguez-Amaya DB (2009) Lycopene epoxides and apo-lycopenals formed by chemical reactions and autoxidation in model systems and processed foods. J Food Sci 74: C674–C682
203. Marty C, Berset C (1988) Degradation products of trans-β-carotene produced during extrusion cooking. J Food Sci 53:1880–1886
204. Marty C, Berset C (1990) Factors affecting the thermal degradation of all-trans-β-carotene. J Agric Food Chem 38:1063–1067
205. Marty C, Berset C (1986) Degradation of trans-β-carotene during heating in sealed glass tubes and extrusion cooking. J Food Sci 51:698–702
206. Henry LK, Puspitasari-Nienabe NL, Jarén-Galán M, van Breemen RB, Castignani GL, Schwartz SJ (2000) Effects of ozone and oxygen on the degradation of carotenoids in an aqueous system. J Agric Food Chem 48:5008–5013

207. Kanasawud P, Crouzet JC (1990) Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium. 1. β-carotene degradation. J Agric Food Chem 38:237–243
208. Khachik F, Steck A, Niggli UA, Pfander H (1998) Partial synthesis and structural elucidation of the oxidative metabolites of lycopene identified in tomato paste, tomato juice, and human serum. J Agric Food Chem 46:4874–4884
209. Khachik F, Pfander H, Traber B (1998) Proposed mechanisms for the formation of synthetic and naturally occurring metabolites of lycopene in tomato products and human serum. J Agric Food Chem 46:4885–4890
210. Mercadante AZ, Rodriguez-Amaya DB (1998) Influence of ripening, cultivar differences, and processing on the carotenoid composition of mango. J Agric Food Chem 46:128–130
211. Cano MP, de Ancos B (1994) Carotenoid and carotenoid ester composition in mango fruit as influenced by processing method. J Agric Food Chem 42:2737–2742
212. Lee HS, Coates GA (2003) Effect of thermal pasteurization on Valencia orange juice color and pigments. Lebensm-Wiss Technol 36:153–156
213. Dhuique-Mayer C, Tbatou M, Carail M, Caris-Veyrat C, Dornier M, Amiot MJ (2007) Thermal degradation of antioxidant micronutrients in citrus juice: kinetics and newly formed components. J Agric Food Chem 55:4209–4216
214. Hadjal T, Dhuique-Mayer C, Madani K, Dornier M, Achir N (2013) Thermal degradation kinetics of xanthophylls from blood orange in model and real food systems. Food Chem 138:2442–2450
215. Kopec RE, Riedl KM, Harrison EH, Curley RW Jr, Hruszkewycz DP, Clinton SK, Schwartz SJ (2010) Identification and quantification of apo-lycopenals in fruits, vegetables, and human plasma. J Agric Food Chem 58:3290–3296
216. Zeb A, Murkovic M (2013) Determination of thermal oxidation and oxidation products of β-carotene in corn triacylglycerols. Food Res Int 50:534–544
217. Zepka LQ, Mercadante AZ (2009) Degradation compounds of carotenoids formed during heating of a simulated cashew apple juice. Food Chem 117:28–34
218. Kanasawud P, Crouzet JC (1990) Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium. 2. Lycopene degradation. J Agric Food Chem 38:1238–1242
219. Caris-Veyrat C, Schmid A, Carail M, Bohm V (2003) Cleavage products of lycopene produced by in vitro oxidations: characterization and mechanisms of formation. J Agric Food Chem 51:7318–7732
220. Rios JJ, Fernández-García E, Mínguez-Mosquera MI, Pérez-Gálvez A (2008) Description of volatile compounds generated by the degradation of carotenoids in paprika, tomato and marigold oleoresins. Food Chem 106:1145–1153
221. Kobori CN, Wagner R, Padula M, Rodriguez-Amaya DB (2014) Formation of volatile compounds from lycopene by autoxidation in a model system simulating dehydrated foods. Food Res Int 63(Part A):49–54
222. Coria-Cayupán YS, de Pinto MIS, Nazareno MA (2009) Variations in bioactive substance contents and crop yields of lettuce (*Lactuca sativa* L.) cultivated in soils with different fertilization treatments. J Agric Food Chem 57:10122–10129
223. Znidarcic D, Ban D, Sircelj H (2011) Carotenoid and chlorophyll composition of commonly consumed leafy vegetables in Mediterranean countries. Food Chem 129: 1164–1116
224. Acosta-Quezada PG, Raigón MD, Riofrío-Cuenca T, García-Martínez MD, Plazas M, Burneo JI, Figueroa JG, Vilanova S, Prohens J (2015) Diversity for chemical composition in a collection of different varietal types of tree tomato (*Solanum betaceum* Cav.), an Andean exotic fruit. Food Chem 169:327–335
225. Schwartz SJ, Lorenzo TV (1990) Chlorophyll in foods. Crit Rev Food Sci Nutr 29:1–17
226. Heaton JW, Marangoni AG (1996) Chlorophyll degradation in processed foods and senescent plant tissues. Trends Food Sci Technol 7:8–15

227. Schwartz SJ, Woo SL, von Elbe JH (1981) High-performance liquid chromatography of chlorophylls and their derivatives in fresh and processed spinach. J Agric Food Chem 29:533–535
228. Watanabe T, Nakazato M, Mazaki H, Hongu A, Konno M, Saitoh S, Honda K (1985) Chlorophyll a epimer and pheophytin a in green leaves. Biochim Biophys Acta 807:110–117
229. López-Ayerra B, Murcia MA, Garcia-Carmona F (1998) Lipid peroxidation and chlorophyll levels in spinach during refrigerated storage and after industrial processing. Food Chem 61:113–118
230. Murcia MA, López-Ayerra B, Martínez-Tomé M, García-Carmona F (2000) Effect of industrial processing on chlorophyll content of broccoli. J Sci Food Agric 80:1447–1451
231. Turkmen N, Poyrazoglu ES, Sari F, Sedat Velioglu Y (2006) Effects of cooking methods on chlorophylls, pheophytins and colour of selected green vegetables. Int J Food Sci Technol 41:281–288
232. Schwartz SJ, von Elbe JH (1983b) Kinetics of chlorophyll degradation to pyropheophytin in vegetables. J Food Sci 48:1303–1306
233. Canjura FL, Schwartz SJ, Nunes RV (1991) Degradation kinetics of chlorophylls and chlorophyllides. J Food Sci 56:1639–1643
234. Koca N, Karadeniz F, Burdurlu HS (2006) Effect of pH on chlorophyll degradation and colour loss in blanched green peas. Food Chem 100:609–615
235. Belitz HI, Grosch W (1987) Vegetables and their products. Food chemistry (trans: Hadziyev D). Springer, Berlin
236. Matile P, Hörtensteiner S, Thomas H (1999) Chlorophyll degradation. Annu Rev Plant Physiol Plant Mol Biol 50:67–95
237. Takamiya K-I, Tsuchiya T, Ohta H (2000) Degradation pathway(s) of chlorophyll: what has gene cloning revealed? Trends Plant Sci 5:426–431
238. Vergara-Domínguez H, Rios JJ, Gandul-Rojas B, Roca M (2016) Chlorophyll catabolism in olive fruits (var. Arbequina and Hojiblanca) during maturation. Food Chem 212:604–611
239. von Elbe JH, Huang AS, Attoe EL, Nank WK (1986) Pigment composition and color of conventional and Veri-Green canned beans. J Agric Food Chem 34:52–54
240. Gaur S, Shivhare U, Ahmed J (2006) Degradation of chlorophyll during processing of green vegetables a review. Stewart Postharvest Rev 5:14
241. Tapiero H, Townsend DM, Tew KD (2004) The role of carotenoids in the prevention of human pathologies. Biomed Pharmacother 58:100–110
242. Krinsky NI, Johnson EJ (2005) Carotenoid actions and their relation to health and disease. Mol Asp Med 26:459–516
243. Voutilainen S, Nurmi T, Mursu J, Rissanen TH (2006) Carotenoids and cardiovascular health. Am J Clin Nutr 83:1265–1271
244. Rao AV, Rao LG (2007) Carotenoids and human health. Pharmacol Res 55:207–216
245. Riccioni G (2009) Carotenoids and cardiovascular disease. Curr Atheroscler Rep 11:434–439
246. Krinsky NI (2001) Carotenoids as antioxidants. Nutrition 17:815–817
247. Kiokias S, Gordon MH (2004) Antioxidant properties of carotenoids *in vitro* and *in vivo*. Food Rev Int 20:99–121
248. Stahl W, Sies H (2003) Antioxidant activity of carotenoids. Mol Asp Med 24:345–351
249. Stahl W, Ale-Agha N, Polidori MC (2002) Non-antioxidant properties of carotenoids. Biol Chem 383:553–558
250. Pan M-H, Lai C-S, Dushenkov S, Ho C-T (2009) Modulation of inflammatory genes by natural dietary bioactive compounds. J Agric Food Chem 57:4467–4477
251. Agarwal S, Rao AV (2000) Tomato lycopene and its role in human health and chronic diseases. Can Med Assoc J 163:739–744
252. Agarwal M, Parameswari RP, Vasanthi HR, Das DK (2012) Dynamic action of carotenoids in cardioprotection and maintenance of cardiac health. Molecules 17:4755–4769
253. Giovannucci E (2002a) A review of epidemiologic studies of tomatoes, lycopene and prostate cancer. Exp Biol Med 227:852–859

254. Rao AV, Rao LG (2004) Lycopene and human health. Curr Top Nutr Res 2:127–136
255. Singh P, Goyal GK (2008) Dietary lycopene: its properties and anticarcinogenic effects. Comp Rev Food Sci Food Saf 7:255–270
256. Sharoni Y, Linnewiel-Hermoni K, Zango G, Khanin M, Salman H, Veprik A, Danilenko M, Levy J (2012) The role of lycopene and its derivatives in the regulation of transcription systems: implications for cancer prevention. Am J Clin Nutr 96: 1173–1178S
257. Hadley CW, Miller EC, Schwartz SJ, Clinton SK (2002) Tomatoes, lycopene, and prostate cancer: progress and promise. Exp Biol Med 227:869–880
258. Miller EC, Giovannucci E, Erdman JW Jr, Bahnson R, Schwartz SJ, Clinton S (2002) Tomato products, lycopene and prostate cancer risk. Urol Clin N Am 29:83–93
259. Wertz K, Siler U, Goralczyk R (2004) Lycopene: modes of action to promote prostate health. Arch Biochem Biophys 430:127–134
260. Stacewicz-Sapuntzakis M, Bowen PE (2005) Role of lycopene and tomato products in prostate health. Biochim Biophys Acta 1740:202–205
261. Ito Y, Wakai K, Suzuki K, Tamakoshi A, Seki N, Ando M, Nishino Y, Kondo T, Watanabe Y, Ozasa K, Ohno Y, for the JACC Study Group (2003) Serum carotenoids and mortality from lung cancer: a case-control study nested in the Japan Collaborative Cohort (JACC) Study. Cancer Sci 94:57–63
262. Nkondjock A, Ghadirian P, Johnson KC, Krewski D, the Canadian Cancer Registries Epidemiology Research Group (2005) Dietary intake of lycopene is associated with reduced pancreatic cancer risk. J Nutr 135:592–597
263. Erhardt JG, Meisner C, Bode JC, Bode C (2003) Lycopene, β-carotene, and colorectal adenomas. Am J Clin Nutr 78:1219–1224
264. Franceschi S, Bidioli E, La Vecchia C, Talamini R, D'Avanzo B, Negri E (1994) Tomatoes and risk of digestive-tract cancers. Int J Cancer 59:181–184
265. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE (2001) Macular pigment in donor eyes with and without AMD: a case-control study. Invest Ophthalmol Vis Sci 42:235–240
266. Landrum JT, Bone RA (2001) Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys 385:28–40
267. Moeller SM, Jacques PF, Blumberg JB (2000) The potential role of dietary xanthophylls in cataract and age-related macular degeneration. J Am Coll Nutr 19:522S–527S
268. Moeller SM, Parekh N, Tinker L, Ritrenbaugh C, Blodi B, Wallace RB, Mares JA (2006) Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the Carotenoids in Age-related Eye Disease Study (CAREDS): ancillary study of the Women's Health Initiative. Arch Opthalmol 124:1151–1162
269. Gale CR, Hall NF, Phillips DIW, Martyn CN (2003) Lutein and zeaxanthin status and risk of age-related macular degeneration. Invest Opthalmol Vis Sci 44:2461–2465
270. Delcourt C, Carriere I, Delage M, Barbenger-Gateau P, Schalch W (2006) Plasma lutein and zeaxanthin and other carotenoids as modifiable risk factors for age-related maculopathy and cataract; the POLA Study. Invest Opthalmol Vis Sci 47:2329–2335
271. Tan JSL, Wang JJ, Flood V, Rochtchina E, Smith W, Mitchell P (2008) Dietary antioxidants and the long-term incidence of age-related macular degeneration – The Blue Mountain Eye Study. Opthalmology 115:334–341
272. Carpentier S, Knaus M, Suh M (2009) Associations between lutein, zeaxanthin, and age-related macular degeneration. Crit Rev Food Sci Nutr 49:313–326
273. Gale CR, Hall NF, Phillips DIW, Martyn CN (2001) Plasma antioxidant vitamins and carotenoids and age-related cataract. Ophthalmology 108:1992–1998
274. Dherani M, Murthy GVS, Gupta SK, Young IS, Maraini G, Camparini M, Price GM, John N, Chakravarthy U, Fletcher AE (2008) Blood levels of vitamin C, carotenoids and retinol are inversely associated with cataract in a North Indian population. Invest Ophthalmol Vis Sci 49:3328–3335

275. Ma L, Dou H-L, Wu Y-Q, Huang Y-M, Huang Y-B, Xu X-R, Zou Z-Y, Lin X-M (2012) Lutein and zeaxanthin intake and the risk of age-related macular degeneration: a systematic review and meta-analysis. Br J Nutr 107:350–359
276. Olmedilla B, Granado F, Blanco I, Vaquero M, Cajigal C (2001) Lutein in patients with cataracts and age-related macular degeneration: a long-term supplementation study. J Sci Food Agric 81:904–909
277. Olmedilla B, Granado F, Blanco I, Vaquero M (2003) Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-year double-blind, placebo-controlled pilot study. Nutrition 19:21–24
278. Krinsky NI, Landrum JT, Bone RA (2003) Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr 23:171–201
279. Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, Shircore A, Hama-Levy S, Hough G, Wang X, Drake T, Merz CNB, Fogelman AM (2001) Oxygenated carotenoid lutein and progression of early atherosclerosis: The Los Angeles Atherosclerosis study. Circulation 103:2922–2927
280. Xu X-R, Zou Z-Y, Huang Y-M, Xiao X, Ma L, Lin X-M (2012) Serum carotenoids in relation to risk factors for the development of atherosclerosis. Clin Biochem 45:1357–1361
281. Karppi J, Kurl S, Mäkikallioi TH, Ronkainen K, Laukkanen JA (2013) Serum β-carotene concentrations and the risk of congestive heart failure in men: a population-based study. Int J Cardiol 168:1841–1846
282. Connor SL, Ojeda LS, Sexton G, Weidner G, Connor WE (2004) Diets lower in folic acid and carotenoids are associated with coronary disease epidemic in central and eastern Europe. J Am Diet Assoc 104:1793–1799
283. Ghosh D, Konishi T (2007) Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. Asia Pac J Clin Nutr 16:200–208
284. Pojer E, Mattivi F, Johnson D, Stockley CS (2013) The case for anthocyanin consumption to promote human health: a review. Compr Rev Food Sci Food Saf 12:483–508
285. Gowd V, Jia Z, Chen W (2017) Anthocyanins as promising molecules and dietary bioactive components against diabetes – a review of recent advances. Trends Food Sci Technol 68:1–13
286. Hou D-X (2003) Potential mechanisms of cancer chemoprevention by anthocyanin. Curr Mol Med 3:149–159
287. Wang LS, Stoner GD (2008) Anthocyanins and their role in cancer prevention. Cancer Lett 269:281–290
288. Li D, Wang P, Luo Y, Zhao M, Chen F (2017) Health benefits of anthocyanins and molecular mechanisms: update from recent decade. Crit Rev Food Sci Nutr 57:1729–1741
289. Wallace TC (2011) Anthocyanins in cardiovascular disease. Adv Nutr 2:1–7
290. Tesoriere L, Allegra M, Butera D, Livrea MA (2004) Absorption, excretion, and distribution of dietary antioxidant betalains in LDLs: potential health effects of betalains in humans. Am J Clin Nutr 80:941–945
291. Clifford T, Howatson G, West DJ, Stevenson EJ (2015) The potential benefits of red beetroot supplementation in heath and disease. Forum Nutr 7:2801–2822
292. Gengatharan A, Dykes GA, Cho WS (2015) Betalains: Natural plant pigments with potential application in functional foods. LWT- Food Sci Technol 64:645–649
293. Balder HF, Vogel J, Jansen MC, Weijenberg MP, van den Brandt PA, Westenbrink S, van der Meer R, Goldbohm RA (2006) Heme and chlorophyll intake and risk of colorectal cancer in the Netherlands cohort study. Cancer Epidemiol Biomark Prev 15:717–725
294. Dashwood RH (1997) Chlorophylls as anticarcinogens. Int J Oncol 10:721–727
295. Tajmir-Riahi HA, Neault JF, Diamantoglou S (2004) DNA adducts with chlorophyll and chlorophyllin as antimutagenic agents: synthesis, stability, and structural features. Methods Mol Biol 274:159–171
296. De Vogel J, Jonker-Termont DS, van Lieshout EM, Katan MB, van der Meer R (2005) Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon. Carcinogenesis 26:387–393

Lycopene: Metabolism and Functional Aspects

31

Soma Srivastava

Contents

Abstract

Lycopene is the most abundant carotenoid found in human serum and has been recognized as the most effective antioxidant among all the carotenoids. Lycopene has 11 conjugated double bonds in its structure. However, lycopene occurs naturally in the all trans form in the dietary sources, found in as many as 18 different isometric forms in human serum and prostate cells mostly in cis form. However, the absolute concentrations of individual carotenoids within specific lipoprotein classes have not been reported; relative distribution of β-carotene, α-carotene, and lycopene among the very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) was similar, with 58–73% in LDL, 17–26% in HDL, and 10–16% in VLDL when separated by conventional sequential flotation ultracentrifugation and quantified by high-performance liquid chromatography. Lycopene has also been found helpful in

S. Srivastava (✉)
Division of Agricultural Engineering and Renewable Energy,
Central Arid Zone Research Institute, Jodhpur, India
e-mail: soma.sriv8@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_92

elimination of xenobiotics through stool or urine. Lycopene alters hormone and growth factor signalling including IFG-1 (31.5% decrease in serum IFG-1 levels) which is associated with cell proliferation. However its stability is a critical factor for its functional aspects. Physical and chemical factors like elevated temperature, exposure to oxygen and light, metallic ions (e.g., $Cu2^{+}$ and $Fe3^{+}$), extreme in pH, and active surfaces affect its stability.

Keywords
Lycopene · Metabolism · Functional · Antioxidant · Carotenoids

Abbreviations

BV	Biological value
CDP-ME	Methylerythritol cytidyl diphosphate
CDP-MEP	4-Diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate
CTP	Cytidine 5′-triphosphate
DMAPP	Dimethylallyl pyrophosphate
DXR/IspC	DXP reductoisomerase
HDL	High-density lipoprotein
HMBPP	4-Hydroxy-3-methyl-butenyl 1-diphosphate
IFG-1	Growth factor-1
IPP	Isopentenyl pyrophosphate
IspD	CDP-ME synthetase
LDL	Low-density lipoprotein
LOOHs	Hydroperoxides
MEcPP	2-C-Methyl-D-erythritol-2,4-cyclodiphosphate
MEP	2C-methyl-D-erythritol 4-phosphate
MVA	Mevalonic acid
PUFAs	Polyunsaturated fatty acids
VLDL	Very low-density lipoprotein

1 Introduction

Fruits and vegetables play a significant role in human nutrition, especially as sources of vitamins, minerals, and dietary fiber. Tomatoes are important for both, its large consumption and richness in health-related food components. It is one of the most versatile vegetable crops, ranking second around the world after potato [1]. Lycopene, the carotenoid pigment responsible for the red color, is the most distinctive compound present in tomatoes and has been recognized as the most effective antioxidant among the carotenoids. The unique quality about the composition of tomatoes and tomato products with respect to other fruits and vegetables is their high content of lycopene, the acyclic carotenoid containing 11 conjugated double bonds. There is a small amount of lycopene in few other fruits such as watermelon, pink guava, pink grapefruit, strawberry, and papaya, but tomato products are the major source of lycopene in human diet. Varietal effect is also prominent, and lycopene

content depends upon the variety, climatic conditions, and harvesting stage of tomato.

In addition to lycopene, violaxanthin, neoxanthin, lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, α-carotene, β-carotene, γ-carotene, neurosporene, phytoene, phytofluene, cyclolycopene, b-carotene 5, and 6-epoxide are other carotenoids commonly cited in tomato and tomato-derived products [2]. Lycopene is the most abundant carotenoid found in human serum and, therefore, most important in terms of net antioxidant activity [3]. However, lycopene occurs in all *trans* form in dietary sources; it is always present in *cis* form in human tissues. The reason for particular interest in lycopene is its pharmacological effect and potential use in prevention of various metabolic diseases. Therefore, it is now regarded as a neutraceutical or functional food ingredient.

2 Biosynthesis of Lycopene

Lycopene belongs to the carotenoid family which are the red, yellow, and orange pigments found in dietary sources principally in fruits and vegetables. It was first isolated by Millardet in 1876 who called it "solanorubine" [4]. It was again isolated by Schunck in 1903 in pure form and named as "lycopene" [4]. It has an aliphatic hydrocarbon chain with 13 double bonds in its structure, and the molecular formula is $C_{40}H_{56}$ (Fig. 1). Lycopene has 11 conjugated double bonds in its structure, which makes possible to assume 2048 geometrical configurations theoretically. However naturally only 72 *cis* isomers of lycopene are found and are structurally favorable ingredients. Scientists are conducting clinical trials to know the exact pharmacokinetics of lycopene in the human body.

Carotenoid synthesis in plants occurs by various biosynthetic precursors naturally found in plants. It is basically de novo synthesis of carotenoids in plants which is affected by the presence of these biosynthetic precursrs. Maturation or ripening in fruits and fruit vegetables is generally accompanied by enhanced "carotenogenesis," the carotenoids increasing markedly both in number and quantity. Exposure to sunlight and high temperature enhance carotenoid biosynthesis, so the tropical foods are generally colored by carotenoids contrary to fruits of colder regions that are mostly colored due to anthocyanins [5, 6].

Isopentenyl pyrophosphate (IPP), an isoprenoid, is the biological precursor for all carotenoids including lycopene. Isopentenyl pyrophosphate is synthesized from acetyl-coenzyme A by a well-established pathway via mevalonic acid (MVA) (Fig. 2). Isomerized form of IPP which is known as dimethylallyl pyrophosphate (DMAPP) acts as a starter molecule for chain elongation. When the DMAPP is produced after isomerization of IPP, it again condenses with a molecule of IPP to form geranyl pyrophosphate (C_{10}) which further condenses with a molecule of IPP to form farnesyl pyrophosphate (C_{15}) and finally produces geranylgeranyl pyrophosphate (C_{20}) by similar elongation. The presence of enzymes which influence the chain elongation is not clear, but the mevalonic kinase and 5-phosphokinase which are present in the chloroplast fragments are

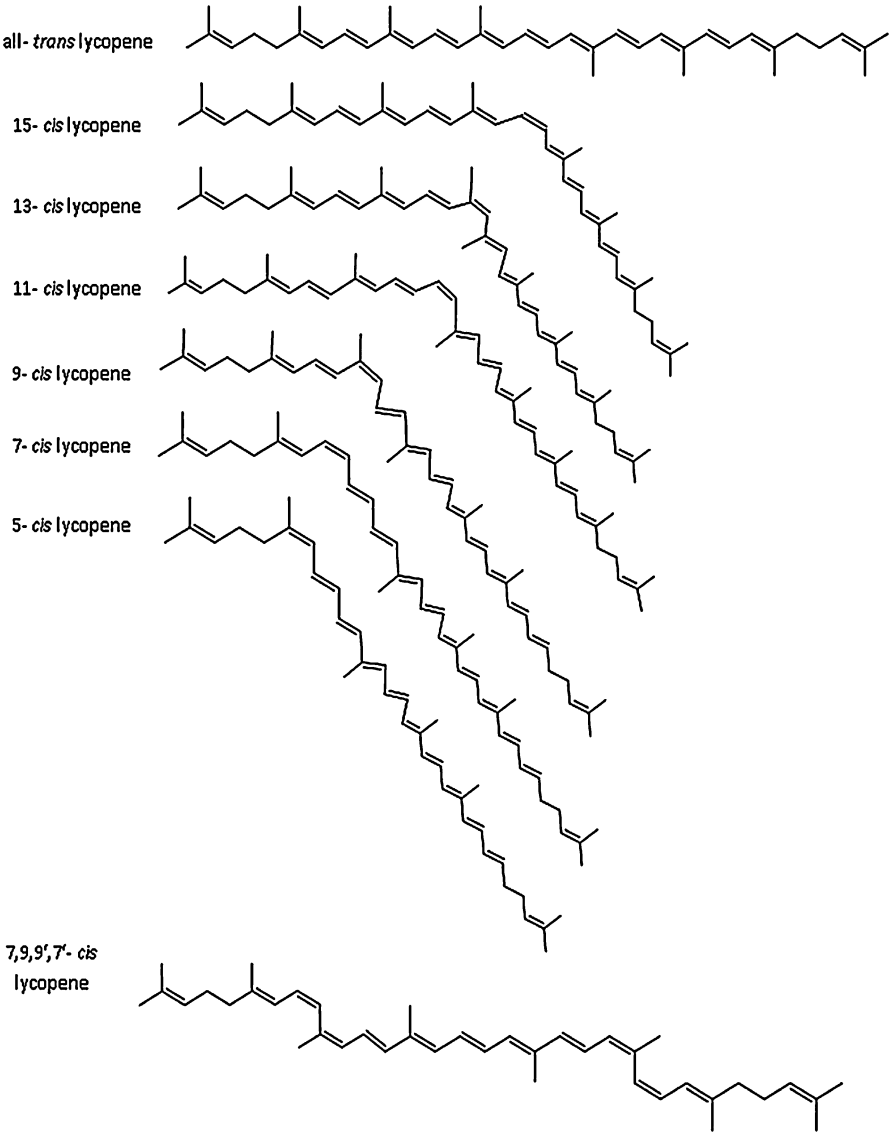


Fig. 1 Common lycopene isomers

believed to be the core enzymes which affect this chain elongation process [7]. Phytoene which is the first (C_{40}) compound is believed to be a carotenoid precursor. Phytoene is stepwise dehydrogenated to phytofluene, ζ-carotene, and neurosporene (Fig. 3). Cyclization begins at neurosporene level, and it leads to the formation of various cyclic carotenes including lycopene (Fig. 4).

During postharvest transport or storage, carotenoid biosynthesis may continue, raising the carotenoid content, provided that the fruit, vegetable,

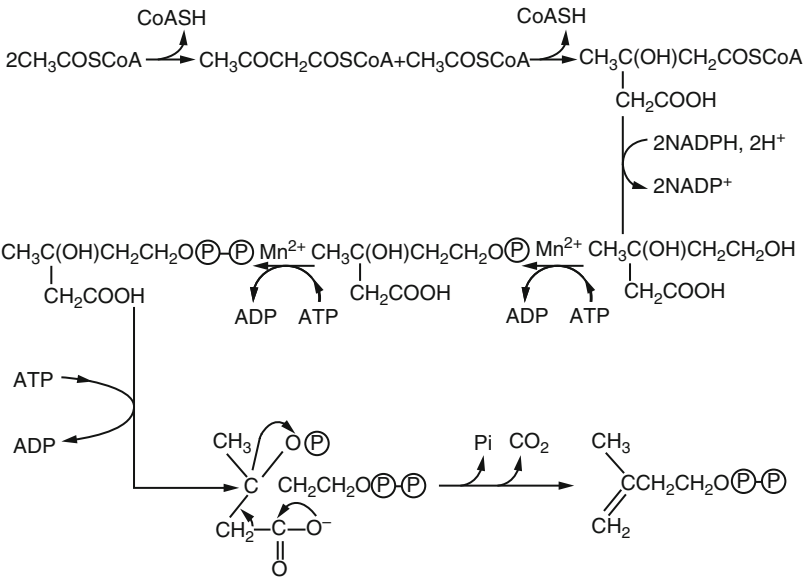


Fig. 2 Conversion of acetyl-CoA into IPP

or root is kept intact, preserving the enzyme system responsible for "carotenogenesis" [6].

For decades, the MVA pathway was thought to be the only pathway for the biosynthesis of IPP and DMAPP. However, the 2C-methyl-D-erythritol 4-phosphate (MEP) pathway was discovered in the 1990s [8–10]. This pathway is initiated with a thiamin diphosphate-dependent condensation between D-glyceraldehyde 3-phosphate and pyruvate to produce DXP which is then reductively isomerized to MEP by DXP reductoisomerase (DXR/IspC). Subsequent coupling between MEP and cytidine 5′-triphosphate (CTP) is catalyzed by CDP-ME synthetase (IspD) and produces methylerythritol cytidyl diphosphate (CDP-ME). An ATP-dependent enzyme (IspE) phosphorylates the C2 hydroxyl group of methylerythritol cytidyl diphosphate, and the resulting 4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate (CDP-MEP) is cyclized by IspF to 2-C-methyl-D-erythritol-2,4-cyclodiphosphate (MEcPP). IspG catalyzes the ring opening of the cyclic pyrophosphate and the C3-reductive dehydration of MEcPP to 4-hydroxy-3-methyl-butenyl 1-diphosphate (HMBPP). The final step of the MEP pathway is catalyzed by IspH and converts (HMBPP) to both IPP and DMAPP. Thus, unlike the MVA pathway, IPP/DMAPP isomerase (IDI) is not essential in many MEP pathway-utilizing organisms [8].

The 2C-methyl-D-erythritol 4-phosphate (MEP) pathway, in charge of the essential biosynthesis of isoprenoids, represents a promising and selective target for developing new drugs against tuberculosis. To date, only fosmidomycin, a molecule that targets the second enzyme of the MEP pathway, has reached clinical trials, but recent advances elucidating the structure and kinetics of the MEP enzymes are likely to change this scenario [11, 12].

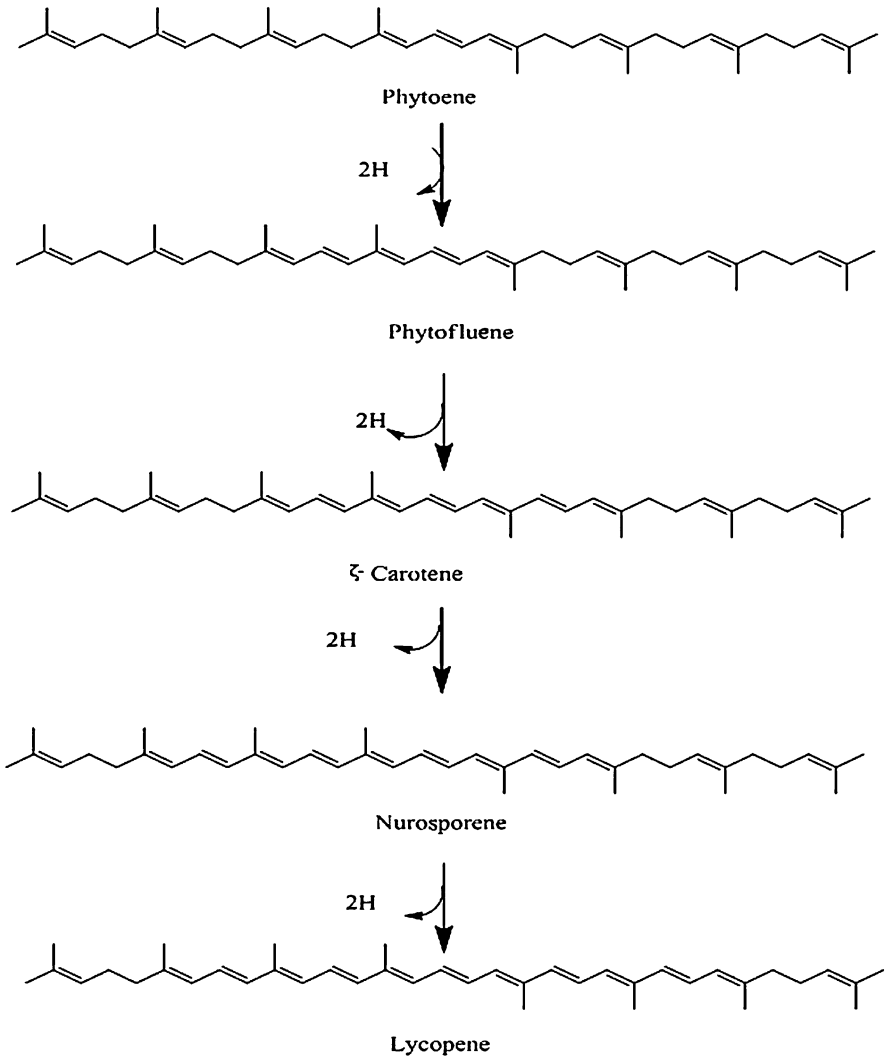


Fig. 3 Stepwise dehydrogenation of phytoene to lycopene

3 Absorption and Metabolism Aspect of Lycopene in the Human Body

As it has been discussed earlier that, however, lycopene occurs naturally in the all *trans* form in the dietary sources, it is mostly found in *cis* form in the human tissues. This supports the hypothesis that it is readily absorbed in *cis* form and present in the same configuration in human tissues. Lycopene was found in as many as 18 different

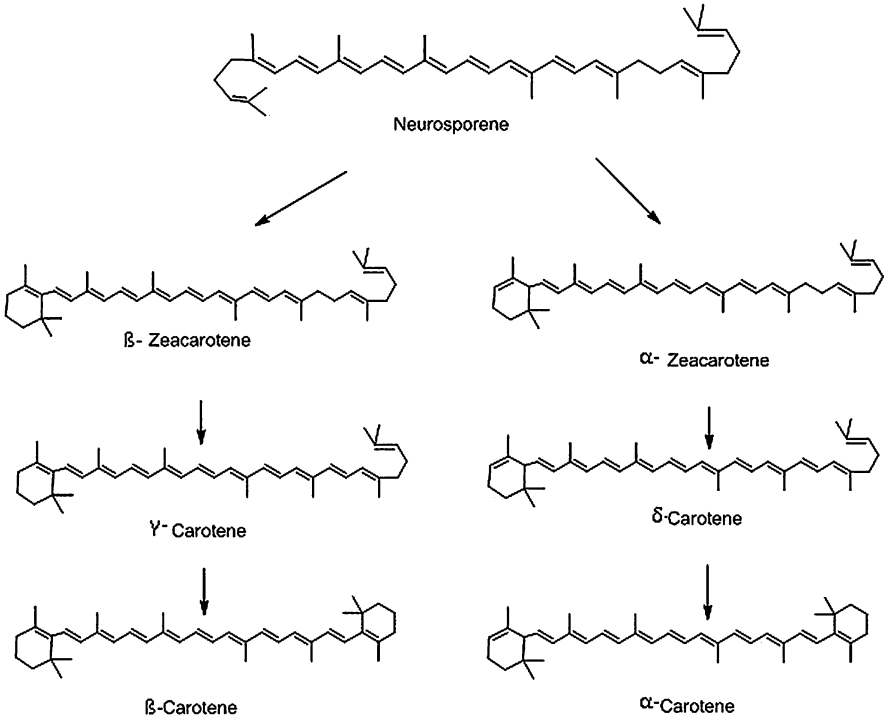


Fig. 4 Conversion of neurosporene to α-carotene and β-carotene

isometric forms in human serum and prostate cells. According to the study of Boileau et al. [13] when ferrets were given oral dose of only 9% *cis* isomers, the *cis-trans* isomer ratios showed that *cis* isomers are more readily taken up by micelles and making them more readily absorbed. However, the diet was containing only 9% *cis* isomers, mucosa (58.8%), lymph (77.4%), and blood (52%), and tissues contained significantly more *cis*-lycopene isomers than the stomach (6%) or intestinal content (17.5%). Studies by Boileau et al. [13] also suggested that mucosal cells contain 41% more *cis*-lycopene. In-vitro the mucosal cells were having more of *cis*-lycopene, in vitro trials were conducted to understand the mechanism of lycopene isomer uptake by bile acid micelles. However the complete mechanism of preferential absorption of *cis* isomers of lycopene is not clearly understood it was hypothesized that the introduction of one or more double bonds into a lycopene molecule reduces its length and thus fits well into micelles with greater ease. Alternatively, it has also been suggested that linear all *trans* isomers may more readily aggregate within the intestine and form crystals greatly reducing their uptake by micelles [14]. The hypothesis was also supported by the study of [15] when male F344 rats were fed lycopene-containing diet for 8 weeks; they also achieved similar lycopene concentration in tissues.

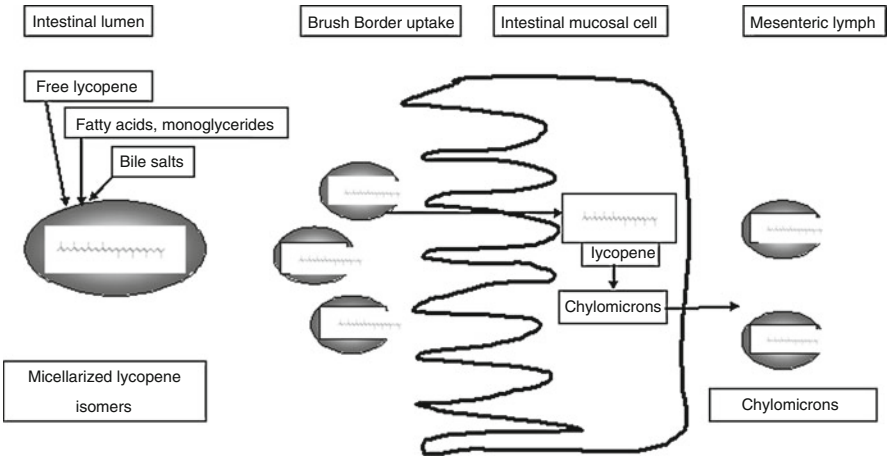


Fig. 5 Uptake of lycopene through mucosal cells

All the carotenoids appear to be absorbed by duodenal mucosal cells by passive diffusion, similar to that of cholesterol and triglyceride lipolysis (Fig. 5). Physical matrix in which they are ingested is the critical and important factor which affects their bioavailability and their dissolution in bulk lipids. Studies by Hernell et al. [16] indicated that the ultimate structure produced during lipid digestion is a discoidal mixed lipid micelle composed largely of bile salts, free fatty acids, monoglycerides, and phospholipids with a diameter of 80 Å. The carotenoids are included in micelles according to their structure and micellar lipid composition. Carotenoids are taken up by the intestinal mucosal cells through the bile acid micelles. Bile acid micelle formation is influenced by the fat intake, and hence lycopene or some other carotenoid absorption is increased when it is taken with fat or a meal containing fat [17].

Lycopene in the mixed lipid micelles is then taken up by the duodenal brush border enzymes through passive diffusion. Different carotenoids and fat-soluble vitamins like vitamin E compete with each other during absorption. Canthaxanthin and lycopene reduced the 0–24-h plasma β-carotene response when given concurrently compared to administration of β-carotene alone [18]. Lycopene exits the mucosal cell in chylomicrons, which are secreted via the mesenteric lymph system into the blood. The distribution of carotenoids among the various lipoproteins classes therefore appears to be determined by the physical characteristics of the individual carotenoid and the lipid composition of the lipoproteins. However, the absolute concentrations of individual carotenoids within specific lipoprotein classes have not been reported; relative distribution of β-carotene, α-carotene, and lycopene among the very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) was similar, with 58–73% in LDL, 17–26% in HDL, and 10–16% in VLDL when separated by conventional

sequential flotation ultracentrifugation and quantified by high-performance liquid chromatography [19].

4 Lycopene and Its Role in Detoxification Pathway in Humans

Cytochrome P-450 enzyme in animals constitutes one of the primary defense systems against natural toxic chemicals from plants which are the major sources of dietary toxins. The induction of cytochrome P-450 enzyme prevents acute toxic effects from foreign chemicals but also results in oxidant by-products that damage DNA. Cytochrome P-450 enzyme helps in detoxification via two different pathways called phase I and phase II detoxification pathways. The study showed lycopene significantly induced phase I enzymes in a dose-dependent manner and doubled hepatic quinone reductase (QR), a phase II enzyme. Lycopene has also been found helpful in elimination of xenobiotics through stool or urine. Xenobiotics are pharmacologically, endocrinologically, and toxicologically active substances that must be metabolized and degraded to such compounds so that it can be eliminated through human excreta. Xenobiotics are also metabolized by cytochrome P-450 enzyme phase I and phase II pathways.

Lipid oxidation is a normal biological process by which we obtain energy from fat. Deleterious lipid oxidation occurring in the body is called peroxidation of polyunsaturated fatty acids (PUFAs) and produces hydroperoxides (LOOHs). Uncontrolled oxidation of lipids in biological membranes is a major contributor in several diseases such as heart disease, cancer, and neurodegeneration. The elevated level of LOOHs was observed during instances of cellular injury and found correlated to the disruption of cellular membranes, inactivation of enzymes, and damage to DNA and protein molecule. Initiation and propagation of lipid peroxidation was studied in isolated microsomes of the liver. The reaction was catalyzed by iron and microsomal NADPH-cytochrome P-450 reductase enzyme. This enzyme is reported to produce superoxide anion formed by addition of an extra electron onto the diatomic oxygen molecule. Aust and Svingen [20] suggested that lipid peroxidation in microsomes of the liver occurs in two stages. In initiation stage cytochrome P-450 reductase catalyzes reduction of ADP-Fe + 3 which is subsequently reacting with oxygen molecule to form a ADP-perferyl radical. The perferyl radical is then responsible for initiating lipid peroxidation and formation of lipid peroxides.

Oxidative DNA damage could be a major risk factor for the development of tumors, so that dietary antioxidants are able to decrease such damage in vivo which would be expected to have cancer prevention effects. Hence, antioxidant substances when present in foods at low concentrations compared with those of oxidizable substrate markedly reduce, delay, or prevent the oxidation of the substrate [21]. Due to the safety concerns over synthetic compounds, research has been focused on novel antioxidant components present in foods.

5 Mechanism Responsible for Health Benefits of Lycopene

There are five mechanisms that researchers proposed which may account for the beneficial effects of tomato phytochemicals and their metabolite. Firstly, lycopene is the strongest antioxidant compared to other commonly consumed carotenoids. Decreased DNA damage has been reported in white blood cells after 15 days of supplementation of tomato and tomato juice. It accounts for about 50% of carotenoids in human serum. Among the common dietary carotenoids, lycopene has the highest singlet oxygen quenching capacity in vitro (Table 1). Another outstanding feature is its high concentration in the testes, adrenal gland, and prostate. Lycopene uptake varied with individuals, but peak serum concentrations were always reached between 24 and 48 h. The carotenoid was eliminated from serum with a half-life of 2–3 d. The increase in peak serum concentrations was dose-dependent but not linear with the dose.

Secondly, lycopene alters the biotransformation of xenobiotics; cooked tomatoes and lycopene alter hormone and growth factor signaling in prostate cells. This includes alterations in insulin-like growth factor-1 (IFG-1) activity. IFG-1 stimulates cellular proliferation and decreases apoptosis, which is a mechanism by which normal cell death happens. Eating cooked tomatoes was associated with a 31.5% decrease in serum IFG-1 levels in a case-controlled study of 112 men. Beneficial alterations of IFG-1 concentrations and its ability to stimulate cell division have also been found in rats and healthy men. An in vitro study showed lycopene and tomato polyphenols including quercetin, kaempferol, and rutin, to interfere with IGF-1 signaling, thus preventing the growth factor from stimulating cell proliferation. In a number of cancer cell line including breast cancer cells and endometrial and prostate cancer cells, lycopene halted cellular replication in vitro. Lastly, lycopene and its metabolites may help fight some cancers by increasing connexin 43 levels. Connexin 43 is a molecule involved in cell-to-cell communication, which is important in the regulation of uncontrolled, rapid cell growth. In a metastatic prostate cancer cell line, lycopene did not increase connexin 43; however, it did in another prostate cancer cell line, a breast cancer cell line, and oral cancer cells. The inhibition

Table 1 Comparison of antioxidant capacities of carotenoids

Carotenoid	Rate constant for quenching of singlet oxygen $K_q \times 10^9$ (mol^{-1} s^{-1})
Lycopene	31
γ-Carotene	25
β-Carotene	19
α-Carotene	14
Lutein	8
Astaxanthin	24
Bixin	14
Canthaxanthin	21
Zeaxanthin	10

Source: Data from Di Masio et al. [22] and Miller et al. [23]

of connexin 43 in these cell lines was associated with an inhibition of cell growth, suggesting that upregulation of connexin 43 may be important to the anticancer action of lycopene. Since a synergistic effect appears to exist between tomato phytochemicals, recommending the consumption of supplements made from whole tomatoes and/or the consumption of two to four or more servings per week of tomato products may reduce the incidence of prostate cancer and health-care costs in our aging population.

In contrast to other carotenoids, its serum values are not regularly reduced by smoking or alcohol consumption but by increasing age. Remarkable inverse relationships between lycopene intake or serum values and risk have been observed in particular for cancers of the prostate, pancreas, and to a certain extent of the stomach. In some of the studies, lycopene was the only carotenoid associated with risk reduction. Its role in cancer risk reduction still needs to be clarified. Patients with HIV infection, inflammatory diseases, and hyperlipidemia with and without lipid-lowering treatment may have depleted lycopene serum concentrations [24].

6 Stability and Functional Aspects of Lycopene

Stability is a critical aspect of lycopene functionality. As a highly conjugated polyene, lycopene can undergo two types of changes: isomerization and oxidation. Physical and chemical factors known to contribute to the degradation of other carotenoids are also reported to affect in case of lycopene. These include elevated temperature, exposure to oxygen and light, metallic ions (e.g., Cu^{2+} and Fe^{3+}), extreme in pH, and active surfaces [25, 26].

Cis and *trans* isomers of lycopene have distinct bioactivity and biostability. In general *cis* isomers are more soluble in oil and hydrocarbon solvents than their all *trans* counterparts. They are less prone to crystallization than *trans* isomers due to their kinked structures. They are less intense in color which affect consumer's perception of food quality. All *trans* configurations predominate in fresh tomatoes and gradually isomerize to *cis* configurations upon processing and storage. Isomerization of lycopene has been shown to take place both in food systems and in model systems. *Trans*-to-*cis* isomerization typically occurs during isomerization. The storage of processed foods favors reversion from *cis* to *trans* because the *cis* isomers are in a relatively unstable state compared to the *trans* isomer, which is relatively a stable ground state. Mostly the 5-*cis*, 9-*cis*, and 15-*cis* isomers are found in human serum when assessed using nuclear magnetic resonance (NMR) spectroscopy. With its acyclic structure, a large array of conjugated double bonds and important hydrophobicity lycopene exhibits a range of unique and distinct biological properties. Of these properties its antioxidant potential is of particular interest. The system of conjugated double bonds allows lycopene molecule to quench the singlet oxygen and also other free radicals. In an in vitro study by Boileau et al. [13], it was found an effective singlet oxygen quencher. Under appropriate conditions, it has been found effective as an antioxidant at lower concentrations not only against O_2^- but also against lipid peroxidation and a highly destructive hydroxyl radical HO.

The antioxidant properties of lycopene most likely contribute to limit the risk of some diseases [3].

Various factors such as heat, processing conditions, heat, oxygen, light, dehydration, and storage conditions affect the lycopene stability and *cis*-to-*trans* isomerization of lycopene isomers. Time-temperature combination in heat processing of lycopene is crucial and affects its isomerization and degradation. Besides time-temperature combination reaction medium, physical matrix and environmental conditions also affect the stability of lycopene. Basically two types of changes occur, isomerization and degradation, due to the parameters described above. As is has been discussed in earlier sections that majority of lycopene is present naturally in the all-*trans* form in fruits and vegetables. However, the absorption and bioavailability of the *cis*-lycopene is better than the *trans* isomers. During the heat processing, *trans* isomers gradually change into the *cis* form. It has been observed that all *trans*-lycopene isomerizes into mono- or poly-*cis* form due to changes in seven of the conjugated double bonds initially during the thermal processing followed by degradation of lycopene content. It has been reported by Hakette et al. [27] that degradation of lycopene started at a temperature as low as 25 °C. At this temperature degradation of lycopene occurs mainly due to oxidation, and isomerization occurs at a very low rate. Isomerization of lycopene from *trans* to *cis* form occurs on a faster rate at a temperature above 75 °C [27, 28]. According to the study of Lee and Chen [29], no significant change occurs in the lycopene content during the first 12 h of heating at 50 °C. However, at 100 °C heating for 9 h, isomerization of lycopene from all *trans* to mono-*cis* form followed by degradation of mono-*cis* to di-*cis* and poly-*cis* form of lycopene occurs. Thus it can be concluded that the isomerization of mono-*cis*-lycopene to di-*cis*-lycopene is the main phenomenon during heating in initial phase followed by conversion to poly-*cis* form and subsequent degradation. At the temperature above 100 °C, lycopene was found highly unstable, and severe deduction was noted in the lycopene content at higher temperature above 100 °C. No lycopene was detected after 10 min at heating over 100 °C.

The exposure of light also induced similar kind of changes in the all *trans*-lycopene. According to the study of Lee and Chen [29] and Shi et al. [30, 31], all *trans*-lycopene first changes into 9-*cis*, 13-*cis*, and 15-*cis* isomers, and then degradation of lycopene occurs. It has been also found during the study that *cis* isomers are less stable than all *trans* isomers under light exposure. Lee and Chen [29] studied the stability of lycopene standard at 25 °C for 6 days at illumination intensity (2000–3000 lx). All *trans*-lycopene was found to decrease with an increase in illumination time, and total loss of lycopene was measured as 94% after 144 h of illumination exposure.

Lycopene stabilization was found three times higher in the presence of oxygen than under inert conditions. Vacuum and N_2^- packaging of tomato powder were found highly effective to reduce the oxidation changes of lycopene. According to the study of Sharma and Le Maguer [32], vacuum pack and dark storage combination showed the lowest lycopene loss. Auto-oxidation of lycopene is irreversible and leads to fragmentation of molecule. During the storage *cis-trans* reversion of lycopene is also reported. During storage *cis-trans* re-isomerization and auto-oxidation

of lycopene are the main reasons of loss in lycopene content. Auto-oxidation produce acetone methylheptenone, levulinic aldehyde and glycoxal also which is responsible for loss in color and typical hay like odor [33].

7 The Linkage of Health and Food Choice

Health is one reason consumers mention when they are asked about factors that influence their food choices correspondingly; nutrition experts emphasize that the degree of healthy eating needs to be judged on the level of diet. Whereas no single food product can be categorized as health promoting or not, this is true that from a nutrition point of view, information about fat, vitamin, and other nutraceutical contents of food tend to be categorized, for example, all fruits and vegetables are rich sources of vitamin C and carotenoids or other pigment contents which are of health-promoting value. Functional food is regarded somewhere between medicine and conventional food, but there is no common shared definition of functional food. Japan has its own legislation of "food for specified health uses," called FOSHU, in which functional foods are clearly regarded as food products that are eaten as part of an ordinary diet. In Europe, the definition suggested by an EU-funded concerted action project has widely been used. According to this definition, functional foods are "satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of diseases" [34].

Traditional nutritional education has emphasized the role of a health-promoting diet. Following nutritional guidelines will result in reduced risk to develop obesity and other chronic lifestyle-related diseases, such as cardiovascular disease, diabetes, and cancer. The message on the benefit of a healthy diet may be hard to convey to the consumer, as the end result is unsure and achieving the possible reward takes several years or even decades. Functional foods offer a new kind of positive health benefit to the people. Instead of avoiding certain kind of foods, these food products promise positive physiological effect on bodily functions or even a reduction on risk level of diseases through eating a single product. Functional food effects are easier to understand for the consumer since they contain results that can be instrumentally measured, such as lowering the level of cholesterol in the blood, decreasing the blood pressure, or increasing the density of bone mass [35, 36].

The beneficial health effect of functional foods is due to the presence of a myriad of bioactives that render their effects via a number of mechanisms. The diseases of concern include coronary heart disease, certain types of cancer, type 2 diabetes, brain health and mental disorders, immune response, inflammation, obesity, and arthritis, in association with oxidative stress and metabolic syndrome. The substances that may influence such diseases often originate from plant sources and sometimes animal sources and microorganisms. Examples include carotenoids such as alpha- and beta-carotene; lutein; astaxanthin; lycopene; fucoxanthin; dietary fiber; beta-glucan; soluble fiber; long-chain omega-3 fatty acids; phenolics such as phenolic acid; phenylpropanoids; catechins; anthocyanidins; flavones; flavanones;

proanthocyanidins; lignans; sterols and stanols; pre-, pro-, and syn-biotics; and soy isoflavones. Some proteins and biologically active peptides also come under the category of functional foods.

8 Regulatory Aspects of Functional Foods

The success of a functional food depends to a large extent on the regulatory framework in which it is allowed to be marketed. From country to country, the method of regulating the labeling and composition of functional foods varies enormously. The most important issue that must be considered related to functional foods is "the nature of the ingredient." What specific health benefit does it actually confer on the consumer? A whole host of the products in the market profess to be functional, ranging from vitamin- and mineral-enriched products to the products containing added fiber ingredients and pre- and probiotics. Is there really a distinction between vitamin added for fortification and a vitamin added for its perceived functional qualities?

Health claims of a product describe the relationship of a diet to the specific disease, and only 11 health claims are approved by the FDA. An example is healthful diet with adequate folate may reduce a women's risk of having a child with brain or spinal cord defect. Health claims traditionally have been approved based on the concept of "significant scientific agreement" which the FDA has recently defined in a guidance document as an agreement among qualified scientific experts that a substance-disease relationship exists based on a sound body of scientific evidence. The level of scientific evidence must be strong enough that it would unlikely to be reversed by the further study [37].

Nutritional claims describe the level of a nutrient or dietary substances in a product using terms such as free, high, and low, or they compare the level of a nutrient in a food to that of another food, using terms such as more reduced, etc. An accurate quantitative statement (e.g., 200 mg of sodium) that does not characterize the nutrient level may be used to describe any amount of a nutrient present [37]. Structural and functional claims are statements of health-promoting or nutritional benefit allowed on dietary supplement labels. They are not allowed to mention disease conditions; they must describe the support or maintenance of the normal functioning of the body. "Cranberry supports the health of urinary tract" is an example of a model structure or functional claim.

9 Conclusion

Lycopene not only has the pharmacological and nutritional effect in humans and animals but also has promising health benefit too. However its potential health benefits have been known since the late 1950s; recently clinical and functional aspects of lycopene and its metabolic fate have been stimulated their potential use in the prevention of the chronic diseases. There has been a growing interest in the

role of lycopene in a variety of ailments in humans including some cancers and heart diseases. Recent studies have shown that consumption of lycopene-rich foods reduces the risk of such diseases. Lycopene is also a more potent antioxidant than all other carotenoids found in human serum. However, it has no pro-vitamin A activity, but it efficiently quenches the singlet oxygen and prevents degeneration of proteins and DNA. Heat treatment has shown improved bioavailability of lycopene after cooking, which means it is more easily absorbable by the body in the heat-processed tomato products. Heat processing affects *trans-cis* isomerization of tomato products. The loosely bound lycopene in tomato products is released during heat processing, while the degree of isomerization is directly correlated with temperature and duration of heat processing. Recent scientific and epidemiological data has proven the anticancer properties of lycopene. The US National Research Council of the Academy of Sciences, World Cancer Research Fund International, American Cancer Research Institute, and WHO have strong documentary proofs and made similar recommendations for possibility of reducing cancer risks. Considering the possible health benefits of lycopene and functionality, it has a brighter scope for the functional food domain and food processing industry.

References

1. Gopalkrishnan TR (2007) Horticultural science series-vegetable crops, vol 4. New India Publishing Agency, New Delhi, pp 1–45, 87–100
2. Khachik F, Goli MB, Beecher GR, Holden J, Lusby WR, Tenorio MD, Barrera MR (1992) Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. J Agric Food Chem 40:390–398
3. Stahl W, Sies H (1996) Perspectives in biochemistry and biophysics. Lycopene: a biologically important carotenoid for humans. Arch Biochem Biophys 336:1–9
4. Vogele AC (1937) Effect of environmental factors upon the color of the tomato and the watermelon. Plant Physiol 12:929–955
5. Rodriguez-Amaya DB (1993) Nature and distribution of carotenoids in foods. In: Charalambous G (ed) Shelf-life studies of foods and beverages; chemical, biological, physical and nutritional aspects. Elsevier Science Publishers, Amsterdam
6. Rodriguez-Amaya DB (1997) Carotenoids and food preparation: the retention of provitamin carotenoids in prepared, processed and stored foods. USAID, OMNI project. John Snow, Arlington, 88 p
7. Rogers LJ, Shah SPJ, Goodwin TW (1966) Intracellular localization of mevalonate activating enzymes in plant cells. J Biochem 99:381–388
8. Rohmer M, Knani M, Simonin P, Sutter B, Sahm H (1993) Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. Biochem J 295(Pt 2):517–524
9. Lishan Z, Chang W, Xiao Y, Liu H, Liu P (2013) Methylerythritol phosphate pathway of isoprenoid biosynthesis. Annu Rev Biochem 82:497–530
10. Fraser PD, Bramley PM (2004) The biosynthesis and nutritional uses of carotenoids. Prog Lipid Res 43:228–265
11. Obiol-Pardo C, Rubio-Martinez J, Imperial S (2011) The methylerythritol phosphate (MEP) pathway for isoprenoid biosynthesis as a target for the development of new drugs against tuberculosis. Curr Med Chem 18(9):1325–1338
12. Wiemer AJ, Hsiao CH, Wiemer DF (2010) Isoprenoid metabolism as a therapeutic target in gram-negative pathogens. Curr Top Med Chem 10:1858–1871

13. Boileau AC, Merchen NR, Wasson K, Atkinson CA, Erdman JW (1999) *cis*-lycopene is more bioavailable than *trans*-lycopene in vitro and in vivo in lymph-cannulated ferrets. J Nutr 129:1176–1181
14. Britton G (1995) Structure and properties of carotenoids in relation to function. FASEB J 9:1551–1558
15. Boileau TWM, Clinton SK, Erdman JW Jr (2000) Tissue lycopene concentrations and isomer patterns are affected by androgen status and dietary lycopene concentration in male F344 rats. J Nutr 130:1613–1618
16. Hernell O, Staggers JE, Carey MC (1999) Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. Biochemist 29:2041–2056
17. Stahl W, Sies H (1992) Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. J Nutr 122:2161–2166
18. White WS, Stacewicz-Sapuntzakis M, Erdman JW Jr, Bowen PF (1994) Pharmacokinetics of β-carotene and canthaxanthin after ingestion of individual and combined doses by human subjects. J Am Coll Nutr 13:665–671
19. Reddy PP, Clevidence BA, Berlin E, Taylor PR, Biery JG, Smith JC (1989) Plasma carotenoid and vitamin E profile of lipoprotein fractions of men fed a controlled typical US diet. FASEB J 3(4):A955p
20. Aust SD, Swingen BA (1982) In: Pryor WA (ed) Free radicals in biology, vol 5. Academic, New York, pp 1–28
21. Halliwell B (1999) Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic Res 31:261–272
22. Di Mascio P, Kaiser S, Sies H (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538
23. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA (1996) Antioxidant activities of carotenes and xanthophylls. FEBS Lett 484(3):240–242
24. Gerster H (2013) The potential role of lycopene for human health. J Am Coll Nutr 16 (2):109–126
25. Scita G (1992) Stability of beta-carotene under different laboratory conditions. Methods Enzymol 213:175–185
26. Henry LK, Catignani GL, Schwartz SJ (1998) Oxidative degradation kinetics of lycopene, lutein, and 9-*cis* and all-*trans* β-carotene. J Am Oil Chem Soc 75(7):823–829
27. Hackett MM, Lee JH, Francis D, Schwartz SJ (2004) Thermal stability and isomerization of lycopene in tomato oleoresins from different varieties. J Food Sci 69:536–541
28. Mayer-Meibach E, Schuchmann HP, Regier M, Behsnilian D (2005) Thermal processing of carrots: lycopene stability and isomerization with regard to antioxidant potential. Food Res Int 38:1103–1108
29. Lee MT, Chen BH (2002) Stability of lycopene during heating and illumination in a model system. Food Chem 78:425–432
30. Shi J, Wu Y, Bryan M, Maguer LM (2002) Oxidation and isomerization of lycopene under thermal treatment and light irradiation in food processing. Nutraceut Food 7:179–183
31. Shi J, Dai Y, Kakuda Y, Mittal G, Xue SJ (2008) Effect of heating and exposure to light on the stability of lycopene in tomato puree. Food Control 19(5):514–520
32. Sharma SK, Le Maguer M (1996) Kinetics of lycopene degradation in tomato pulp solids under different processing and storage conditions. Food Res Int 29:309–315
33. Cole ER, Kapur NS (1957) The stability of lycopene. I.- degradation by oxygen. J Sci Food Agric 8:360–365
34. Diplock AT, Aggett PJ, Ashwell M, Bornett F, Fern EB, Robetfroid MB (1999) Scientific concepts of functional foods in Europe: consensus document. Br J Nutr 81(4):S1–S27
35. Marttila-Sandholm T, Maria S (2003) Functional dairy products. In: CRC Woodhead publishing house. New York, Washington, DC
36. Stahl W, Sies H (1992) Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. J Nutr 122:2161–2166
37. Shi J, Mazza G, Le Maguer M (2002) Functional foods: biochemical and processing aspects, vol 2. CRC Press, Hoboken

Sweet Potato: Bioactive Compounds and Health Benefits

32

Remya Mohanraj

Contents

Abstract

Sweet potato, a delicious root vegetable, possesses high nutritional value. It is reported to exhibit anticancer, antidiabetic, and anti-inflammatory activities and to be a natural alternative to estrogen therapy. Sweet potatoes are a rich source of phytochemical compounds, and plant-derived compounds always have been an important source of several clinically useful biomolecules. This chapter aims to focus on the health benefits and phytochemical composition of sweet potato with special emphasis on 4-ipomeanol. 4-Ipomeanol, produced by infected sweet potatoes, is a potential anticancer agent. Earlier studies revealed that bioactivation of 4-ipomeanol to a cytotoxic metabolite occurred particularly in tissues that are abundant in specific P450 mixed function oxidase enzymes. Based on the above

R. Mohanraj (✉)
Houston Community College, Houston, TX, USA

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_62

rationale, 4-ipomeanol was the first agent to undergo clinical development as an anticancer agent especially against lung cancer. 4-Ipomeanol as a potential prodrug for P450-directed gene therapy of liver and brain cancers has also been investigated. Recent findings suggest that ^{18}F-labelled 4-ipomeanol could be used in imaging tumors and monitoring enzyme/prodrug interactions.

Keywords
Sweet potato · 4-Ipomeanol · Anti-cancer activity · Phytochemical composition

1 Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam, Convolvulaceae) (Fig. 1) is a root vegetable rich in starch and sweet to taste [1, 2]. It ranks as the seventh important staple crop in the world and in developing countries, it is ranked as the fifth following rice, wheat, maize, and cassava [3]. The skin may be red, purple, brown, or white in color. The flesh color ranges from white, yellow, and orange to purple [4].

Sweet potatoes are native to South America. Columbus introduced it to Europe from where it eventually spread to other parts of the world [5]. From time immemorial, this root tuber that possesses an abundance of pharmacologically active ingredients occupied a significant position in human nutrition and animal feeding. Several reports point out to the use of sweet potatoes in traditional medicine. The leaves are used by Akan tribes of Ghana to treat type 2 diabetes [6]; in Brazil, they are used in the treatment of inflammatory and/or infectious oral diseases [7]. In regions of Kagawa, Japan, sweet potatoes are used to treat anemia, hypertension, and diabetes [8]. The stems are used for treatment of prostatitis [9]. In addition to the above, sweet potatoes are reported to be alterative, aphrodisiac, astringent,

Fig. 1 Purple-skinned sweet potatoes

bactericide, demulcent, fungicide, laxative, and tonic. It is a folk medicine for asthma, bugbites, burns, catarrh, ciguatera, convalescence, diarrhea, dyslactea, fever, nausea, renosis, splenosis, stomach distress, tumors, and whitlows [10]. In Mexico, the leaves are used for the treatment of inflammatory tumors, and their decoction is used in baths and gargles for tumors of the mouth and throat [11].

Reed [12] described the plant as a tuberous-rooted perennial that is usually grown as an annual. Slender, prostrate stems form a running vine up to 4 m long and produce milky juice. Lateral stem branches that arise from the short stem are usually not branched. Leaves are ovate-cordate and borne on long petioles, palmately veined, angular or lobed, green or purplish depending on the variety. Fowers white or pale violet, axillary, funnel-shaped, borne singly or in cymes on short peduncles. Pods are round with 1–4 flattened, hard-coated, angular seeds per pod [12].

Keeping the above background in mind, this chapter aims at providing an insight into the health benefits and recent advances in phytochemical composition of sweet potato tubers with special emphasis on 4-ipomeanol, an anticancer agent.

2 Health Benefits

Sweet potato is a staple food in many parts of the world and is a drought-tolerant crop that holds promise in improving food and nutrition security [13]. Sweet potatoes are especially significant because of their abundant nutraceutical components. This tuberous root is a rich source of carbohydrates, dietary fiber, vitamin A (as β-carotene), vitamin B6, vitamin C, manganese, copper, potassium, and iron [14]. Red sweet potatoes have been reported to have 12.98% of glucose and therefore gain recognition as a good energy source. Besides this, sweet potatoes contain 2.78% crude fiber as an added advantage for the consumers. In essence, sweet potatoes are not only a good food diversification but also a food alternative that bestow food security and promote health [15].

Sweet potatoes offer a near-balanced diet for the human body in that they possess significant amounts of carbohydrates in comparison with other starchy foods such as rice, maize, and sorghum porridge [16]. They encompass a broad range of macro- and micronutrients, generous amount of vitamin C, reasonable amounts of vitamin B complex (vitamins B1, B2, B5, and B6) and folic acid, as well as an adequate amount of vitamin E [17, 18].

A colossal advantage is that the sweet potato crop could be made accessible throughout the year in tropical and subtropical areas where warm conditions abound. This offers a leverage in case drought poses a challenge for staple crops such as cereals. The competence of sweet potatoes in being drought tolerant after establishment raises its yield potential higher than that of other staple crops [13].

The US Food and Drug Administration summates the nutritional facts of sweet potatoes as follows [19]:

Serving size (gram weight/ounce weight)	1 medium (130 g/4.6 oz)
Calories	100
Calories from fat	0
Total fat	0
Sodium	70 mg
	3% DV
Potassium	440 mg
	13% DV
Total carbohydrate	23 g
	8% DV
Dietary fiber	4 g
	16% DV
Sugars	7 g
Protein	2 g
Vitamin A	120% DV
Vitamin C	30% DV
Calcium	4% DV
Iron	4% DV

Percent Daily Values (% DV) are based on a 2,000 calorie diet

In the class of different varieties of sweet potatoes, orange-fleshed sweet potato is reported to possess high amounts of β-carotene, a precursor for vitamin A. It has been recorded that the amount of β-carotene is directly proportional to the intensity of orange color of the sweet potato flesh [17, 20, 21].

Leighton [21] tested for β-carotene in different South African orange-fleshed sweet potato varieties and concluded that β-carotene concentration varied with depth of color.

Alam et al. [22] analyzed the nutritional composition of nine varieties of orange-fleshed sweet potatoes. They also analyzed the total carotenoids and total polyphenol content. Each variety showed a significant variation in the nutritional composition. The quantification of total carotenoids and total polyphenol content was done using spectrophotometry, and the proximate composition was done using AOAC. The results obtained indicated that the total polyphenol content varied from 94.63 to 136.05 mg gallic acid equivalent/100 g fresh weight. The obtained results also indicated that total carotenoids content ranged from 0.38 to 7.24 mg/100 g fresh weight. Dark orange-colored flesh varieties had a higher content of total carotenoids as compared to their light-colored counterparts. These findings suggest that the sweet potato varieties studied are rich in proteins and carbohydrates, low in fat, and high in polyphenol and carotenoids. Therefore sweet potatoes could serve as an excellent source of dietary antioxidants which could potentially prevent free radical damage and also prevent vitamin A malnutrition [22].

Sanoussi et al. [23] assessed the mineral composition of ten selected cultivars of sweet potatoes (01 cream, 02 white, 03 yellow, and 04 orange flesh-colored) using standard spectrophotometry, in order to establish a scientific basis for efficient

valorization and sustainable utilization of these crops in Benin. The mineral composition of the tubers on dry weight basis ranged from 0.53 to 0.73 mg/100 g for iron; 0.23 to 0.27 mg/100 g for zinc; 23.04 to 29.97 mg/100 g for calcium; 21.30 to 25.40 mg/100 g for magnesium; 42.00 to 46.33 mg/100 g for phosphorus; 308.67 to 328.67 mg/100 g for potassium; and 29.00 to 34.00 mg/100 g for sodium. The mineral salt recorded in highest amount in all the samples was potassium, which could contribute on an average up to 19.78% and 15.83% of the recommended dietary allowance of children and adults, respectively [23].

2.1 Cardioprotective Effects

Studies from Harvard University School of Public Health reveal that sweet potatoes are a tremendous source of B6 vitamins, which promote breaking down of homocysteine. It is noteworthy that homocysteine contributes to the hardening of blood vessels and arteries. As an exceptional source of potassium that lowers blood pressure and maintains fluid balance, sweet potatoes play a significant role in improving heart health (American Heart Association) [19].

Phytochemical screening of aqueous tuber extract of *Ipomoea batatas* conducted by Shafe et al. [24] showed the presence of tannin, saponin, flavonoid, terpenoid, alkaloid, anthraquinones, reducing sugars, and cardiac glycosides. The administration of tuber extracts lead to a decrease in the activities of serum creatine and lactate dehydrogenase. The results obtained suggest a potential cardioprotective effect of the aqueous tuber extracts of sweet potato [24].

2.2 Regulation of Blood Sugar

According to sources from North Carolina State University, sweet potatoes help regulate the levels of blood glucose. Linus Pauling Institute at Oregon State University reported that sweet potatoes are an excellent source of manganese which helps the body metabolize carbohydrates and thus maintain healthy blood sugar levels [19].

2.3 Immune Booster and Anti-inflammatory Properties

Mercy Margaret et al. [25] prepared the aqueous extract of *I. batatas* and evaluated the in vitro anti-inflammatory activity of the extracts by membrane stabilizing method. Phytochemical analyses of the extracts revealed the presence of phenols, flavonoids, tannins, anthraquinones, and reducing sugars. The results indicated that the anti-inflammatory potential of the extracts could be attributed to the presence of phenols and flavonoids in the extracts [25]. Vitamins A and E present in sweet

potatoes also support a healthy immune system and are powerful disease-fighting antioxidants [19].

2.4 Benefits for the Gastrointestinal System

The high fiber content of sweet potatoes helps in retaining water. Also, magnesium, which is present in sweet potatoes, aids in digestion. They are soothing for the stomach and intestines. B-complex vitamins, vitamin C, beta-carotene, potassium, and calcium present in sweet potatoes are beneficial in curing stomach ulcers. Additionally, the roughage in sweet potatoes alleviates constipation and the resultant acid formation, which diminishes the chance of ulcers [26].

2.5 Benefits for Respiratory System

Sweet potatoes are effective in providing relief from asthma by clearing congestion of the nose, bronchi, and lungs. This property could be attributed to its typical aroma. In addition to harboring vitamin C, iron, and other nutrients, sweet potatoes are capable of warming up the body which help to cure bronchitis [26].

2.6 Cancer Prevention

Many scientific investigations have revealed that eating sweet potatoes have the potential to decrease the risk of breast, colorectal, gallbladder, and kidney cancer. In a 10-year study for evaluating the risk factors for kidney cancer death, 47,997 males and 66,520 females aged 40 years and older were included. Researchers concluded that eating sweet potatoes and potatoes regularly was associated with a decreased risk of kidney cancer [27]. The various health benefits of sweet potatoes are summarized in Fig. 2.

3 Bioactive Compounds from Sweet Potato

Sweet potatoes harbor an immense amount of bioactive compounds which contribute to the health benefits conferred by them. Earlier research studies have pointed out that bioactive compounds are different in orange-fleshed sweet potato and white-fleshed sweet potato. Phytochemical screening conducted by Shekar et al. [28] revealed high percentage of carbohydrate, reducing sugar, and phenolics in white-fleshed sweet potato and increased levels of total protein, flavonoids, anthocyanins, and carotenoids in orange-fleshed sweet potato. In orange-fleshed sweet potato, it was also observed that the rate of starch and cellulose degradation was lesser during storage, which pointed out to a strict regulation of gene(s) involved in starch

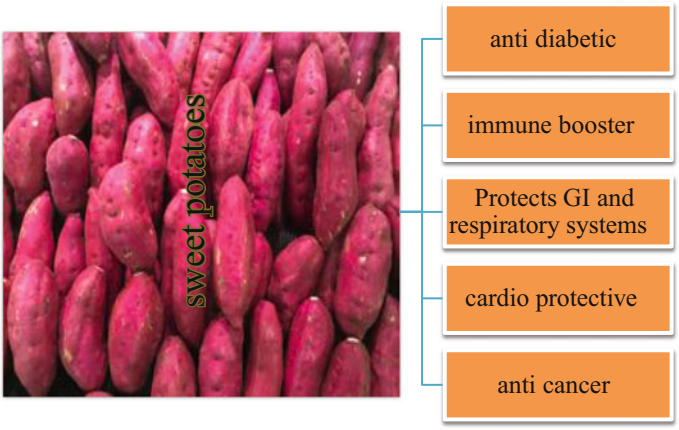


Fig. 2 Health Benefits of Sweet Potatoes

degradation. These researchers also conducted comparative proteomics which displayed a cultivar-dependent expression of proteins along with evolutionarily conserved proteins [28].

Phenolic compounds and carotenoids have been reported to be present in sweet potatoes. Phenolic compounds, such as phenolic acids and anthocyanins, are quite predominant in the purple-flesh variety. In orange fleshed varieties, α-carotene, β-carotene, and β-5 cryptoxanthin are predominant. Sweet potato is an abundant source of dietary fiber, minerals, vitamins, beta-carotene, phenolic acids, and anthocyanins. These bioactive compounds confer distinctive flesh colors to sweet potatoes such as cream, yellow, orange, and purple [29].

The tubers of sweet potato are rich in polyphenols such as anthocyanins and phenolic acids. They also possess vitamins A, B, and C [30]. Sucharitha et al. [30] reported that the root of *Ipomoea batatas* contains phytoconstituents like flavonoids, carbohydrates, and tannins.

Phytochemical components such as alkaloids, saponin, tannins, steroids, anthocyanins, flavonoids, and anthraquinones were extracted by Anbuselvi and Muthumani [14] using different solvents like ethyl acetate, methanol, chloroform, and acetone.

Park et al. [31] determined the phytochemical diversity, including carotenoids, flavonoids, anthocyanins, and phenolic acids, in sweet potatoes of varying flesh colors (white, orange, and purple). They also made an attempt to identify the hydrophilic primary metabolites from the different varieties. As a result of their study, they reported that in orange-fleshed varieties, carotenoid content was considerably higher among which β-carotene was the most plentiful. Only purple-fleshed sweet potatoes showed the presence of anthocyanins. The levels of phenolic acids and flavonoids were higher in purple-fleshed varieties as compared to the other two varieties [31].

Oluyori et al. [32] conducted phytochemical screening of the peels of sweet potatoes and reported the presence of tannins/phenolic compounds, terpenoids, reducing sugar, cardiac glycosides, alkaloids, and lipids.

Rosas-Ramírez and Pereda-Miranda [33] carried out the purification of the chloroform-soluble resin glycosides from the roots of yellow-skinned sweet potatoes. This was done using preparative-scale HPLC, and six oligosaccharides, batatin VII, and batatinosides VII–IX, with novel structures were collected, along with previously known resin glycosides pescaprein I and batatinoside IV. Each structure was characterized using high-field NMR spectroscopy and FAB mass spectrometry [33].

Studies carried out by Kang et al. [34] indicated that sweet potato extracts inhibited excessive production of pro-inflammatory mediators such as NO, iNOS, COX-2, and TNF-α and thereby attenuated neuroinflammatory responses in LPS-activated BV-2 microglia. The results suggested that the anti-neuroinflammatory potential of sweet potato extracts may be related to its strong antioxidant properties and its regulatory actions on pro-inflammatory cytokine such as TNF-α. These results suggest the sweet potato extracts might be developed as a promising candidate for the treatment of neuroinflammation-mediated neurological disorders [34].

3.1 Anthocyanins

It has been reported that the purple-fleshed sweet potato anthocyanins protects against acetaminophen induced hepatotoxicity in mice [35].

Cuevas Montilla et al. [36] compared different Japanese purple sweet potato cultivars and reported a remarkable variation of anthocyanin profile. Ten major pigments with non-, mono-, or diacylated structures of 3-*O*-(2-*O*- -D-glucopyranosyl- -D-glucopyranoside)-5-*O*- -D-glucosides of cyanidin and peonidin were characterized by ESI-MS^n and NMR analyses.

Aldi et al. [37] reported that purple sweet potato peel has high levels of anthocyanins, which are powerful antioxidants. They conducted a study aimed at determining immunostimulatory effect of purple sweet potato peel. They observed parameters like the activity and capacity of peritoneal macrophages, the total number of leukocytes, and the percentage of leukocytes and spleen weights relative. The results indicated that the ethanol extract of purple sweet potato peel posses immunostimulatory effect that increased the activity and capacity of peritoneal macrophage cells, the total number of leukocytes, and number of neutrophil segments [37].

3.2 Phenolics

Jung et al. [38] used chromatography to isolate different polyphenolic compounds possessing potent antioxidant activities, from methanolic and hydromethanolic extracts of sweet potato tuber flour. The isolated compounds

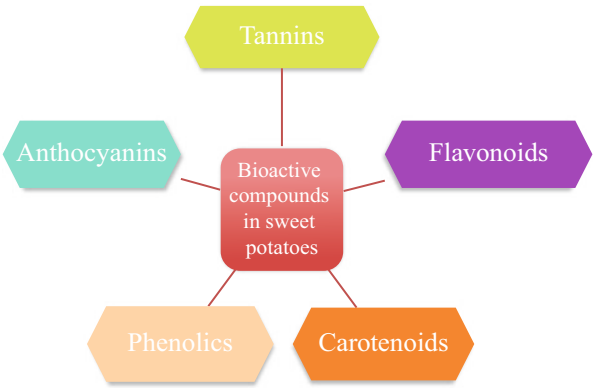


Fig. 3 Bioactive components of sweet potatoes

were 4-*O*- caffeoylquinic acid, 1,3-di-*O*-caffeoylquinic acid, and 3,5-di-*O*-caffeoylquinic acid [38].

Pochapski et al. [39] carried out phytochemical screening that showed positive results for triterpenes/steroids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and phenolic acids. Total contents of alkaloids, anthraquinones, and phenolic compounds in 100 g of the dry sample were reported to be 345.65, 328.44, and 662.02 mg, respectively [39].

Hesam et al. [40] reported that sweet potato contains endogenous amylases along with the high starch content. The two predominant amylases are α- and β-amylases. Since sweet potato is a promising source of β-amylase, they focused their investigation on the properties of β-amylase from white-flesh sweet potato grown in Iran as a potential source for industrial applications [40]. The various bioactive components of sweet potatoes are given in Fig. 3.

4-Ipomeanol a furanoterpenoid produced from infected sweet potatoes has been proven to possess anticancer properties. The following is a detailed description of this wonderful yet less known compound from sweet potatoes.

3.3 4-Ipomeanol

4-Ipomeanol is a stress metabolite produced in response to general damage to sweet potatoes [41]. Clark et al. observed that organisms like *Plenodomus destruens*, *Diaporthe batatatis*, *Diplodia tubericola*, *Fusarium solani*, and *Ceratocystis fimbriata* induced accumulation of relatively high concentration of 4-ipomeanol (5–236 μg/g) in sweet potato [42]. However, it has been recently reported that 4-ipomeanol can also be produced in vivo in the root tubers of *I. batatas* without specifically being infected [43, 44].

3.3.1 Physical and Chemical Properties

The various physical and chemical properties of 4-ipomeanol is given in Table 1 [42, 45]. The structure of 4-ipomeanol is shown in Fig. 4 [46].

Table 1 Various physical and chemical properties of 4-ipomeanol [42, 45]

S. No.	Properties	Data
1	Molecular formula	$C_9H_{12}O_3$
2	Molecular weight	168.18978 g/mol
3	H-bond donor	1
4	H-bond acceptor	3
5	Rotatable bond count	4
6	Topological polar surface area	50.4
7	Description	Colorless to yellowish color oil in state
8	Specific optical rotation	+7.86 deg at 25 deg C/D
9	Flash point	>150 °C
10	Maximum absorption	211 nm
11	Rf value	0.65 [methanol/benzene (1:10)]
12	GC-MS NIST number	131689

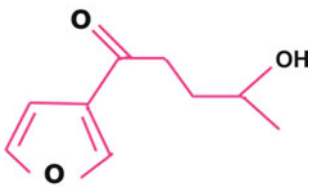


Fig. 4 Structure of 4-ipomeanol [46]

3.3.2 In Vitro Production

A procedure for isolation of 4-ipomeanol from infected sweet potatoes was described by Boyd and coworkers [47]. 4-Ipomeanol was also synthesized chemically from diethyl 3,4-furandicarboxylate in a five-step process [48]. Krauss et al. [49, 50] used another approach for the chemical synthesis of 4-ipomeanol in four steps starting from commercially available furan-3-carbaldehyde. They also synthesized analogues of 4-ipomeanol from furan-3-carbaldehyde and furan-2-carbaldehyde [49, 50]. A protocol for in vitro bioproduction of 4-ipomeanol from the rhizogenic callus of *I. batatas* was reported for the first time by Remya and Subha [43, 44].

3.3.3 Covalent Binding Property

The cytochrome P450 system present in animal bronchial Clara cells activates 4-ipomeanol. Localized cytotoxicity is elicited by the resulting metabolites by their binding to specific macromolecules [51]. Possibility of an influence by differences in species and strain in the covalent binding of 4-ipomeanol was studied by Dutcher and Boyd [52]. Investigation of three strains of rat, Hartley guinea pigs, albino New Zealand rabbits, golden Syrian hamsters, and six strains of mouse divulged that lungs were the main target for 4-ipomeanol covalent binding and toxicity. In addition to pulmonary damage, liver and kidney necrosis was reported in the hamster and the mouse strains. Principal site of damage was found to be the

lung in rats, guinea pig, and rabbits. In all the six mouse strains studied, covalent binding of 4-ipomeanol was approximately twice as high in kidney as in lung; there was some lung damage and remarkable renal toxicity [52]. Though birds (Japanese quail, chickens), devoid of Clara cells in their respiratory tracts, do not exhibit pulmonary toxicity after 4-ipomeanol, they develop severe hepatic injury [53].

Following [14C] 4-ipomeanol administration to rats, Boyd et al. demonstrated that radioactivity was concentrated in the lungs, and 90% was covalently bound [54]. Outcome of another study by Boyd showed that electron-dense granules which later became necrotic were found specifically localized over Clara cells following the administration of [14C] 4-ipomeanol to rats, mice, and hamsters. On the contrary, it was observed that the adjacent ciliated bronchiolar cells and other major pulmonary parenchymal cells were neither radiolabeled nor necrotic. When animals were pretreated with piperonyl butoxide, an inhibitor of cytochrome P450, there was a considerable reduction in covalently bound radioactivity, and there was no necrosis in the Clara cells [55]. Covalently bound 4-ipomeanol was most heavily concentrated over the apical cap of Clara cells where the cytochrome P450 enzymes are concentrated [56, 57]. Studies implied that formation of a reactive metabolite was essential for covalent binding and that it was localized in lung proteins. It was also observed that pretreatment with diethylmaleate helped increase covalent binding of the reactive metabolite of 4-ipomeanol in both the lung and liver and reduced the LD50 value. The "vital macromolecules" to which the reactive metabolite of 4-ipomeanol is covalently bound are tissue proteins and not nucleic acids. This was revealed by studies that showed that hot trichloroacetic acid or perchloric acid dislodged nucleic acids and the insoluble material left behind was mainly protein [56, 58, 59].

3.3.4 Therapeutic Potential

4-Ipomeanol has been proven to have exhibited both in vitro and in vivo antitumor activity against non-small cell lung cancer (NSCLC), and therefore was suggested as an antitumor agent specific to lung cancer [60, 61]. The clinical development of 4-ipomeanol as a lung cancer-specific agent was based on its organ-specific toxicity in preclinical studies in mammals [62]. In animals like rabbits, rats, guinea pigs, female mice, and dogs, preferential activation of 4-ipomeanol occurs in the lung Clara cells. To a lesser extent the compound is also activated by type II pneumocytes abundant in cytochrome P450 isoenzymes [55]. It could be observed that toxicity is predominantly pulmonary, due to the binding of active intermediate to nucleophilic macromolecules, leading to bronchiolar epithelial necrosis preferentially involving Clara cells [53, 55, 63]. In preclinical toxicity studies, 4-ipomeanol has produced dose-dependent pulmonary toxicity [63]. The primary sites of 4-ipomeanol binding in mammals are lungs and proximal renal cortical tubules [51, 64]. In male rats administered with radiolabeled 4-ipomeanol, radioactivity was highest in lung tissue followed successively by intestines, liver, and kidney [54].

Rowinsky et al. [65] observed cytotoxic effects in the human lung, liver, and kidney, presumably because all these organs contain the isoforms of cytochrome P450 that could metabolically activate 4-ipomeanol. These results suggested that

clinical evaluation of 4-ipomeanol in humans should be extended to include liver cancers, and renal cancers, in addition to lung cancers [65]. Phase I study on 4-ipomeanol in patients with non-small cell lung cancer (NSCLC) was performed by Kasturi et al. [66].

It was noticed that 4-ipomeanol may be preferentially activated in the human liver rather than the lung, and these results formed the basis for evaluating the clinical activity and for evaluating the toxicity of 4-ipomeanol in patients with advanced hepatocellular carcinoma [67].

Studies on the human CYP4B1 isozyme revealed that its metabolic activity toward 4-ipomeanol is <1% of the rate of rabbit CYP4B1 [68]. Expression of rabbit CYP4B1 in rat and human glioma cell lines made these cells highly susceptible to 4-ipomeanol [69]. Mohr et al. demonstrated that the CYP4B1/4-ipomeanol prodrug-activating system is effective in inducing cell death of hepatocellular carcinoma cells at low 4-ipomeanol concentrations. It was proposed that this system might be useful for augmentation of standard chemotherapy or gene therapy [70].

Constanze et al. demonstrated that a proline residue at position 427 in human CYP4B1 is important for 4-ipomeanol bioactivation. They developed a novel human suicide gene system that could be used for adoptive cellular therapies by modifying the human CYP4B1 enzyme for efficient activation of 4-ipomeanol [71].

Cytotoxicity induction by 4-ipomeanol (4-IM) in combination with ionizing radiation in cells transfected with a fusion protein of rabbit cytochrome CYP4B1 under influence of EGR1 (radiation inducible promoter) was investigated by Hsu et al. [72]. The results indicated that EGR1-CYP4B1/4-IM system is a feasible radiation-gene therapy system that may allow effective control of cytotoxicity by therapeutic radiation fields.

Cytotoxic properties of cytochrome P450 4B1 (CYP4B1)-activated 4-ipomeanol for prodrug-activated gene therapy was reported by Jang et al. [73].

Roellecke et al. recently conducted a study that was aimed at developing a clinically relevant self-inactivating lentiviral vector for systematic co-expression of CYP4B1 as an ER-located protein. The study lead to the development of a novel human suicide gene systems that is based on human CYP4B1 and 4-ipomeanol. 4-Ipomeanol was found to induce apoptosis in primary T cells co-expressing mutant CYP4B1 and the divergently located MACS selection and chimeric antigen receptor genes [74].

Recent studies on 4-ipomeanol have revealed its potential to be used as an imaging agent as well for gene prodrug activation therapy. (18)F-Labeled 4-ipomeanol could be used to image tumors and monitor enzyme-activating anticancer prodrugs [75].

4 Conclusion

Sweet potatoes are rich source of nutrients and bioactive components. They have been proven to exhibit anticancer, antidiabetic, cardioprotective, antimicrobial, immune boosting, and hepatoprotective properties. Among the various secondary

metabolites from sweet potatoes, 4-ipomeanol holds promise as a potential anticancer agent and offers prospects for further research. Further research in the direction of utilizing 4-ipomeanol to its maximum potential in the treatment of different types of cancers through effective bioactivation, enhancement of metabolic stability, structure modification, inclusion of biomarkers, evaluation of its utility in imaging of specific types of tumors, and other techniques is essential.

References

1. Purseglove JW (1972) Tropical crops: dicotyledons, vol 1. Longman, London
2. Woolfe JA (1992) Sweet potato–past and present. Cambridge University Press, Cambridge
3. Loebenstein G, Fuentes S, Cohen J, Salazar LF (2003) Sweet potato. In: Loebenstein G, Thottappilly G (eds) Virus and virus-like diseases of major crops in developing countries. Kluwer, Dordrecht
4. Zhao G, Kan J, Li Z, Chen Z (2005) Characterization and immunostimulatory activity of an (1→6)-a-D-glucan from the root of *Ipomoea batatas*. Int Immunopharmacol 5:1436–1445
5. Parle M, Monika (2015) Sweet potato as a super food. Int J Res Ayurveda Pharm 6:557–562
6. Abel C, Busia K (2005) An exploratory ethno botanical study of the practice of herbal medicine by the Akan peoples of Ghana. Altern Med Rev 10:112–122
7. Pochapski MT, Fosquiera EC, Esmerino LA, Santos EB, Farago PV, Santos FA et al (2011) Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from *Ipomoea batatas* (L.) Lam. Pharmacogn Mag 7:165–170
8. Ludvik B, Neuffer B, Pacini G (2004) Efficacy of *Ipomoea batatas* (Caiapo) on diabetes control in type 2 diabetic subjects treated with diet. Diabetes Care 27:436–440
9. Emmanuel N (2010) Ethno medicines used for treatment of prostatic disease in Foumban, Cameroon. Afr J Pharm Pharmacol 4:793–805
10. Duke JA, Wain KK (1981) Medicinal plants of the world. Computer index with more than 85,000 entries, 3 vols, Plants genetics and germplasm Institute. Agriculture Research Service, Beltsville, Maryland
11. Diaz JL (1976) Usos de las Plantas Medicinales de Mexico. Monografias Cientificas II. Instituto Mexican para el Estudio de las Plantas Medicinales, A.C., Mexico
12. Reed CF (1976) Information summaries on 1000 economic plants. Typescripts submitted to the USDA
13. Motsa NM, Modi AT, Mabhaudhi T (2015) Sweet potato (*Ipomoea batatas* L.) as a drought tolerant and food security crop. S Afr J Sci 111:11–12
14. Anbuselvi S, Muthumani S (2014) Phytochemical and antinutritional constituents of sweet potato. J Chem Pharm Res 6:380–383
15. Panigoro R, Dhianwaty D (2014) Total glucose and crude fiber in local red sweet potato [*Ipomoea batatas* L. (Lam)] tuber. Int J Pharm Pharm Sci 6:147–149
16. Woolfe J (1992) Sweet potato an untapped food resource. Cambridge University Press, Cambridge, UK
17. Bovell-Benjamin AC (2007) Sweet potato: a review of its past, present and future roles in human nutrition. Adv Food Nutr Res 52:1–59
18. Walter WM, Catignani GL, Yow LL, Porter DH (1983) Protein nutritional value of sweet potato flour. J Agric Food Chem 31:947–949
19. https://www.livescience.com/46016-sweet-potato-nutrition.html
20. Laurie SM, Van Den Berg AA, Magoro MD, Kgonyane MC (2004) Breeding of sweet potato and evaluation of imported cultivars in South Africa. Afr Crop Sci J 12:189–196
21. Leighton CS (2007) Nutrient and sensory quality of orange-fleshed sweet potato. MSc dissertation, University of Pretoria, Pretoria

22. Alam MK, Rana ZH, Islam SN (2016) Comparison of the proximate composition, total carotenoids and total polyphenol content of nine orange-fleshed sweet potato varieties grown in Bangladesh. Foods 5:64
23. Sanoussi F, Adjatin A, Dansi A, Adebowale A, Sanni LO, Sanni A (2016) Mineral composition of ten elites sweet potato (*Ipomoea batatas* [L.] Lam.) Landraces of Benin. Int J Curr Microbiol App Sci 5:103–115
24. Shafe MO, Eze ED, Ubhenin AE, Tende JA (2016) Effects of aqueous tuber extract of *Ipomea batatas* on cardiac enzymes, lipid profile and organ weights in Wistar rats. J Basic Appl Res 4:414–417
25. Mercy Margaret T, Krishna P, Revathi B, Eswar Tony D, Sathish Kumar M, Narendra Babu A (2013) Assessment of in vitro anti inflammatory activity of aqueous extract of *Ipomoea batatas* tubers. Asian J Res Biol Pharm Sci 1:47–53
26. https://www.organicfacts.net/health-benefits/vegetable/health-benefits-of-sweet-potatoes.html
27. Washio M, Mori M, Sakauchi F, Watanabe Y, Ozasa K, Hayashi K et al (2005) Risk factors for kidney cancer in a Japanese population: findings from the JACC study. J Epidemiol 15:S203–S211
28. Shekhar S, Mishra D, Buragohain AK, Chakraborty N (2015) Comparative analysis of phytochemicals and nutrient availability in two contrasting cultivars of sweet potato (*Ipomoea batatas* L.) Food Chem 173:957–965
29. Nwosisi S, Nandwani D, Ravi R (2017) Bioactive compounds in organic sweetpotato. J Adv Mol Biol 1:81–90
30. Sucharitha M, Kotesh M, Devika K, Naresh Y, Kiran M (2016) Evaluation of diuretic activity of aqueous extract of *Ipomoea batatas* (L). Sch J Appl Med Sci 4:1902–1905
31. Park SY, Lee SY, Yang JW, Lee J-S, Oh S-D, Oh S, Lee SM, Lim M-H, Park SK, Jang J-S, Cho HS, Yeo Y (2016) Comparative analysis of phytochemicals and polar metabolites from colored sweet potato (*Ipomoea batatas* L.) tubers. Food Sci Biotechnol 25:283
32. Oluyori PA, Olatunji GA (2016) Antimicrobial and antioxidant activity of peels' extracts from *Ipomoea Batatas* L. Phytochem Anal 6:157–164
33. Rosas-Ramírez D, Pereda-Miranda R (2013) Resin glycosides from the yellow-skinned variety of sweet potato (*Ipomoea batatas*). J Agric Food Chem 61:9488–9494
34. Kang H, Kwak Y-G, Koppula S (2014) Protective effect of purple sweet potato (*Ipomoea batatas* Linn, Convolvulaceae) on neuroinflammatory responses in lipopolysaccharide-stimulated microglial cells. Trop J Pharm Res 13:1257–1262
35. Choi JH, Choi CY, Lee KJ, Hwang YP, Chung YC, Jeong HG (2009) Hepatoprotective effects of an anthocyanin fraction from purple-fleshed sweet potato against acetaminophen-induced liver damage in mice. J Med Food 12:320–326
36. Cuevas Montilla E, Hillebrand S, Winterhalter P (2010) Anthocyanins in purple sweet potato (*Ipomoea batatas* L.) varieties. Fruit Veg Cereal Sci Biotechnol 5:19–24
37. Aldi Y, Dillasamola D, Florina T, Friardi D (2016) Activity and capacity test of macrophage peritoneal cell and number leukocyte of ethanol extract purple sweet potato peel *Ipomoea batatas* (L.) Lam. Res J Pharm Biol Chem Sci 7:178–186
38. Jung JK, Lee SU, Kozukue N, Levin CE, Friedman M (2011) Distribution of phenolic compounds and antioxidative activities in parts of sweet potato (*Ipomoea batata* L.) plants and in home processed roots. J Food Compos Anal 24:29–37
39. Pochapski MT, Fosquiera EC, Esmerino LA, Dos Santos EB, Farago PV, Santos FA, Groppo FC (2011) Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from *Ipomoea batatas* (L.) Lam. Pharmacogn Mag 26:165–170
40. Hesam F, Taheri Tehrani R, Balali GR (2015) Evaluation of β-amylase activity of sweet potato (*Ipomoea batatas*) cultivated in Iran. J Food Biosci Technol 5:41–48
41. Wilson BJ, Yang DTC, Boyd MR (1970) Toxicity of mould-damaged sweet potatoes (*Ipomoea batatas*). Nature 227:521–522
42. Clark CA, Lawrence A, Martin FA (1981) Accumulation of furanoterpenoids in sweet potato tissue following inoculation with different pathogens. Phytopathology 71:708–711

43. Remya M, Subha S (2015) Production of 4-ipomeanol, an anticancer agent from the root tubers and rhizogenic callus of *Ipomoea batatas* Lam. – a comparative study. Indian J Exp Biol 53:297–304
44. Remya M, Subha S (2017) Optimization of process parameters for bioproduction, isolation and purification of 4-ipomeanol, an anticancer agent from cell suspension cultures of *Ipomoea batatas* (L.) Lam. Indian J Exp Biol 55:191–196
45. https://pubchem.ncbi.nlm.nih.gov/compound/4-Ipomeanol
46. Remya M, Subha S (2014) Sweet potato [*Ipomoea batatas* (L.) Lam.] – a valuable medicinal food: a review. J Med Food 17(7):733–741
47. Boyd MR, Burka LT, Harris TM, Wilson BJ (1974) Lung-toxic furanoterpenoids produced by sweet potatoes (*Ipomoea batatas*) following microbial infection. Biochim Biophys Acta 337:184–195
48. Boyd MR, Wilson BJ, Harris TM (1972) Confirmation by chemical synthesis of the structure of 4-ipomeanol, a lung-toxic metabolite of the sweet potato, *Ipomoea batatas*. Nat New Biol 236:158–159
49. Krauss J, Bracher F, Unterreitmeier D (2005) A new approach towards (±)-4-ipomeanol and its 2-furyl regioisomer. Turk J Chem 29:635–639
50. Krauss J, Unterreitmeier D (2005) Synthesis of new lipophilic ipomeanol analogues and their cytotoxic activities. Arch Pharm 338:44–48
51. Boyd MR, Reznik-Schuller H (1984) Metabolic basis for the pulmonary clam cells as a target for pulmonary carcinogenesis. Toxicol Rather 12:56–61
52. Dutcher JS, Boyd MR (1979) Species and strain differences in target organ alkylation and toxicity by 4-ipomeanol: predictive value of covalent binding in studies of target organ toxicities by reactive metabolites. Biochem Pharmacol 28:3367–3372
53. Buckpitt AR, Statham CN, Boyd MR (1982) In vivo studies on the target tissue metabolism, covalent binding, glutathione depletion, and toxicity of 4-ipomeanol in birds, species deficient in pulmonary enzymes for metabolic activation. Toxicol Appl Pharmacol 65:38–52
54. Boyd MR, Burka LT, Wilson BE (1975) Distribution, excretion and binding of radioactivity in the rat after intraperitoneal administration of the lung-toxic fur [14C] 4-ipomeanol. Toxicol Appl Pharmacol 32:147–157
55. Boyd MR (1977) Evidence for the Clara cells as a site of cytochrome P450 dependent mixed function oxidase activity in lung. Nature (Land) 269:713–715
56. Boyd MR, Burka LT (1978) In vivo studies on the relationship between target organ alkylation and the pulmonary toxicity of a chemically reactive metabolite of 4-ipomeanol. J Pharmacol Exp Ther 207:687–697
57. Serabjit-Singh CJ, Nisho SJ, Philpot RM, Plopper CG (1988) The distribution of cytochrome P450 monooxygenase in cells of the rabbit lung: an ultrastructural immunochemical characterization. Mol Pharmacol 33:279–289
58. Brooks P, Lawley PW (1964) Evidence for the binding of polynuclear aromatic hydrocarbons to the nucleic acids of mouse skin: relation between carcinogenic power of hydrocarbons and their binding to deoxyribonucleic acid. Nature (London) 202:781–784
59. Boyd MR (1980) Biochemical mechanisms in chemical-induced lung injury: roles of metabolic activation. Crit Rev Toxicol 7:103–176
60. McLemore TL, Liu MC, Blacker PC, Gregg M, Alley MC, Abbotl BJ, Shoemaker RH, Bohlman ME, Liltersl CC, Hubbard WC, Brennan RH, McMahon JB, Fine DL, Eggleston JC, Mayo JG, Boyd MR (1987) Novel intrapulmonary model for orthotopic propagation of human lung cancers in athymic nude mice. Cancer Res 47:5132–5140
61. McLemore T, Coudert B, Adelberg S, Liu MC, Hubbard WC, Litters CC, Eggleston JC, Boyd MR (1988) Metabolic activation of 4-ipomeanol by human pulmonary carcinoma cells propagated in vitro and intrabronchially in nude mice. Clin Res 36:498A
62. Christian MC, Wittes RE, Leyland-Jones B, McLemore TL, Smith AC, Grieshaber CK, Chabner BA, Boyd MR (1989) Ipomeanol: a novel investigational new drug for lung cancer. J Natl Cancer Inst 81:1133–1143

63. Buckpitt AR, Boyd MR (1982) Metabolic activation of 4-ipomeanol by avian tissue microsomes. Toxicol Appl Pharmacol 65:53–62
64. Wolf CR, Statham CN, McMenamin MG et al (1982) The relationship between the catalytic activities of the lung-specific toxicity of the furan derivative, 4-ipomeanol. Mol Pharmacol 22:738–744
65. Rowinsky EK, Noe DA, Ettinger DS, Christian MC, Lubejko BG, Fishman EK, Sartorius SE, Boyd MR, Donehower RC (1993) Phase I and pharmacological study of the pulmonary cytotoxin 4-ipomeanol on a single dose schedule in lung cancer patients: hepatotoxicity is dose limiting in humans. Cancer Res 5:1794–1801
66. Kasturi VK, Dearing MP, Piscitelli SC, Russell EK, Sladek GG, O'Neil K, Turner GA, Morton TL, Christian MC, Johnson BE, Kelley MJ (1998) Phase I study of a five-day dose schedule of 4-ipomeanol in patients with non-small cell lung cancer. Clin Cancer Res 4:2095–2102
67. Lakhanpal S, Donehower RC, Rowinsky EK (2001) Phase II study of 4-ipomeanol, a naturally occurring alkylating furan, in patients with advanced hepatocellular carcinoma. Invest New Drugs 19:69–76
68. Czerwinski M, McLemore TL, Philpot RM et al (1991) Metabolic activation of 4-ipomeanol by complementary DNA expressed human cytochromes P-450: evidence for species specific metabolism. Cancer Res 51:4636–4638
69. Rainov NG, Dobberstein KU, Sena-Esteves M et al (1998) New prodrug activation gene therapy for cancer using cytochrome P450 4B1 and 2-aminoanthracene/4-ipomeanol. Hum Gene Ther 9:1261–1273
70. Mohr L, Rainov NG, Mohr UG, Wands JR (2000) Rabbit cytochrome P450 4B1: a novel prodrug activating gene for pharmacogene therapy of hepatocellular carcinoma. Cancer Gene Ther 7:1008–1014
71. Wiek C, Eva MS, Katharina R, Marcel F, Mariko N, Edward JK, Wolfgang K, Vladimir YY, Christof MK, Allan ER, Hanenberg H (2015) Identification of amino acid determinants in CYP4B1 for optimal catalytic processing of 4-ipomeanol. Biochem J 1:103–114
72. Hsu H, Rainov NG, Quinones A, Eling DJ, Sakamoto KM, Spear MA (2003) Combined radiation and cytochrome CYP4B1/4-ipomeanol gene therapy using the EGR1 promoter. Anticancer Res 23:2723–2728
73. Jang SJ, Kang JH, Lee TS, Kim SJ, Kim KI, Lee YJ, Cheon GJ, Choi CW, Lim SM (2010) Prodrug-activating gene therapy with rabbit cytochrome P450 4B1/4-ipomeanol or 2-aminoanthracene system in glioma cells. Nucl Med Mol Imaging 44:193–198
74. Roellecke K, Virts EL, Einholz R, Edson KZ, Altvater B, Rossig C, von Laer D, Scheckenbach K, Wagenmann M, Reinhardt D, Kramm CM, Rettie AE, Wiek C, Hanenberg H (2016) Optimized human CYP4B1 in combination with the alkylator prodrug 4-ipomeanol serves as a novel suicide gene system for adoptive T-cell therapies. Gene Ther 23:615–626
75. Moon BS, Jang SJ, Lee TS, Chi DY, Lee BC, Kang JH, Kim SE (2013) Synthesis and evaluation of a 18F-labeled 4-ipomeanol as an imaging agent for CYP4B1 gene prodrug activation therapy. Cancer Biother Radiopharm 28:588–597

Anticancer Properties of Lycopene

33

Kazim Sahin, Cemal Orhan, Nurhan Sahin, and Omer Kucuk

Contents

Abstract

Lycopene is an acyclic isomer of beta-carotene, found in red-colored fruits and vegetables, including tomatoes, and their processed products, watermelon, papaya, guava, carrots, red grapefruit, and sweet potatoes. It is synthesized by plants or autotrophic bacteria but not by animals. This work provides an up-to-date

K. Sahin (✉) · C. Orhan · N. Sahin
Department of Animal Nutrition, Faculty of Veterinary Science, Firat University, Elazig, Turkey
e-mail: nsahinkm@yahoo.com; corhan@firat.edu.tr; nsahin@firat.edu.tr

O. Kucuk
Winship Cancer Institute of Emory University, Atlanta, GA, USA
e-mail: omer.kucuk@emory.edu

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_88

overview of mechanisms linking lycopene in the human diet and cancer, considering epidemiological, clinical studies, and experimental data. Dietary lycopene supplementation may reduce the risk of cancers of many organs such as prostate and at the same time retard the growth of tumors. The main protection properties of lycopene against cancer include antioxidant, anti-inflammatory, anti-inhibitory of cancer cell proliferation, anti-apoptotic, increased gap-junctional communication, interferences in insulin-like growth factor 1 receptor signaling pathways, and cell cycle progression and, the ability to improve the metabolic profile. In this context, lycopene has been shown to exert a protective effect in humans or animals with cancers including prostate, breast, gastric, colon, pancreatic, renal, and several other cancers in many studies, although the obtained results are sometimes inconsistent, which warrants further studies focusing on its bioactivity. In this chapter, lycopene supplementation in cancer prevention is reviewed and possible mechanisms of action are discussed in detail.

Keywords

Cancer · Nutrition · Prevention · Lycopene · Molecular mechanism

Abbreviations

4-NQO	4-Nitroquinoline-1-oxide
5-LOX	5-Lipoxygenase
ABCA1	ATP-binding cassette transporter 1
ACF	Aberrant crypt foci
AOM	Azoxymethane
ARE	Antioxidant response element
BRCA	Breast cancer
CAT	Catalase
CDK	Cyclin-dependent kinases
CI	Confidence interval
COX-2	Cyclooxygenase-2
Cx43	Connexin 43
DEN	Diethylnitrosamine
DMBA	7,12-Dimethyl-benz[a]anthracene
DMH	1,2-Dimethylhydrazine
ERK1	Extracellular signal-regulated kinase 1
GJC	Gap-junctional intercellular communication
GSK-3β	Glycogen synthase kinase-3β
HCC	Hepatocellular carcinoma
HepG2	Human hepatocellular liver carcinoma cell line
HFD	High-fat diet
IGF-1	Insulin-like growth factor
IGFBP-3	Insulin like growth factor binding protein 3
iNOS	Inducible nitric oxide synthase
Keap-1	Kelch-like ECH-associated protein 1-

LXRα	Liver X receptor alpha
MCF-7	Human breast adenocarcinoma cell line
MMP-2	Matrix metalloproteinase 2
MMP-7	Matrix metalloproteinase 7
MMP-9	Matrix metalloproteinase 9
MNU	*N*-methyl-*N*-nitrosourea
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NNK	4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridal)-1-butanone
Nrf2	Nuclear factor-E2-related factor 2
ORs	Odds ratios
PCB	Polychlorinated biphenyls
PCNA	Proliferating cellular nuclear antigen
p-mTOR	Phosphorylated mammalian target of rapamycin
PPARγ	Peroxisome proliferator-activated receptor gamma
PSA	Prostate-specific antigen
RCC	Renal cell carcinoma
ROS	Reduction oxidative stress
SOD	Superoxide dismutase
TNF-α	Tumor necrosis factor-alpha

1 Introduction

There is a relationship between diet-related factors, especially nutritional factors in foods and the risk of cancer [1–3]. Consumption of many bioactive substances naturally found in foods, especially antioxidant compounds such as lycopene, is related to a lower risk of cancer [4]. Lycopene, a natural pigment synthesized by plants and microorganisms, is mostly found in tomato products, watermelon, papaya, guava, and pink grapefruit [5]. Lycopene prevents the growth of tumors and inhibits tumorigenesis through a variety of mechanisms, such as cell cycle arrest and/or apoptosis stimulation, modulation of growth factor signaling, modulation of redox status, alterations in cell growth-related enzymes, improvement of gap junctional communication, and inhibition of inflammation [5]. Moreover, in studying the relation of lycopene to cancer prevention, we have found that lycopene improved the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 expression and reduced NF-κB/cyclooxygenase-2, preventing the inflammatory cascade and stimulating antioxidant signaling. Lycopene treatment also reduced carcinogenesis associated rises in the phosphorylated mammalian target of rapamycin (p-mTOR), phosphorylated p70 ribosomal protein S6 kinase 1 (S6 K1), phosphorylated 4E-binding protein 1 (p-4E-BP1), and protein kinase B [6]. In this chapter, we review the latest data from in vitro, animal, and human studies to evaluate the protective effects of lycopene in cancer prevention and its mechanisms of action.

Table 1 Dietary source of lycopene [7–9]

Source	μg/g wet weight
Raw tomato	8.8–42
Tomato juice	86–100
Tomato sauce	63–131
Tomato ketchup	124
Pink grapefruit	3.6–34
Pink guava	54
Watermelon	23–72
Papaya	20–53
Rosehip puree	7.8
Apricot	<0.1

2 Lycopene

Lycopene, found in tomatoes, red fruits, and vegetables, including carrots, watermelons, apricot, papayas, guava, and strawberries, and discovered in 1999 by Nguyen and Schwartz, is a tetra-terpene from the carotenoid family [7]. Processed tomato products such as tomato sauce, tomato paste, and ketchup are more concentrated lycopene sources than unprocessed tomatoes. The lycopene-rich sources are shown in Table 1 [7–9].

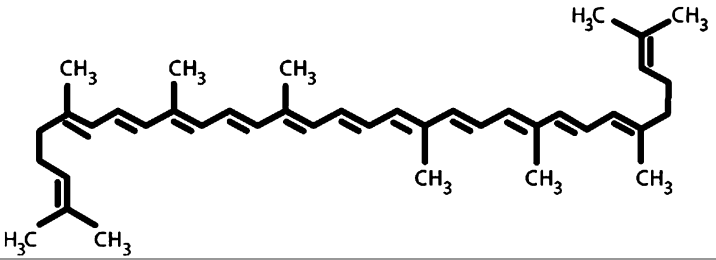
Lycopene's chemical name is 2,6,10,14,19,23,27,31-octamethyl 2,6,8,10,12,14,16,18,20,22,24,26,30-dotriacontatridecaene. Its formula and molecular weight are C40H56 and 536.85 [8, 9]. Physicochemical properties of lycopene are shown in Table 2. Its structural formula is shown in Fig. 1.

The lycopene consists of eight isoprene units comprising 40 carbon and 56 hydrogen atoms [10]. Lycopene is an unsaturated hydrocarbon with 11 conjugates and 2 unconjugated double bonds, which are highly reactive with oxygen and free radicals [11, 12]. Lycopene in natural plants presents mainly in trans configuration. Conversely, induced via chemical reactions, light, and thermal energy, it can also form cis-trans isomers including 15-, 13-, 11-, 9-, 7-, 5-cis isomers [13]. A study presented that the 5-cis isomer of lycopene is the greatest stable one followed by the all-trans, 9-cis, 13-cis, 15-cis, 7-cis, and 11-cis isomers [14]. Heat processes applied to tomato and tomato products increase isomerization to lycopene cis form and increase bioavailability [13–15].

3 Molecular Targets/Mechanisms of Action of Lycopene in Cancer Prevention

Various studies have shown that lycopene has a protective influence against oxidative stress, an important factor in cancer formation [9]. This may be because lycopene is the most effective singlet oxygen quencher among carotenoids

Table 2 Physicochemical properties of lycopene [9].

Molecule	Lycopene
Structure	
IUPAC name	(6E,8E,10Z,12Z,14E,16E,18E,20Z,22Z,24E,26E)-2,6,10,14,19,23,27,31-Octamethyldotriaconta-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene
Formula	$C_{40}H_{56}$
Molecular weight (g/mol)	536.87264
Solubility	Ethanol and methanol, soluble in chloroform, hexane, benzene, carbon disulfide, acetone, petroleum ether and oil, insoluble in water
Melting point (°C)	172–175
Stability	Sensitive to high temperature, acids, catalyst, light, oxygen, metal ions. Store at −70 °C. Combustible. Incompatible with strong oxidizing agents

[16]. Moreover, it was reported that lycopene is an antioxidant 10 times more potent than alpha-tocopherol and twice as potent as beta-carotene [17]. Consumption of tomato or tomato products reduces oxidative DNA damage [18], oxidative stress sensitivity in lymphocytes [19], and lipid peroxidation [20]. Lycopene intake also increases the production of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx) enzymes [21, 22]. In an animal model, animals reared under the stress conditions had lower lycopene concentration in sera and tissue samples, SOD, CAT, and higher MDA levels than unstressed birds. Variations were observed in serum and tissue lycopene and MDA concentrations and antioxidant enzymes as dietary lycopene level increased [21]. Lycopene may also scavenge peroxynitrite, resulting in oxidized lycopene products [23].

Lycopene also induces phase II detoxification enzymes to protect cells from ROS [24, 25]. It activates the transcription system of the antioxidant response elements/electrophile response elements (ARE) transcription system by disturbing cytosolic connections between the main ARE-activating Nrf2 and the inhibitor Keap1 [24]. In another study, increased lycopene in the diet inhibited Keap1 expression in muscle and improved Nrf2 expression, which was augmented by 150% and reduced by 40%, in response to stress [21].

Besides antioxidant effects, other important mechanisms that explain the anticarcinogenic effect of lycopene are: (i) upregulation of gap-junctional gene connexin 43 (Cx43), (ii) the inhibition of cancer cell proliferation and stimulation of differentiation by regulating the expression of cell cycle regulatory proteins, (iii) regulation of the IGF-1/IGFBP-3, (iv) inhibition of 5-lipoxygenase (5-LOX), (v) regulation

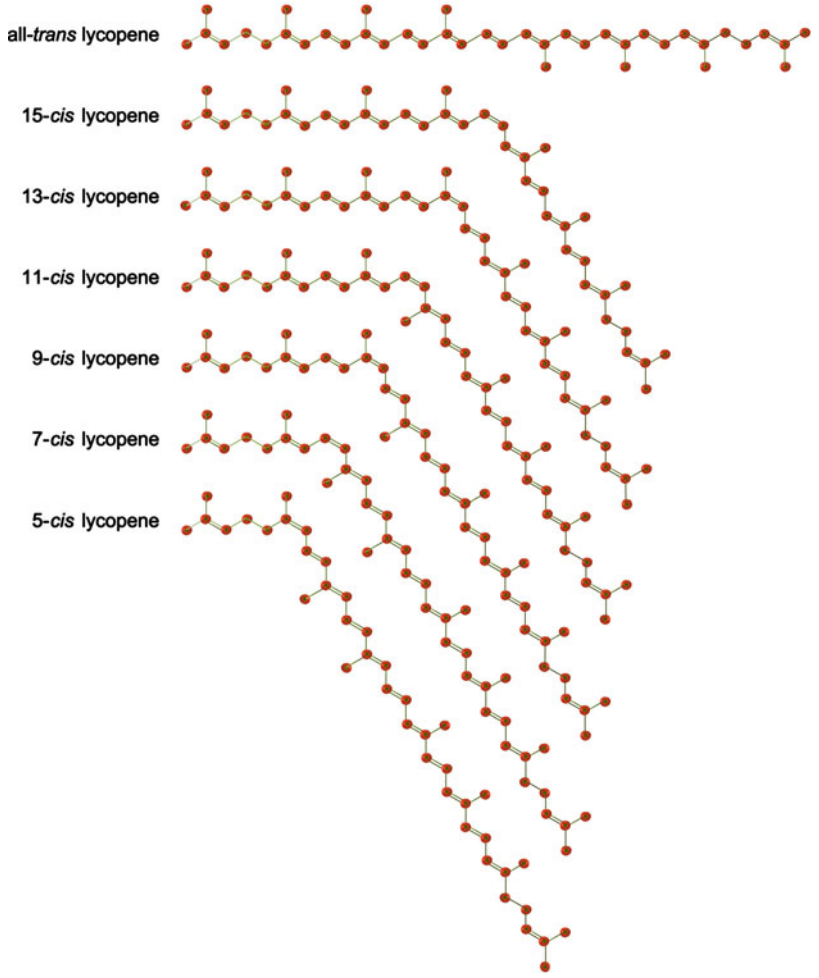


Fig. 1 Structure formula of lycopene

of carcinogenic metabolizing enzymes, (vi) modulation of immunity, (vii) regulation of carcinogen-metabolizing enzymes, and (viii) decrease of oxidative stress by reducing ROS-producing enzymes (Figs. 2 and 3).

Improved Cx43 expression and rises in gap-junctional intercellular communication (GJC) have been observed to arise after human cells were treated in culture with various carotenoids including lycopene [26]. This effect is intensely associated with the capacity of these carotenoids to suppress neoplastic transformation in model cell culture [26, 27], which is an effect shared by retinoid [28]. Diminished expression of connexins, considered as tumor suppressor genes, has been demonstrated in human tumors compared to normal tissue [29–31]. Stahl et al. [32] reported that lycopene

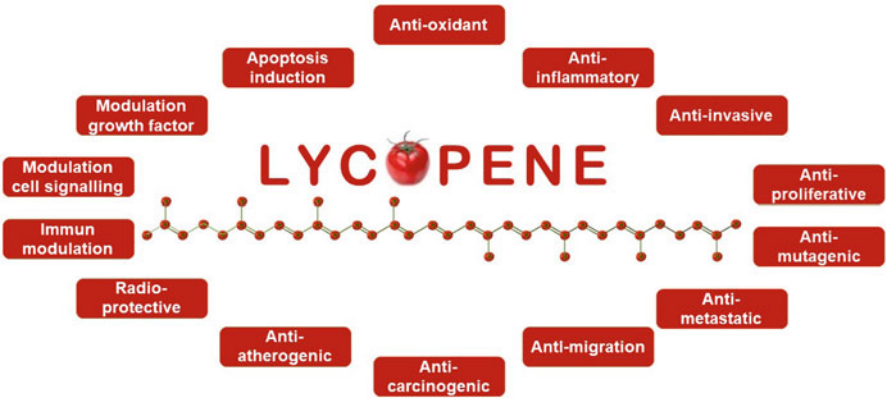


Fig. 2 Mechanism of lycopene in the prevention of chronic diseases

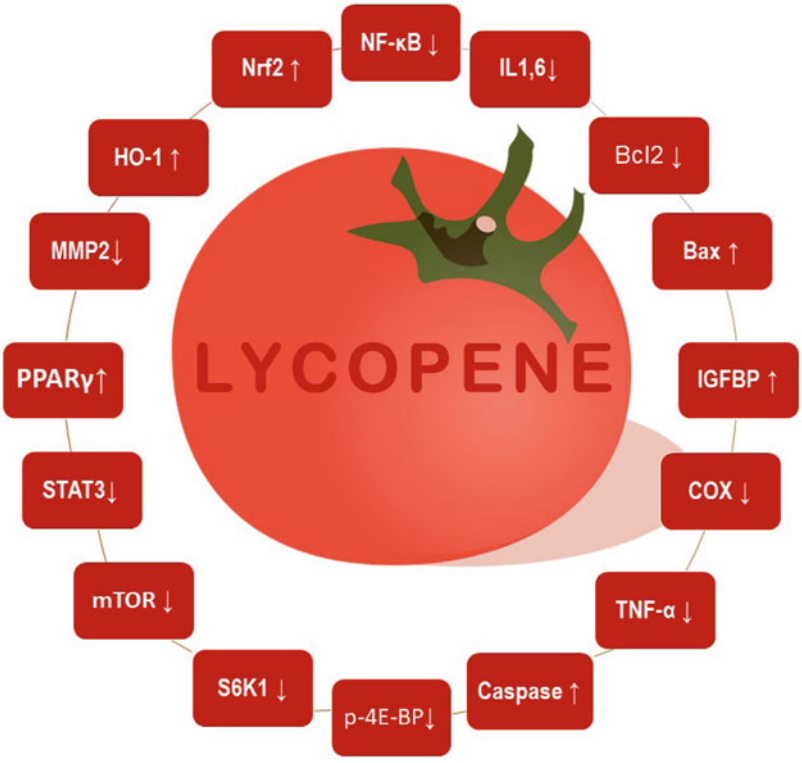


Fig. 3 Molecular targets of lycopene

improved GJC fetal skin fibroblasts after 1 and 3 days of treatment. In addition, Cx-43 expression was basically upregulated by lycopene administration in KB-1 human oral tumor cells, and to a much-reduced extent with β-carotene, thus preventing proliferation and increasing GJC [33, 34]. On the other hand, lycopene acts at multiple points to inhibit VEGF-mediated angiogenesis and suppresses Akt activation by preventing phosphorylation of the molecule [35]. Lycopene also inhibits the NF-κB pathway by inhibiting nuclear translocation and NF-κB DNA binding [36]. Lycopene also acts to inhibit invasion and metastasis via reducing plasma levels and activity of MMP-2 and -9 [37].

Lycopene treatment inhibits tumor formation by affecting various cellular processes such as cell cycle progression and signal transduction pathways [38]. Nahum et al. [39] reported that reduction of cyclin D1 expression by lycopene supplementation and consequent inhibition of cell cycle progression in the G0/G1 phase are significant mechanisms for the decrease of mitogenic effect of IGF-1. Lycopene supplementation significantly reduced serum IGF-1 levels in humans with colon cancer [40]. One study reported that accumulation of lycopene and vitamin E in the tumor and macroscopic estimation of tumors by magnetic resonance imaging presented a significant increase in necrotic area with lycopene plus vitamin E in a prostate cancer model [41].

Lycopene acts via its anti-inflammatory effects to prevent cancer. For example, lycopene inhibits the mRNA and protein expression of the proinflammatory cytokine IL-8 through the NF-κB inactivation. Moreover, lycopene administration was revealed to result in reduced p38MAPK ERK1/2 and JNK phosphorylation and increased PPARγ expression, resulting in improved PTEN activity and the inactivation of AKT [42]. Lycopene treatment has been shown to induce NF-κB inactivation by inhibiting the phosphorylation of IKKα and IKBα. It was also reported that lycopene inhibits TNFα, COX-2, iNOS, and IL-6 secretion [37, 43] (Fig. 3).

Lycopene treatment may suppress levels of nonphosphorylated β-catenin protein and Akt activation, and increase the phosphorylation of β-catenin, which were linked with decreased cyclin D1 expression [35]. Therefore, lycopene treatment inhibits Wnt/β-catenin pathways through the linking along the Akt/GSK3β/β-catenin [44]. The 5-LOX signaling pathway is another potential pathway for lycopene anticancer activity. In one study, it was estimated that the acyclic tomato carotene lycopene and its natural dihydroxy analog lycophyll is bound by the high affinity in the superficial cleft at the interface of the beta-barrel and the catalytic domain of 5-LOX [45].

4 Lycopene in Cancer Prevention

4.1 Breast Cancer

In the etiology of breast cancer, which is most common in women, environmental factors, nutrition, and exercise play an important role. Studies have reported that carotenoids including lycopene reduce the risk of breast cancer perhaps due to their

capability to scavenge DNA damaging free radicals, inhibit cell proliferation, induce apoptosis, and suppress angiogenesis [46, 47]. For example, in an *in vitro* study, treatment with a lycopene-rich extract from red guava (LEG) influenced the viability of human breast cancer cells (MCF-7) but not murine fibroblast cells (NIH-3T3). Additionally, LEG showed low cytotoxicity against BALB/c peritoneal macrophages and no hemolytic activity. LEG triggered a decrease in the cell proliferation and induced cell cycle arrest, DNA fragmentation, alterations in the mitochondrial membrane potential, and morphologic variations linked to granularity and size in MCF-7 cells; nevertheless, it failed to cause any noteworthy damage to the cell membrane or display necrosis or apoptosis [48]. In another cell study, mammary tumor cell lines were cycle-arrested at G1/S phase after treatment with 10 μm lycopene for 48 h by increasing breast cancer-1 (BRCA1) and breast cancer-2 (BRCA2) expression in estrogen-receptor (ER) positive cell lines (MCF-7 and HBL-100) and decreasing them in ER-negative cell lines (MDA-MB-231) [49]. In addition, expression of bcl-2 was reduced by 88% and cell cycle progress was blocked at G(2)/M phase after treatment with lycopene in MCF-7 cell line [50]. Peng et al. [51] also reported that lycopene caused MCF-7 cell contractility and breakage, suggesting that its aggressiveness has decreased in a dose- and time-dependent manner. Additionally, lycopene treatment caused a reduction in cell proliferation and a rise in apoptosis, and it could also upregulate p53 and Bax expression in the cells. Furthermore, lycopene could also increase the expression of p53 and Bax mRNAs in MCF-7 cells [51]. Nahum et al. [52] reported that lycopene treatment inhibits cell cycle progression by inhibiting the level of cyclin D and retention of p27 in cyclin E-cdk2, and inhibiting CDK activities. Assar et al. [36] found that lycopene inhibits breast cancer cell growth at physiologically applicable concentrations of 1.25 μM. This also resulted in a 30–40% decrease in the inhibitor of IκB phosphorylation in cells. In addition, inhibition was detected at the same rate as lycopene for NF-κB activity [36].

In animal studies, it was shown that lycopene inhibits the occurrence and growth of the chemically induced breast cancer [53–55]. In a study, our research group has presented data showing inhibition of mammary cancer incidence, tumor weight and tumor volume by lycopene (70, 48 and 18%), genistein (60, 61, and 35%) and their combination (40, 67 and 65%) were observed in rats, respectively [54]. Combination of lycopene and genistein treatment was more effective in preventing DMBA-induced mammary tumors and regulating the apoptosis-associated protein expression than the treatment by each agent alone [54].

Numerous case-control trials have found associations between the lycopene and mammary cancer in humans. Nevertheless, the results remain inconsistent. Eliassen et al. [56] reported that significant preventive effects with breast cancer were detected for α-carotene, β-carotene, lutein+zeaxanthin, lycopene, and total carotenoids. When the data obtained from case control studies, lycopene decreased breast cancer by 29.0% [46, 57]. A case-control study with 508 breast cancer cases and 508 controls nested in the same cohort was performed and their plasma lycopene levels were not changed. In another nested case control study, 1452 breast cancer cases and 5239 women showed age-adjusted relative risks for breast cancer

increased by lycopene intake 1.0, 1.15, 0.93, 0.97, and 1.01 [58]. In a study comparing lycopene levels in mammary adipose tissue, lycopene was found to be inversely associated with breast cancer risk when adjusted for age, smoking status, and menopausal status [59, 60].

4.2 Lung Cancer

Lung cancer, one of the most common cancers in the world, will be the main cause of cancer deaths in 2020. Studies show that lung cancer patients have lower retinol, α-tocopherol, β-carotene, lycopene, β-cryptoxanthin, selenium, and zinc concentrations [61]. Epidemiological studies have shown that the lung cancer incidence is inversely correlated with fruit and vegetable intake [62, 63]. Tomato and tomato products have also been associated with a lower risk of lung cancer (Table 3) [14]. Lycopene, a bioactive compound of tomato, has been postulated to prevent lung tumorigenesis by cell cycle arrest and/or apoptosis induction, modulation of growth factor signaling, modulation of redox status, cellular changes, alterations in cell growth-related enzymes, and increase in GJC [64].

In an in vitro study, apo-10′-lycopenoic acid has been reported to inhibit the normal human bronchial epithelial cells (NHBE), immortalized normal bronchial epithelial cells (BEAS-2B), and nonsmall cell lung cancer cells (A549) by reducing cyclin E levels, inhibiting cell cycle progression from G (1) to the S phase, and improving cell cycle regulators p21 and p27 proteins [65]. In the same study, pulmonary tumor growth was reduced from a mean of 16 tumors per mouse to an average of 10, 7, and 5 tumors, in a dose-dependent manner, in groups supplemented with apo-10′-lycopenoic acid at 0, 40 and 120 mg /kg [65]. In addition, apo-10′-lycopenoic acid treatment results in nuclear transcription factor Nrf2 accumulation in BEAS-2B human bronchial epithelial cells [66]. In these cells, Nrf2 activation involves the induction of phase II detoxifying/antioxidant enzymes including HO-1, NAD(P)H: quinone oxidoreductase1, glutathione S-transferases, and glutamate-cysteine ligases.

Clinical trials have shown that high intake of lycopene reduces lung cancer risk by approximately 20–30% [67, 68]. In a study in which human plasma and isolated LDL and lycopene solutions were treated with cigarette smoke, depletion of all (E)-lycopenin was higher in plasma than 5 (Z)-lycopenin or beta-carotene. However, LDL was found to be more sensitive to both all (E)- and 5 (Z)-lycopenin than to beta-carotene [69]. Shareck et al. [70] reported that the ORs linked with upper versus lower tertiles of intake were 0.66 for β-carotene, 0.70 for α-carotene, 0.65 for β-cryptoxantin, 0.75 for lycopene, and 0.74 for C vitamins. In a study conducted in Spanish women (130 cases, 206 control), high lycopene consumption was found to be inversely correlated with lung cancer when smoking status, E and C vitamins, and total flavonoid consumption and other carotenoids were adjusted [71]. In another case-control study, a significant inverse relationship between serum lycopene levels and lung cancer mortality after adjusting for cigarettes and serum levels of other

Table 3 Summary of the effect of lycopene in different types of cancer

Cancer	In vitro/in vivo studies	Lycopene formulation	Mechanistical effects	References
Breast cancer	Human breast carcinoma cell line MCF-7	Synthetic 0, 2, 4, 8, and 16 mM 200 mL culture medium	Reduced cell proliferation and increased apoptosis,upregulated the expression of p53 and Bax mRNAs in MCF-7 cells	Peng et al. [51]
Breast cancer	Female Wistar rats	Synthetic 20 mg/kg	Inhibited of tumor growth and expression of apoptosis associated proteins	Sahin et al. [54]
Breast cancer	female Wistar rats	Synthetic 15 mg/kg BW	Decreased tumor proliferation and increased survival rate of treated animals	Jain et al. [184]
Breast cancer	MCF-7 breast cancer cells	0, 2, 4, 6, 8, and 10 μM of lycopene	Modulated cell cycle proteins such as beta tubulin, CK8/18, CK19, and heat-shock proteins in MCF-7 breast cancer cells	Uppala et al. [185]
Breast cancer	Female rats	Synthetic 50 mg/kg of diet	MDA↓, NO↓, SOD↑, CAT↑, GPx↑	Al-Malki et al. [186]
Lung cancer	Mouse model	10, 40, and 120 mg/kg diet of apo-10′-lycopenoic acid	Inhibited the growth of normal human bronchial epithelial (NHBE) cells	Lian et al. [65]
Lung cancer	BEAS-2B human bronchial epithelial cells	10 mM, Apo-10′-lycopenoic acid, apo-10′-lycopenol and apo-10′-lycopenal	Induced both the nuclear accumulation of Nrf2 protein and the induction of phase II detoxifying/antioxidant enzymes,	Lian and Wang [66]
Colon cancer	Human colon cancerHT-29 cells	Synthetic 0, 0.1, 0.5, 1, and 2 μM	Inhibited the phosphorylation of Akt, glycogen synthase kinase-3β (GSK-3β), and ERK 1/2 proteins in human colon cancer HT-29 cells	Lin et al. [44]
Gastric cancer	Male Wistar rats	Synthetic 50, 100, and 150 mg/kg BW	Increased blood IL-2, IL-4, IL-10, TNF-α levels and reduced IL-6 level and enhance blood IgA, IgG, and IgM levels in gastric cancer rats Decreased MDA and increased blood and gastric antioxidant parameters (SOD, CAT and GSH-Px)	Luo et al. [86]

(*continued*)

Table 3 (continued)

Cancer	In vitro/in vivo studies	Lycopene formulation	Mechanistical effects	References
Liver (hepato carcinoma)	SK-Hep-1 cell line	Synthetic 10 μM	Decreased the activities and protein expression of MMP-2 and -9; increased the protein expression of nm23-H1 and the tissue inhibitor of MMP (TIMP)-1 and -2; suppressed protein expression of Rho small GTPases; inhibited focal adhesion kinase-mediated signaling pathway, such as ERK/p38 and PI3K-Akt axis	Yang et al. [187]
Pancreas	Rat pancreatic acinar AR42J cells	Synthetic 2, 10, 20,100 nmol/L	Inhibited the decrease in Ku70 in whole-cell extracts and nuclear extracts, induced by G/GO in AR42J cells	Seo et al. [188]
Renal cancer	Male Sprague-Dawley rats	Synthetic 10 mg/kg	Improved against renal damage caused by Mtx, presumably via antioxidant and anti-inflammatory activities	Oguz et al. [189]
Prostate cancer	Human	Natural (derived from tomato, red watermelon, pink grapefruit, papaya, guava, rose hip canned)	Decreased the risk of prostate cancer	Giovannucci et al. [190]
Prostate cancer	Human	Synthetic 15 mg (twice daily)	Reduced IGF-1 level; increased IGFBP-3 level; decreased tumor growth	Kucuk et al. [161]
Prostate cancer	TRAMP:Bco2 mice	Tomato powder,384 and 462 mg lycopene·kg diet	Lowered incidence of prostate cancer	Tan et al. [191]
Prostate cancer	Human study	Natural, 30 mg of lycopene daily	Lowered PSA level in patients with nonmetastatic prostate cancer	Paur et al. [163]
Prostate ccancer	LNCaP cells	Synthetic 2.5–10 μM	Involved the activation of the PPARγ-LXRα-ABCA1 pathway, leading to reduced cellular total cholesterol levels	Yang et al. [192]
Prostate cancer	LNCaP cancer cells	Synthetic 10 μM	Inhibited DU145 cell proliferation via PPARγ-LXRα-ABCA1 pathway	Yang et al. [151]

Prostate cancer	DU145 prostate cancer cells	Lycopene and apo-12′-lycopenal	Unaltered the levels of the gap junction protein, connexin 43, decreased cell apoptosis rates	Ford et al. [156]
Ovarian cancer	NOD/SCID mice	2.0 ve 5.0 μM lycopene	Decreased the expression of the ovarian cancer biomarker, CA125	Holzapfel et al. [177]
Ovarian cancer	Laying hens	200 or 400 mg of lycopene	Reduced the expression of NF-κB while increasing the expression of nuclear factor erythroid 2 and its major target protein, heme oxygenase 1	Sahin et al. [176]
Photocarcinogenesis (exposed to UV-A radiation)	Human dermal fibroblasts GT-F	Synthetic, 0.5–1.0 μM	Suppressed the increase of collagenase metalloproteinase 1 (MMP-1) mRNA expression	Offord et al. [193]
Skin cancer	Human foreskin fibroblasts and metastatic melanoma	Synthetic, 10 μM	Inhibited PDGF-BB induced fibroblasts migration, attenuated PDGF-BB induced phosphorylation, and reduced PDGF-BB induced signaling.	Chiang et al. [194]
Skin cancer	Albino rats	Lycopene extract from tomatoes vehiculated in nanoemulsions	As lycopene is involved in the synthesis of prostaglandins and phospholipids components of cell membrane	Butnariu and Giuchici [195]
Skin cancer	NMRImice (ears)	(~ 0.05%) lycopene extract	Reduced inflammatory infiltrate in higher extension, which supports the beneficial potential of lycopene.	Ascenso et al [196]
Skin cancer	ICR mice	Synthetic 10–40 mg/kg BW	Reduced the tumor incidence and volume, inhibited the formation of ROS and MDA, protected against the loss of GSH, affected the activities of several oxidant enzymes in skin, translocated higher levels of NF-E2-related factor 2	Shen et al. [134]

(*continued*)

Table 3 (continued)

Cancer	In vitro/in vivo studies	Lycopene formulation	Mechanistical effects	References
Skin cancer	SENCAR mice	Synthetic	Decreased the epidermal thickness, reduced the number of mice with mutation in 61 codon of Ha-ras (anti-initiation effect)	Kowalczyk et al. [197]
Skin cancer	SKH-1 hairless and immunocompetent mice	Red tomato powder	Lowered tumor numbers, metabolomic analyses elucidated compounds derived from tomato glycoalkaloids (including tomatidine and hydroxylated-tomatidine) as significantly different metabolites in skin after tomato exposure	Cooperstone et al. [133]
Skin cancer	SKH-1 hairless mice	Synthetic	Attenuated UVB-induced cell hyper-proliferation and promoted apoptosis, accompanied by decreased cyclin-dependent kinase 2 (CDK2) and CDK4 complex in both human keratinocytes	Chen et al. [132]

carotenoids were observed [72]. On the other hand, there was no significant relationship between lycopene and lung cancer in VITAL study [73].

4.3 Gastric Cancer

Gastric cancer, the fourth most common cancer in the world, is the second leading cause of cancer death. Regular and high intake of fruits and vegetables, and the bioactive substances contained therein such as lycopene, can decrease the gastric cancer risk [74]. These bioactive compounds including lycopene have been observed to increase in the serum and tissues of those who consume them, and high serum levels of lycopene and other carotenoids decrease risk of gastric cancer [75]. In another study, it was reported that lycopene inhibits gastric tumorigenesis by upregulating GSH-dependent hepatic detoxification systems including GSH, GPx, GST, and GR [76]. The combined use of S-allylcysteine, found in garlic, and lycopene decreased gastric cancer via induction of apoptosis by regulating Bcl-2, Bax, and caspases [77]. In an *in vitro* study, lycopene exhibited antiproliferative action in HGC-27 cell lines by enhancing LC3-I, p-ERK expression. In addition, lycopene treatment significantly decreased tumor weight in gastric cancer nude mice model [78]. In clinical trials, lycopene supplementation did not affect the risk of gastric cardiac cancer but reduced the risk of gastric noncardiac cancer by $<33\%$ [79]. In a cohort study, the overall relative risk of gastric cancer for fruits and vegetables were 0.82 and 0.88 [80]. In another case-control study, it was reported that consumption of tomato and tomato products had strong preventive effects on stomach cancer development and its incidence [81]. Moreover, serum lycopene concentrations were lower in patients with *H. pylori* [82]. In a case-control study with 723 gastric cancer patients and 2.879 matched controls in Italy, a significant trend was observed in the reduction of cancer risk due to raw tomato consumption after adjusting for age, gender, education, calorie consumption, alcohol intake, and smoking [83]. In a study involving 191 cases and 570 age-matched controls, the odds ratio for gastric cancer in the highest versus lowest quartile of prediagnostic lycopene was found to be 0.55 [84]. However, Zhou et al. [85] reported that only β-carotene and α-carotene consumption reduced gastric cancer risk. Luo and Wu [86] showed that lycopene treatment to gastric cancer-induced rats mainly up-regulated the redox status and immunity with reduction the risk of gastric carcinoma.

4.4 Liver Cancer

Liver cancer is the fifth most common cancer type in the world. [87]. The most important risk factors for this cancer are hepatitis B and hepatitis C viruses, chronic liver diseases, alcoholism and long-term aflatoxin exposure and other chronic infections [88, 89]. Phytochemicals are powerful potential therapeutic agents for a

multitude of chronic diseases such as cancer, diabetes, and neurodegenerative diseases. It has been reported that supplemental phytochemicals including lycopene and resveratrol are beneficial in the liver cancer treatment [87]. In addition, there is a noteworthy contrary link between serum lycopene, retinol, and retinol-binding protein 4 (RBP4) levels with fibrosis phase in the liver. Serum β-carotene and lycopene are also closely related with their relevant liver levels [90]. In addition, lycopene treatment inhibited metastasis both in vitro and in nude mice in hepatoma SK-Hep-1 cells [91, 92], while the Nrf2-ARE system in HepG2 cells was active [93]. In an experimental study, it was reported that lycopene decreased the number of liver tumor-bearing mice by 49.7% and decreased the average number of tumors per mouse by 88% [94]. Hepatocytes treated with lycopene and beta-carotene are protected from the effects of carcinogenic aflatoxin at both cellular and molecular levels [95]. Conversely, in a study conducted with Long-Evans Cinnamon (LEC) rats, it was stated that lycopene treatment did not diminish spontaneous liver cancer for 70 weeks. In another study, it was found that NADPH oxidase 4 (NOX4) expression and intracellular ROS levels in SK-Hep-1 cells inhibited by 64.3% with lycopene treatment at the highest level in 2.5 μM [96]. In a study, we reported that lycopene supplementation improved the liver CAT, SOD, GSHPx and decreased the NF-κB/COX-2. In addition, lycopene treatment decreased the rises in p-mTOR, p-S6 K1, p-4EBP-1, and protein kinase B [6]. Lycopene treatment is effective against the preneoplastic foci in the liver [97]. Lycopene treatment prevents liver tumor formation in rats spontaneously developing liver tumors and in rats with induced tumor formation with DEN and high fat diet [98]. Cheng et al. [99] showed that treatment of apo-10′-lycopenoic acid in a dose dependent is effective in preventing migration and invasion, suppression of angiogenesis and of liver cells by inhibition of MM-2 expression and activation.

Lycopene supplementation for 26 weeks inhibited the tobacco carcinogen 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced expressions of α7 nicotinic acetylcholine receptor in the lung and NF-κB and CYP2E1 in the liver and reduced the NNK-induced mortality and pathological lesions in both the lungs and livers [100]. Moreover, lycopene treatment for 24 weeks reduced liver NK-κB p65 and STAT3, IL6, and inflammatory foci in mice. Conversely, lycopene treatment in BCO2-KO were related with decreased hepatic endoplasmic reticulum stress-mediated unfolded protein response through reducing ER(UPR)-mediated protein kinase RNA-activated like kinase-eukaryotic initiation factor 2α activation, and inositol-requiring 1α-X-box-binding protein 1 signaling. Lycopene in BCO2-KO mice inhibited Met mRNA, β-catenin, and mTOR complex 1 activation related with augmented liver microRNA (miR)-199a/b and miR214 levels [101]. In another study, it was reported that lycopene decreased levels of ALT, AST, ALP, LDH in mice during initial stages of *N*-nitrosodiethylamine (NDEA)-induced liver cancer. Lycopene treatment of NDEA mice also resulted in decrease of AFP, HIF-1α, VEGF, CD31, MMP-2, and MMP-9 expression in comparison with NDEA group alone [102].

4.5 Pancreatic Cancer

Pancreatic cancer is the eighth most common cause of cancer death in Europe and the fourth in the United States [103]. Chronic pancreatitis is thought to be related with high intake of alcohol, tobacco smoke exposure and obesity, consumption of animal protein and fat and antioxidant deficiencies [104]. Fruits and vegetables may have a role in the inhibition of pancreatic cancer because they comprise potentially protective substances such as carotenoids, tocopherols, and other phytochemicals [103, 105]. These bioactive complexes play a protective role contrary to free radical damage to DNA, improve immune properties, and inhibit IGF by binding to IGF receptors [103, 106]. In a case-control study, there was a substantial contrary relationship between lycopene consumption and pancreatic cancer after adjustment for age, body mass index, smoking, calorie intake, and education [107]. Huang et al. [108] reported an inverse link between lycopene uptake and risk of pancreatic cancer in Caucasians; this association was found to be insignificant in the mixed population. Another meta-analysis study showed that alpha-carotene and lycopene could reduce the risk of prostate cancer [109]. In another case-control study (44 matched controls and 22 pancreatic cancers), it was reported that lycopene levels were lower in cases than in controls, after adjusting for smoking, education, and serum levels of other carotenoids [110]. However, Jeurnink et al. [103] reported that contrary relations with pancreatic cancer risk were detected for serum β-carotene, zeaxanthin, and α-tocopherol [103].

4.6 Colorectal Cancer

There is an inverse relationship between the lycopene intake or tomato products and colorectal cancer [111, 112]. For example, in in vitro study, it was reported an important decrease in the number of viable cells in human colon adenocarcinoma cells (HT-29) and human colon carcinoma cells (T-84) after 48 h of treatment by lycopene. In the same study, it was also demonstrated that lycopene induced cell cycle arrest followed by decreased cell viability in the common of cell lines after 96 h as compared to controls. Moreover, lycopene has been shown to prevent cell proliferation in HT-29 cells with IC50 value of 10 μM. Lycopene also inhibited Akt activation and β-catenin levels in human colon cancer cells [113]. Lycopene inhibited leptin-mediated cell invasion and MMP-2 expression in HT-29 cells [44]. In addition, lycopene plays an important role in leptin-mediated MMP-7 expression and cell invasion of MAPK /ERK and PI3K/Akt signaling pathways. Lycopene could effectively inhibit the phosphorylation of Akt, glycogen synthase kinase-3 (GSK-3p), and ERK1/2 proteins. Huang et al. [114] tested the lycopene and gold nanoparticles (AN) effects on the inhibition of the HT-29 cells. They reported that their combination (AN 10 ppm plus lycopene 12 μM) and nanoemulsion (AN 0.16 ppm plus lycopene 0.4 μM) caused in a 5- and 15-fold rise in early

apoptotic cells of HT-29 compared with the control treatment. Also, the nanoemulsion decreased the caspases 8, 3, and 9, PARP-1, and Bcl-2, while Bax expression was improved. A fivefold decrease in the migration ability of HT-29 cells was detected for this nanoemulsion when compared to control, with the invasion-related markers being reversed through the upregulation of the epithelial marker E-cadherin and downregulation of Akt, NFκB, MMP-2, and MMP-9 [114].

In an animal study, we presented that 5% of the dietary tomato powder decreased the aberrant crypt foci (ACF) ratio and also decreased adenocarcinoma growth and azoxymethane (AOM)-induced colorectal cancer formation in rats. Moreover, tomato powder supplementation shows chemopreventive action by modulation Nrf2/HO-1 pathway in colon tissue while preventing COX-2 levels and inducing apoptosis via the NF-κB pathway [115]. In an in vivo study, lycopene has been shown to be correlated with anticancer effects by suppression of COX-2, PGE2, and phosphorylated ERK1/2 proteins [116]. However, postinitiation treatment with lycopene did not reduce the development of ACF in male B6C3F1 mice [117]. In contrast, lycopene treatment throughout the postinitiation stage decreased ACF growth but not colon tumors in female Fischer 344/NSIc rats [118].

A meta-analysis showed that an inverse relationship was detected between lycopene intake and the colon cancer [112]. In a clinical study, the serum lycopene level was detected to be lower in the patients with colorectal cancer than in the control subjects [119]. However, a study stated that no important relationship was determined between colorectal cancer risk and lycopene consumption in a cohort study, middle-aged men smokers [120]. Case-control studies have reported a 60% decline in the risk of colorectal cancer linked with higher intake of tomatoes [121–123].

4.7 Skin Cancer

UV light penetrates different skin layers and can cause skin damage by affecting specific biological structures at each level. These effects depend on penetration depth, absorption, wavelength, and structural properties affecting reflection and light scattering, molecular patterns, and pigmentation [124]. Carotenoids including lycopene can be used to reduce the adverse effects of UV light that cause damage to the skin. A linear relationship between lycopene consumption and a prevention of UV-induced erythema formation and a reduced risk of nonmelanoma skin cancer were shown [9, 125–127]. In addition, plasma lycopene levels were associated with lycopene levels in skin and a positive effect was found between the β-carotene in the serum and the β-carotene in the skin. However, plasma-skin associations were lower and not significant for lutein, zeaxanthin, and β-cryptoxanthin. More skin lycopene is destroyed compared to β-carotene when skin is exposed to UV light [128]. Consumption of products rich in lycopene and synthetic lycopene for 10–12 weeks decreased the sensitivity toward UV-induced erythema in volunteers [129, 130]. Andreassi [131] reported that lycopene intake could protect skin against the effects of UV-B radiation, particularly when linked with vitamins C and E. Another

study reported that lycopene did not affect the apoptotic status and necrotic and viable cells in unirradiated cells. Nevertheless, irradiated cells were subjected to a rise with lycopene in both dead and viable subpopulations compared to unexposed irradiated cells [127] by resulting in overexpression of Bax gene.

Chen et al. [132] demonstrated that lycopene pretreatment reduced UVB-induced cell hyperproliferation with apoptosis and reduced cyclin-dependent kinase 2 (CDK2) and CDK4 complex both in human keratinocytes and SKH-1 hairless mice. While FOXO3a is phosphorylated in response to UVB irradiation and is involved in cytoplasm, lycopene treatment rescues this sensitization. Gene ablation of FOXO3a showed a lycopene-induced reduction in cell hyperproliferation, CDK2 and CDK4 complexes, a critical role for FOXO3a in the lycopene-stimulated antiproliferative effect of keratinocytes during UVB irradiation. Furthermore, loss of AKT induced more enhanced lycopene-induced FOXO3a dephosphorylation, while the loss of mTORC2 by transfection with RICTOR siRNA induced levels of AKT phosphorylation comparable to those obtained with lycopene. Conversely, overexpression of AKT or mTORC2 decreased the lycopene effects on AKT phosphorylation as well as on the expression of FOXO3a, suggesting that lycopene is due to negative modulation of mTORC2/AKT signaling [132]. In an animal study of mice exposed to 2240 J/m2 UV-B irradiation and fed with red tomato powder diet for 35 weeks, the number of tumors was found to be significantly lower in mice consuming red tomato diet [133].

Shen et al. [134] reported that pretreatment with lycopene for oxidative stress and skin cancer induced by DMBA/12-O-tetradecanoylphosphoryl-13-acetate (TPA) in female ICR mice significantly delayed tumor growth and decreased the tumor incidence and volume. In addition, lycopene prevented the production of ROS and MDA, thereby inhibiting the loss of glutathione and activating antioxidant enzymes in the skin of mice. Furthermore, animals treated with lycopene showed higher levels of Nrf2 into the nucleus compared with control animals [134]. On the other hand, there are also studies reporting that lycopene has no effect on skin photocarcinogenesis [135, 136]. The reason for these conflicting reports could be the lycopene levels, the UVB radiation dosage, the different experimental conditions and models [132].

4.8 Head and Neck Cancer

Numerous reports have suggested that lycopene and tomato products may have useful properties in the head and neck cancer therapy [13, 33, 137, 138]. For instance, Lodi et al. [139] reported that lycopene significantly reduced multiple forms of HNSCC including laryngeal, oral, and pharyngeal cancer. Lycopene and β-carotene (5–40 μM) dose- and time-dependently reduced the viability of the EC109 human esophageal squamous carcinoma cells. Lycopene and β-carotene treatments upregulated the expression of PPARγ and p21WAF1/CIP1 and downregulated the expression of cyclin D1 and COX-2. These modulatory effects of the carotenoid treatments were suppressed by GW9662, an irreversible PPARγ

antagonist, suggesting that the inhibition of EC109 cell viability by lycopene and β-carotene involves PPARγ signaling pathways and the modulation of p21WAF1/CIP1, cyclin D1, and COX-2 expression [140]. Mayne et al. [138] found that only serum lycopene levels were significantly inversely related with total mortality and mortality in nonsmoking patients. In a clinical trial (754 oral cancer cases and 1775 control), lycopene intake was not significantly linked with oral cancer risk [141]. In another study, high tomato consumption reported that OR was 0.49 for oral cancer [142]. In addition, lycopene inhibited the proliferation of human oral cancer cell line KB-1 by enhanced the expression of the gap junctional protein connexin 43 [33].

In an experimental study, an inverse relationship was detected between lycopene at 2.5 mg/ kg lycopene dose and squamous cell carcinomas with buccal cuts in hamsters. In a study on hamsters, consumption of lycopene 2.5 mg/kg resulted in an inverse relationship between buccal pouch squamous cell carcinomas [143]. El-Rouby [144] found that lycopene treatment at a dose of 2.5 mg/kg BW once a day with intragastric intubation decreased the incidence of laryngeal cancer and increased E-cadherin and β-catenin expression in the lycopene-treated group compared to the control group. Another study showed no carcinomas in lycopene or mixed carotenoid groups [99]. In a study of 228 cases and 491 hospital-based controls, tomato consumption was linked with a decrease in the risk of 0.30, while tomato sauce-rich foods had a protecting effect of 0.57 [145]. The nutrient group consisting of raw and tomato-rich foods presented a strong inverse association with head and neck cancer. Lycopene was also related with a reduced risk of 0.22.

4.9 Prostate Cancer

Prostate cancer is the second most common cancer in men in the world and the fifth most common cancer death in men [146]. There are several ways in which lycopene treatment can protect against prostate cancer, including the prevention of DNA damage by scavenging free radicals [147], modulating gene expression related to prostate cancer [148] and slowing of cancer cell growth [149, 150], inhibiting prostate cancer cell proliferation via the PPARγ-LXRα-ABCA1 pathway, [151], and progression of prostate cancer is hampered by apoptosis induction and angiogenesis suppression [149, 152]. The lycopene intake was significantly linked with decreased PCa risk, and dose-response studies shows a significant linear effect for the dietary lycopene and PCa risk, thus PCa reduced by 1% for every 2 mg of lycopene intake. In addition, for each 10 μg dL-1 circulating lycopene in the linear and nonlinear models, the PCa risk decreased by 3.5% to 3.6% [153]. A substantial inverse association between PC and serum lycopene levels has been found between the highest and lowest quintiles of intake [154–156]. It has been reported that upregulation of miR-let-7f-1 targeted AKT2 and AKT2 in PC3 cells may improve the properties caused by miR-let-7f-1 [157]. Van Hoang et al. [150] reported that the risk of PC reduced by lycopene consumption, tomatoes, tomato products, and carrots. There was no statistically significant correlation for the consumption of α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and other carotenoids.

In a case-control study [158, 159], it was reported that in patients with high-concentration lycopene, the risk of aggressive prostate cancers was reduced. In particular, a significant relationship has been established between high plasma lycopene concentration and a strong reduction in aggressive prostate cancers. Similarly, a meta-analysis found an inverse relationship between lycopene consumption or serum lycopene concentrations and the risk of prostate cancer, with a stronger relationship, detected for the circulating lycopene compared to the highest to the lowest level [109]. However, Morgia et al. [160] reported that Se and lycopene were not related with higher prostate cancer risk.

In a randomized two-arm studies, plasma prostate-specific antigen (PSA) concentration were stated to be reduced by 18% in the intervention group and by 14% in the control group. Eleven of fifteen patients had no extra prostatic tissue involvement with surgical limitations and/or cancer in the intervention group, compared with 2 of the 11 patients in the control group. In the lycopene treatment group, 12 of 15 patients had tumors measured 4 cc or less, 5 of 11 in the control group [161]. In the same study, Cx43 in the malignant part of the prostate gland was higher in the group using lycopene than in the control group. Phase II randomized clinical trial of 15 mg of lycopene intake twice a day for 3 days before radical prostatectomy found a reduction in IGF-I concentration, but no significant change in Bax and Bcl-2 [162]. Another study reported that the median PSA of the tomato group was lower in postoperative-moderate-risk patients than in the control group [163]. Patients with the highest rise in serum lycopene, Se, and C20: 5 n-3 fatty acid had a 1% reduction in median PSA values compared to the lowest increase in lycopene, Se and C20: 5 n-3 fatty acids. Additionally, PSA was reduced in patients with the highest increase in lycopene group. It has also been shown that neither prediagnosis nor post-diagnosis dietary lycopene was associated with PC-specific mortality [164]. Consumption of either low or high dose of lycopene (4 and 16 mg/kg) and a single dose of β-carotene (16 mg/kg) was reported to strongly inhibit tumor growth and decreased prostate tumor volume and tumor weight in nude mice.

Lycopene and β-carotene at high dose levels significantly decreased PCNA expression in tumor tissues and the plasma IGF-binding protein-3 levels. Soares et al. [166] found a significant reduction in primary prostate cancer cell viability upon with lycopene treatment obtained tomato-based food products. Moreover, lycopene, containing tomato extract and tomato sauce, showed a 50-fold increase in the proportion of apoptotic cells by upregulation of TP53 and Bax expression and also downregulation of Bcl-2 compared to the control group [166].

4.10 Renal Cell Carcinoma

It is expected that 63.340 new cases of renal cell carcinoma (RCC) (42.680 in men and 22.660 in women), the most common type of renal cancer in adults, will occur in 2018, and about 14.970 people (10.010 men and 4.960 women) will die from renal cancer. Renal carcinoma is among the 10 most common cancers in both men and women. In general, the risk of developing kidney cancer in men and women is about

1 in 48 and 1 in 83 [167]. Many studies suggest that micronutrients may be used as dietary supplements, including α-carotene, β-carotene, lutein, zeaxanthin, lycopene, vitamin A, vitamin C, and selenium. These agents may inhibit oxidative DNA damage, mutagenesis, and tumor growth [168–170]. Nevertheless, some studies have reported that there is no significant relationship between renal cancer and consumption of antioxidant substances [171], but others have provided evidence that some agents may have a protective effect [169].

Ho et al. (2015) indicated that lycopene intake was inversely linked with the risk of renal cancer; when compared with the lowest quintile of lycopene intake, the highest quartile was related with a 39% lower renal cancer risk. The authors described the potential anticancer effects of lycopene as follows: (i) In contrast to provitamin A carotenoid (α-carotene, β-carotene, and β-cryptoxanthin), it is recommended that lycopene, along with other carotenoids without provitamin A activity (lutein and zeaxanthin), has a different enzymatic affinity. For this reason, absorption and metabolism of lycopene cannot be controlled by vitamin A concentration through intestinal-specific homeobox transcription factors [169, 172]. (ii) Lycopene, one of the most hydrophobic carotenoids, has the ability to affect a variety of transcriptional pathways at the molecular level, unlike other carotenoids. It has also been shown that lycopene has the greatest singlet oxygen quenching activity of any carotenoid [12]. These properties make lycopene a potential anticancer agent. In a study done in the predisposed TSC2 mutant Eker rat model, the incidence of tumor changed from 94% in control rats to 65% in the lycopene-treated rats, but the change was not significant. However, the mean number of tumors was significantly reduced in rats treated with lycopene compared to the controls rats. In the same study, it was also reported that in the lycopene-treated rats, number of tumors reduced and the numbers tended to reduction linearly as lycopene level increased from 0 to 200. Control rats fed only basal diet had a greater length of tumors than rats fed lycopene supplement groups. Furthermore, length of tumor reduced and tumor length inclined to reduction linearly as dietary lycopene level increased from 0 to 200 mg/kg. All tumors presented strong staining with antibodies against mTOR, phospho-S6, and EGFR [173]. However, a clinical trial revealed that RCC risk was inversely associated with β-carotene, α-carotene, β-cryptoxanthin, vitamin A, vitamin C, but not with lycopene or vitamin E [174]. A case-control study also suggested a significant opposite relationship between lycopene and renal in the nonsmoking group, but not in the smoking group [175].

4.11 Ovarian Cancer

Ovarian cancer is the seventh most frequently diagnosed cancer in women and the sixth leading cause of death from cancer among women. Recently, we found significantly decreased tumor incidence and serum MDA levels and increased serum lycopene levels in the hens fed a lycopene-enriched diet compared to control

groups. NF-kB and STAT-3 expression were reduced and Nrf-2, PIAS-3, and HO-1 were improved in the ovarian tissues of lycopene-fed animals [176]. Holzapfel et al. [177] reported that lycopene administration decreased the metastatic load of ovarian cancer-bearing mice, while treatment with lycopene reduced the tumor burden. Lycopene administration also improved the antitumorigenic effects of paclitaxel and carboplatin. Tumor and metastatic tissues for Ki67 revealed that lycopene reduced the number of proliferating cancer cells. They also reported that lycopene reduced the expression of CA125. These effects were complemented by regulation of expression of ITGA5, ITGB1, MMP9, FAK, ILK, and EMT markers, reduced expression of integrin α5.

In a human study, Li and Xu [178] established an insignificant contrary link between lycopene intake and ovarian cancer risk, and subgroup analysis stratified by study design, location, histological type of ovarian cancer, and length of dietary recall presented no significant results. In addition, a case-control study conducted by Cramer et al. [179] indicated a significant inverse relationship between consumption of lycopene and tomato sauce and risk of ovarian cancer mainly in premenopausal women. In another study, it was found that fruit- and vegetable-rich nutrition was related with a reduction in the risk of ovarian cancer, and among the most convincing were tomatoes [180]. Helzlsouer et al. [181] found no correlation between serum lycopene levels and ovarian cancer risk. Nevertheless, Jeong et al. [182] have found an inverse link between serum lycopene levels and ovarian cancer risk. rGO-Ag, a new biomolecule, lycopene, and tricostatin A have been shown to inhibit the viability of the human ovarian cancer cell (SKOV3) in a dose-dependent manner [183].

5 Conclusion

In conclusion, lycopene consumption and higher levels of lycopene in the plasma have been associated with a lower risk of cancer in clinical and experimental studies. Lycopene may lower cancer risk by decreasing oxidative stress through modulation of ROS-producing enzymes including COX-2 and 5-LOX and inducing antioxidant/detoxifying phase II enzymes. These phase II enzymes are also modulated by the Nrf2-ARE system. The NFκB and Nrf2/HO-1 signaling pathway is suggested to be an important primary target for chemoprevention by lycopene. Lycopene can inhibit the proliferation and induce differentiation of cancer cells by modulating the expression of cell cycle regulatory proteins, modulating the IGF- 1/IGFBP-3 system and other mechanisms including the prevention of oxidative DNA damage and modulation of the immune function, as well as the inactivation of growth factors. There is a need for more detailed experimental studies and larger clinical trials investigating the effect of lycopene on cancer prevention and integrative cancer treatment.

Acknowledgments The study was supported in part by Turkish Academy of Sciences (KS).

References

1. National Research Council (US) Committee on Diet and Health (1989) Diet and health: Implications for reducing chronic disease risk. The National Academies Press, Washington, DC
2. American Cancer Society (1984) Nutrition and cancer: Causation and prevention. An American Cancer Society special report. CA Cancer J Clin 34:5–10
3. Lee BM, Park KK (2003) Beneficial and adverse effects of chemopreventive agents. Mutat Res 523–524:265–278
4. Tanaka T, Shinimizu M, Moriwaki H (2012) Cancer chemoprevention by carotenoids. Molecules 17:3202–3242. https://doi.org/10.3390/molecules17033202
5. Palozza P, Simone R, Catalano A, Russo M, Bohm V (2012) Lycopene modulation of molecular targets affected by smoking exposure. Curr Cancer Drug Targets 12:640–657. https://doi.org/10.2174/156800912801784866
6. Sahin K, Orhan C, Tuzcu M, Sahin N, Ali S, Bahcecioglu IH, Guler O, Ozercan I, Ilhan N, Kucuk O (2014) Orally administered lycopene attenuates diethylnitrosamine-induced hepatocarcinogenesis in rats by modulating Nrf-2/HO-1 and Akt/mTOR pathways. Nutr Cancer 66:590–598. https://doi.org/10.1080/01635581.2014.894092
7. Nguyen ML, Schwartz SJ (1999) Lycopene: Chemical and biological properties. Food Technol 53:38–45
8. Gupta S, Jawanda MK, Arora V, Mehta N, Yadav V (2015) Role of lycopene in preventing oral diseases as a nonsurgical aid of treatment. Int J Prev Med 6:70. https://doi.org/10.4103/2008-7802.162311
9. Ascenso A, Ribeiro H, Marques HC, Oliveira H, Santos C, Simões S (2014) Chemoprevention of photocarcinogenesis by lycopene. Exp Dermatol 23:874–878. https://doi.org/10.1111/exd.12491
10. Olson JA, Krinsky NI (1995) Introduction: the colorful, fascinating world of the carotenoids: important physiologic modulators. FASEB J 9:1547–1550
11. van Breemen RB, Pajkovic N (2008) Multitargeted therapy of cancer by lycopene. Cancer Lett 269:339–351. https://doi.org/10.1016/j.canlet.2008.05.016
12. Di Mascio P, Kaiser S, Sies H (1989) Lycopene as the most effective biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538
13. Lu R, Dan H, Wu R, Meng W, Liu N, Jin X, Zhou M, Zeng X, Zhou G, Chen Q (2011) Lycopene: features and potential significance in the oral cancer and precancerous lesions. J Oral Pathol Med 40:361–368. https://doi.org/10.1111/j.1600-0714.2010.00991.x
14. Giovannucci E (2002) A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. Exp Biol Med 227:852–859
15. Schierle J, Bretzel W, Buhler I, Faccin N, Hess D, Steiner K, Schuep W (1997) Content and isomeric ratio of lycopene in food and human blood plasma. Food Chem 59:459–565
16. Viuda-Martos M, Sanchez-Zapata E, Sayas-Barberá E, Sendra E, Pérez-Álvarez JA, Fernández-López J (2014) Tomato and tomato byproducts. Human health benefits of lycopene and its application to meat products: a review. Crit Rev Food Sci Nutr 54(8):1032–1049. https://doi.org/10.1080/10408398.2011.623799
17. Kong KW, Khoo HE, Prasad KN, Ismail A, Tan CP, Rajab NF (2010) Revealing the power of the natural red pigment lycopene. Molecules 15:959–987. https://doi.org/10.3390/molecules15020959
18. Bowen P, Chen L, Stacewicz-Sapuntzakis M, Duncan C, Sharifi R, Ghosh L, Kim HS, Christov-Tzelkov K, van Breemen R (2002) Tomato sauce supplementation and prostate cancer: lycopene accumulation and modulation of biomarkers of carcinogenesis. Exp Biol Med (Maywood) 227:886–893
19. Porrini M, Riso P (2000) Lymphocyte lycopene concentration and DNA protection from oxidative damage is increased in women after a short period of tomato consumption. J Nutr 130:189–192. https://doi.org/10.1093/jn/130.2.189
20. Agarwal S, Rao AV (1998) Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. Lipids 33:981–984

21. Sahin K, Orhan C, Tuzcu M, Sahin N, Hayirli A, Bilgili S, Kucuk O (2016) Lycopene activates antioxidant enzymes and nuclear transcription factor systems in heat-stressed broilers. Poult Sci 95:1088–1095. https://doi.org/10.3382/ps/pew012
22. Pereira BLB, Reis PP, Severino FE, Felix TF, Braz MG, Nogueira FR, Silva RAC, Cardoso AC, Lourenco MAM, Figueiredo AM, Chiuso-Minicucci F, Azevedo PS, Polegato BF, Okoshi K, Fernandes AAH, Paiva SAR, Zornoff LAM, Minicucci MF (2017) Tomato (*Lycopersicon esculentum*) or lycopene supplementation attenuates ventricular remodeling after myocardial infarction through different mechanistic pathways. J Nutr Biochem 46:117–124. https://doi.org/10.1016/j.jnutbio.2017.05.010
23. Pisoschi AM, Pop A (2015) The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem 97:55–74. https://doi.org/10.1016/j.ejmech.2015.04.040
24. Sahin K, Tuzcu M, Sahin N, Ali S, Kucuk O (2010 Oct) Nrf2/HO-1 signaling pathway may be the prime target for chemoprevention of cisplatin-induced nephrotoxicity by lycopene. Food Chem Toxicol 48(10):2670–2674. https://doi.org/10.1016/j.fct.2010.06.038
25. Mein JR, Lian F, Wang XD (2008) Biological activity of lycopene metabolites: implications for cancer prevention. Nutr Rev 66:667–683. https://doi.org/10.1111/j.1753-4887.2008.00120.x
26. Bertram JS (1999) Carotenoids and gene regulation. Nutr Rev 57:182–191
27. Zhang LX, Cooney RV, Bertram JS (1992) Carotenoids up-regulate connexin 43 gene expression independent of their pro-vitamin A or antioxidant properties. Cancer Res 52:5707–5712
28. Hossain MZ, Wilkens LR, Mehta PP, Loewenstein W, Bertram JS (1989) Enhancement of gap junctional communication by retinoids correlates with their ability to inhibit neoplastic transformation. Carcinogenesis 10:1743–1748. https://doi.org/10.1093/carcin/10.9.1743
29. Neveu M, Bertram JS (2000) Gap junctions and neoplasia. In: Hetzberg EL, Bittar EE (eds) Gap Junctions. JAI Press, Greenwich, pp 221–262
30. Lee SW, Tomasetto C, Sager R (1991) Positive selection of candidate tumor suppressor genes by subtractive hybridization. Proc Natl Acad Sci U S A 88:2825–2829
31. Omori Y, Yamasaki H (1998) Mutated connexin43 proteins inhibit rat glioma cell growth suppression mediated by wild-type connexin43 in a dominant- negative manner. Int J Cancer 78:446–453. https://doi.org/10.1002/(SICI)1097-0215(19981109)78:4<446::AID-IJC10>3.0.CO;2-4
32. Stahl W, von Laar J, Martin HD, Emmerich T, Sies H (2000) Stimulation of gap junctional communication: comparison of acyclo-retinoic acid and lycopene. Arch Biochem Biophys 373:271–274. https://doi.org/10.1006/abbi.1999.1510
33. Livny O, Kaplan I, Reifen R, Polak-Charcon S, Madar Z, Schwartz B (2002) Lycopene inhibits proliferation and enhances gap-junction communication of KB-1 human oral tumor cells. J Nutr 132:3754–3759. https://doi.org/10.1093/jn/132.12.3754
34. Erdman JW Jr, Ford NA, Lindshield BL (2009) Are the health attributes of lycopene related to its antioxidant function? Arch Biochem Biophys 483:229–235. https://doi.org/10.1016/j.abb.2008.10.022
35. Tang FY, Shih CJ, Cheng LH, Ho HJ, Chen HJ (2008) Lycopene inhibits growth of human colon cancer cells via suppression of the Akt signaling pathway. Mol Nutr Food Res 52:646–654. https://doi.org/10.1002/mnfr.200700272
36. Assar EA, Vidalle MC, Chopra M, Hafizi S (2016) Lycopene acts through inhibition of IκB kinase to suppress NF-κB signaling in human prostate and breast cancer cells. Tumor Biol 37:9375–9385. https://doi.org/10.1007/s13277-016-4798-3
37. Trejo-Solís C, Pedraza-Chaverrí J, Torres-Ramos M, Jiménez-Farfán D, Cruz Salgado A, Serrano-García N, Osorio-Rico L, Sotelo J (2013) Multiple molecular and cellular mechanisms of action of lycopene in cancer inhibition. Evid Based Complement Alternat Med 2013:705121. https://doi.org/10.1155/2013/705121
38. Kelkel M, Schumacher M, Dicato M, Diederich M (2011) Antioxidant and anti-proliferative properties of lycopene. Free Radic Res 45:925–940. https://doi.org/10.3109/10715762.2011.564168
39. Nahum A, Zeller L, Danilenko M, Prall OW, Watts CK, Sutherland RL, Levy J, Sharoni Y (2006) Lycopene inhibition of IGF-induced cancer cell growth depends on the level of cyclin D1. Eur J Nutr 45:275–282. https://doi.org/10.1007/s00394-006-0595-x

40. Walfisch S, Walfisch Y, Kirilov E, Linde N, Mnitentag H, Agbaria R, Sharoni Y, Levy J (2007) Tomato lycopene extract supplementation decreases insulin-like growth factor-I levels in colon cancer patients. Eur J Cancer Prev 16:298–303. https://doi.org/10.1097/01.cej.0000236251.09232.7b
41. Siler U, Barella L, Spitzer V, Scnorr J, Lein M, Goralczyk R, Wertz K (2004) Lycopene and vitamin E interfere with autocrine/paracrine loops in the Dunning prostate cancer model. FASEB J 18:1019–1021. https://doi.org/10.1096/fj.03-1116fje
42. Simone RE, Russo M, Catalano A, Giovanni M, Kati F, Volker B, Paola P (2011) Lycopene inhibits NF-KB-Mediated IL-8 expression and changes redox and PPARγ signalling in cigarette smoke-stimulated macrophages. PLoS One 6(5):e19652. https://doi.org/10.1371/journal.pone.0019652
43. Feng D, Ling WH, Duan RD (2010) Lycopene suppresses LPS-induced NO and IL-6 production by inhibiting the activation of ERK, p38MAPK, and NF-κB in macrophages. Inflamm Res 59:115–121. https://doi.org/10.1007/s00011-009-0077-8
44. Lin MC, Wang FY, Kuo YH, Tang FY (2011) Cancer chemopreventive effects of lycopene: Suppression of MMP–7 expression and cell invasion in human colon cancer cells. J Agric Food Chem 59:11304–11318. https://doi.org/10.1021/jf202433f
45. Hazai E, Bikadi Z, Zsila S, Lockwood SF (2006) Molecular modeling of the non-covalent binding of the dietary tomato carotenoids lycopene and lycophyl, and selected oxidative metabolites with 5-lipoxygenase. Biorg Medicinal Chem 14:6859–6867. https://doi.org/10.1016/j.bmc.2006.06.045
46. Hu F, Wang Yi B, Zhang W, Liang J, Lin C, Li D, Wang F, Pang D, Zhao Y (2012) Carotenoids and breast cancer risk: a meta-analysis and meta-regression. Breast Cancer Res Treat 131:239–253. https://doi.org/10.1007/s10549-011-1723-8
47. Bae JM (2016) Reinterpretation of the results of a pooled analysis of dietary carotenoid intake and breast cancer risk by using the interval collapsing method. Epidemiol Health 38: e2016024. https://doi.org/10.4178/epih.e2016024
48. Dos Santos RC, Ombredane AS, Souza JMT, Vasconcelos AG, Plácido A, Amorim ADGN, Barbos EA, Lima FCDA, Ropke CD, Alves MMM, Arcanjo DDR, Carvalho FAA, Delerue-Matos C, Joanitti GA, Leite JRSA (2018) Lycopene-rich extract from red guava (Psidium guajava L.) displays cytotoxic effect against human breast adenocarcinoma cell line MCF-7 via an apoptotic-like pathway. Food Res Int 105:184–196. https://doi.org/10.1016/j.foodres.2017.10.045
49. Chalabi N, Le Corre L, Maurizis JC, Bignon YJ, Bernard-Gallon DJ (2004) The effects of lycopene on the proliferation of human breast cells and BRCA1 and BRCA2 gene expression. Eur J Cancer 40:1768–1775. https://doi.org/10.1016/j.ejca.2004.03.028
50. Li Z, Wang Y, Mo B (2002) The effects of carotenoids on the proliferation of human breast cancer cell and gene expression of bcl–2. Zhonghua Yu Fang Yi Xue Za Zhi 36:254–257
51. Peng SJ, Li J, Zhou Y, Tuo M, Qin XX, Yu Q, Cheng H, Li YM (2017) *In vitro* effects and mechanisms of lycopene in MCF-7 human breast cancer cells. Genet Mol Res 16:1–8. https://doi.org/10.4238/gmr16029434
52. Nahum A, Hirsch K, Danilenko M, Watts CK, Prall OW, Levy J, Sharoni Y (2001) Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27 (Kip1) in the cyclin E-cdk2 complexes. Oncogene 20:3428–3436. https://doi.org/10.1038/sj.onc.1204452
53. Sharoni Y, Giron E, Rise M, Levy J (1997) Effects of lycopene-enriched tomato oleoresin on 7,12-dimethyl-benz[a]anthracene-induced rat mammary tumors. Cancer Detect Prev 21:118–123
54. Sahin K, Tuzcu M, Sahin N, Akdemir F, Ozercan I, Bayraktar S, Kucuk O (2011) Inhibitory effects of combination of lycopene and genistein on 7,12-dimethyl benz(a)anthracene-induced breast cancer in rats. Nutr Cancer 63:1279–1286. https://doi.org/10.1080/01635581.2011.606955

55. Singh A, Neupane YR, Panda BP, Kohli K (2017) Lipid Based nanoformulation of lycopene improves oral delivery: formulation optimization, ex vivo assessment and its efficacy against breast cancer. J Microencapsul 34:416–429. https://doi.org/10.1080/02652048.2017
56. Eliassen AH, Hendrickson SJ, Brinton LA, Buring JE, Campos H, Dai Q, Dorgan JF, Franke AA, Gao YT, Goodman MT, Hallmans G, Helzlsouer KJ, Hoffman-Bolton J, Hultén K, Sesso HD, Sowell AL, Tamimi RM, Toniolo P, Wilkens LR, Winkvist A, Zeleniuch-Jacquotte A, Zheng W, Hankinson SE (2012) Circulating carotenoids and risk of breast cancer: pooled analysis of eight prospective studies. J Natl Cancer Inst 104:1905–1916. https://doi.org/10.1093/jnci/djs461
57. Sesso HD, Buring JE, Zhang SM, Norkus EP, Gaziano JM (2005) Dietary and plasma lycopene and the risk of breast cancer. Cancer Epidemiol Biomark Prev 14:1074–1081. https://doi.org/10.1158/1055-9965.EPI-04-0683
58. Terry P, Jain M, Miller AB, Howe GR, Rohan TE (2002) Dietary carotenoids and risk of breast cancer. Am J Clin Nutr 76:883–888. https://doi.org/10.1093/ajcn/76.4.883
59. Sato R, Helzlsouer KJ, Alberg AJ, Hoffman SC, Norkus EP, Comstock GW (2002) Prospective study of carotenoids, tocopherols, and retinoid concentrations and the risk of breast cancer. Cancer Epidemiol Biomark Prev 11:451–457
60. Zhang S, Tang G, Russell RM, Mayzel KA, Stampfer MJ, Willett WC, Hunter DJ (1997) Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects. Am J Clin Nutr 66:626–632. https://doi.org/10.1093/ajcn/66.3.626
61. Klarod K, Hongsprabhas P, Khampitak T, Wirasorn K, Kiertiburanakul S, Tangrassameeprasert R, Daduang J, Yongvanit P, Boonsiri P (2011) Serum antioxidant levels and nutritional status in early and advanced stage lung cancer patients. Nutrition 27:1156–1160. https://doi.org/10.1016/j.nut.2010.12.019
62. Männistö S, Smith-Warner SA, Spiegelman D, Albanes D, Anderson K, van den Brandt PA, Cerhan JR, Colditz G, Feskanich D, Freudenheim JL, Giovannucci E, Goldbohm RA, Graham S, Miller AB, Rohan TE, Virtamo J, Willett WC, Hunter DJ (2004) Dietary carotenoids and risk of lung cancer in a pooled analysis of seven cohort studies. Cancer Epidemiol Biomark Prev 13:40–48
63. Asbaghi S, Saedisomeolia A, Hosseini M, Honarvar NM, Khosravi A, Azargashb E (2015) Dietary Intake and Serum Level of Carotenoids in Lung Cancer Patients: A Case-Control Study. Nutr Cancer 67:893–898. https://doi.org/10.1080/01635581.2015.1055365
64. Palozza P, Simone RE, Catalano A, Mele MC (2011) Tomato lycopene and lung cancer prevention: from experimental to human studies. Cancers (Basel) 3:2333–2357. https://doi.org/10.3390/cancers3022333
65. Lian F, Smith DE, Ernst H, Russell RM, Wang XD (2007) Apo–10′–lycopenoic acid inhibits lung cancer cell growth in vitro, and suppresses lung tumorigenesis in the A/J mouse model in vivo. Carcinogenesis 28:1567–1574. https://doi.org/10.1093/carcin/bgm076
66. Lian F, Wang XD (2008) Enzymatic metabolites of lycopene induce Nrf2–mediated expression of phase II detoxifying/antioxidant enzymes in human bronchial epithelial cells. Int J Cancer 123:1262–1268. https://doi.org/10.1002/ijc.23696
67. Michaud DS, Feskanich D, Rimm EB, Colditz GA, Speizer FE, Willett WC, Giovannucci E (2000) Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. Am J Clin Nutr 72:990–997. https://doi.org/10.1093/ajcn/72.4.990
68. Holick CN, Michaud DS, Stolzenberg-Solomon R, Mayne ST, Pietinen P, Taylor PR, Virtamo J, Albanes D (2002) Dietary carotenoids, serum beta-carotene, and retinol and risk of lung cancer in the alpha-tocopherol, beta-carotene cohort study. Am J Epidemiol 156:536–547
69. Graham DL, Carail M, Caris-Veyrat C, Lowe GM (2010) Cigarette smoke and human plasma lycopene depletion. Food Chem Toxicol 48:2413–2420. https://doi.org/10.1016/j.fct.2010.06.001

70. Shareck M, Rousseau MC, Koushik A, Siemiatycki J, Parent ME (2017) Inverse association between dietary intake of selected carotenoids and vitamin C and risk of lung cancer. Front Oncol 7:23. https://doi.org/10.3389/fonc.2017.00023
71. Garcia-Closas R, Agudo A, Gonzalez CA, Riboli E (1998) Intake of specific carotenoids and flavonoids and the risk of lung cancer in women in Barcelona, Spain. Nutr Cancer 32:154–158. https://doi.org/10.1080/01635589809514734
72. Ito Y, Wakai K, Suzuki K, Tamakoshi A, Seki N, Ando M, Nishino Y, Kondo T, Watanabe Y, Ozasa K, Ohno Y, JACC Study Group (2003) Serum carotenoids and mortality from lung cancer: A case–control study nested in the Japan Collaborative Cohort (JACC) study. Cancer Sci 94:57–63
73. Satia JA, Littman A, Slatore CG, Galanko JA, White E (2009) Long-term use of beta-carotene, retinol, lycopene, and lutein supplements and lung cancer risk: results from the VITamins and Lifestyle (VITAL) study. Am J Epidemiol 169:815–828. https://doi.org/10.1093/aje/kwn409
74. Liu C, Russell RM (2008) Nutrition and gastric cancer risk: an update. Nutr Rev 66:237–249. https://doi.org/10.1111/j.1753-4887.2008.00029.x
75. Yuan JM, Ross RK, Gao YT, Qu YH, Chu XD, Yu MC ((2004)) Prediagnostic levels of serum micronutrients in relation to risk of gastric cancer in Shanghai, China. Cancer Epidemiol Biomark Prev 11(Pt 1):1772–1780
76. Velmurugan B, Bhuvaneswari V, Nagini S (2001) Lycopene, an antioxidant carotenoid modulates glutathione-dependent hepatic biotransformation enzymes during experimental gastric carcinogenesis. Nutr Res 8:1117–1124. https://doi.org/10.1016/S0271-5317(01)00321-9
77. Velmurugan B, Mani A, Nagini S (2005) Combination of S-allylcysteine and lycopene induces apoptosis by modulating Bcl-2, Bax, Bim and caspases during experimental gastric carcinogenesis. Eur J Cancer Prev 14:387–293
78. Zhou S, Zhang R, Bi T, Lu Y, Jiang L (2016) Inhibitory effect of lycopene against the growth of human gastric cancer cells. Afr J Tradit Complement Altern Med 13:184–190. https://doi.org/10.21010/ajtcam.v13i4.24
79. Nouraie M, Pietinen P, Kamangar F, Dawsey SM, Abnet CC, Albanes D, Virtamo J, Taylor PR (2005) Fruits, vegetables, and antioxidants and risk of gastric cancer among male smokers. Cancer Epidemiol Biomark Prev 14:2087–2092. https://doi.org/10.1158/1055-9965.EPI-05-0038
80. Lunet N, Lacerda-Vieira A, Barros H (2005) Fruit and vegetables consumption and gastric cancer: a systematic review and meta-analysis of cohort studies. Nutr Cancer 53:1–10. https://doi.org/10.1207/s15327914nc5301_1
81. De Stefani E, Boffetta P, Brennan P, Deneo-Pellegrini H, Carzoglio JC, Ronco A, Mendilaharsu M (2000) Dietary carotenoids and risk of gastric cancer: a case-control study in Uruguay. Eur J Cancer Prev 9:329–334
82. Persson C, Sasazuki S, Inoue M, Kurahashi N, Iwasaki M, Miura T, Ye W, Tsugane S, JPHC Study Group (2008) Plasma levels of carotenoids, retinol and tocopherol and the risk of gastric cancer in Japan: a nested case-control study. Carcinogenesis 29:1042–1048. https://doi.org/10.1093/carcin/bgn072
83. Franceschi S, Bidoli E, La Vecchia C, Talamini R, D'Avanzo B, Negri E (1994) Tomatoes and risk of digestive-tract cancers. Int J Cancer 59:181–184
84. Yuan JM, Ross RK, Gao YT, Qu YH, Chu XD, Yu MC (2004) Prediagnostic levels of serum micronutrients in relation to risk of gastric cancer in Shangai, China. Cancer Epidemiol Biomark Prev 13:1772–1780
85. Zhou Y, Wang T, Meng Q, Zhai S (2016) Association of carotenoids with risk of gastric cancer: A meta-analysis. Clin Nutr 35:109–116. https://doi.org/10.1016/j.clnu.2015.02.003
86. Luo C, Wu XG (2011) Lycopene Enhances Antioxidant Enzyme Activities and Immunity Function in N-Methyl-N'-nitro-N-nitrosoguanidine-Induced Gastric Cancer Rats. Int J Mol Sci 12:3340–3351. https://doi.org/10.3390/ijms12053340

87. Rawat D, Shrivastava S, Naik RA, Chhonker SK, Mehrotra A, Koiri RK (2018) An overview of natural plant products in the treatment of hepatocellular carcinoma. Anti Cancer Agents Med Chem. https://doi.org/10.2174/1871520618666180604085612
88. Glauert HP, Calfee-Mason K, Stemm DN, Tharappel JC, Spear BT (2010) Dietary antioxidants in the prevention of hepatocarcinogenesis: a review. Mol Nutr Food Res 54:875–896. https://doi.org/10.1002/mnfr.200900482
89. Bosch FX, Ribes J, Diaz M, Cleries R (2004) Primary liver cancer: worldwide incidence and trends. Gastroenterology 127:5–16
90. Kataria Y, Deaton RJ, Enk E, Jin M, Petrauskaite M, Dong L, Goldenberg JR, Cotler SJ, Jensen DM, van Breemen RB, Gann PH (2016) Retinoid and carotenoid status in serum and liver among patients at high-risk for liver cancer. BMC Gastroenterol 16:30. https://doi.org/10.1186/s12876-016-0432-5
91. Hwang ES, Lee HJ (2006) Inhibitory effects of lycopene on the adhesion, invasion, and migration of SK-Hep1 human hepatoma cells. Exp Biol Med (Maywood) 231:322–327. https://doi.org/10.1177/153537020623100313
92. Huang CS, Liao JW, Hu ML (2008) Lycopene inhibits experimental metastasis of human hepatoma SK-Hep–1 cells in athymic nude mice. J Nutr 138:538–543. https://doi.org/10.1093/jn/138.3.538
93. Ben-Dor A, Steiner M, Gheber L, Danilenko M, Dubi N, Linnewiel K, Zick A, Sharoni Y, Levy J (2005) Carotenoids activate the antioxidant response element transcription system. Mol Cancer Ther 4:177–186
94. Gradlet S, LeBon AM, Bergès R, Suschetet M, Astorg P (1998) Dietary carotenoids inhibit aflatoxin B1-induced liver preneoplastic foci and DNA damage in the rat: role of the modulation of aflatoxin B1 metabolism. Carcinogenesis 19:403–411
95. Reddy L, Odhav B, Bhoola K (2006) Aflatoxin B1-induced toxicity in HepG2 cells I nhibited by carotenoids: morphology, apoptosis and DNA damage. Biol Chem 387:87–93. https://doi.org/10.1515/BC.2006.012
96. Jhou BY, Song TY, Lee I, Hu ML, Yang NC (2017) Lycopene Inhibits Metastasis of Human Liver Adenocarcinoma SK-Hep-1 Cells by Downregulation of NADPH Oxidase 4 Protein Expression. J Agric Food Chem 65:6893–6903. https://doi.org/10.1021/acs.jafc.7b03036
97. Astorg P, Gradelet S, Berges R, Suschetet M (1997) Dietary lycopene decreases the initiation of liver preneoplastic foci by diethylnitrosamine in the rat. Nutr Cancer 29:60–68. https://doi.org/10.1080/01635589709514603
98. Wang Y, Ausman LM, Greenberg AS, Russell RM, Wang XD (2010) Dietary lycopene and tomato extract supplementations inhibit nonalcoholic steatohepatitis-promoted hepatocarcinogenesis in rats. Int J Cancer 126:1788–1796. https://doi.org/10.1002/ijc.24689
99. Cheng J, Miao B, Hu KQ, Fu X, Wang XD (2018) Apo-10′-lycopenoic acid inhibits cancer cell migration and angiogenesis and induces peroxisome proliferator-activated receptor γ. J Nutr Biochem 56:26–34. https://doi.org/10.1016/j.jnutbio.2018.01.003
100. Aizawa K, Liu C, Tang S, Veeramachaneni S, Hu KQ, Smith DE, Wang XD (2016) Tobacco carcinogen induces both lung cancer and non-alcoholic steatohepatitis and hepatocellular carcinomas in ferrets which can be attenuated by lycopene supplementation. Int J Cancer 139:1171–1181. https://doi.org/10.1002/ijc.30161
101. Ip BC, Liu C, Ausman LM, von Lintig J, Wang XD (2014) Lycopene attenuated hepatic tumorigenesis via differential mechanisms depending on carotenoid cleavage enzyme in mice. Cancer Prev Res (Phila) 7:1219–1227. https://doi.org/10.1158/1940-6207.CAPR-14-0154
102. Bhatia N, Gupta P, Singh B, Koul A (2015) Lycopene Enriched Tomato Extract Inhibits Hypoxia, Angiogenesis, and Metastatic Markers in early Stage N-Nitrosodiethylamine Induced Hepatocellular Carcinoma. Nutr Cancer 67:1268–1275. https://doi.org/10.1080/01635581.2015.1087040
103. Jeurnink SM, Ros MM, Leenders M, van Duijnhoven FJ, Siersema PD, Jansen EH, van Gils CH, Bakker MF, Overvad K, Roswall N, Tjønneland A, Boutron-Ruault MC, Racine A, Cadeau C, Grote V, Kaaks R, Aleksandrova K, Boeing H, Trichopoulou A, Benetou V,

Valanou E, Palli D, Krogh V, Vineis P, Tumino R, Mattiello A, Weiderpass E, Skeie G, Castaño JM, Duell EJ, Barricarte A, Molina-Montes E, Argüelles M, Dorronsoro M, Johansen D, Lindkvist B, Sund M, Crowe FL, Khaw KT, Jenab M, Fedirko V, Riboli E, Bueno-de-Mesquita HB (2015) Plasma carotenoids, vitamin C, retinol and tocopherols levels and pancreatic cancer risk within the European prospective investigation into cancer and nutrition: a nested case-control study: plasma micronutrients and pancreatic cancer risk. Int J Cancer 136:E665–E676. https://doi.org/10.1002/ijc.29175

104. Nitsche C, Simon P, Weiss FU, Fluhr G, Weber E, Gärtner S, Behn CO, Kraft M, Ringel J, Aghdassi A, Mayerle J, Lerch MM (2011) Environmental risk factors for chronic pancreatitis and pancreatic cancer. Dig Dis 29:235–242. https://doi.org/10.1159/000323933
105. Donaldson MS (2004) Nutrition and cancer: a review of the evidence for an anti-cancer diet. Nutr J 3:19. https://doi.org/10.1186/1475-2891-3-19
106. McCullough ML, Giovannucci EL (2004) Diet and cancer prevention. Oncogene 23:6349–6364. https://doi.org/10.1038/sj.onc.1207716
107. Nkondjock A, Ghadirian P, Johnson KC, Krewski D, Canadian Cancer Registries Epidemiology Research Group (2005) Dietary intake of lycopene is associated with reduced pancreatic cancer risk. J Nutr 135:592–597. https://doi.org/10.1093/jn/135.3.592
108. Huang X, Gao Y, Zhi X, Ta N, Jiang H, Zheng J (2016) Association between vitamin A, retinol and carotenoid intake and pancreatic cancer risk: Evidence from epidemiologic studies. Sci Rep 6:38936. https://doi.org/10.1038/srep38936
109. Wang Y, Cui R, Xiao Y, Fang J, Xu Q (2015) Effect of carotene and lycopene on the risk of prostate cancer: A systematic review and dose-response meta-analysis of observational studies. PLoS One 10:e0137427. https://doi.org/10.1371/journal.pone.0137427
110. Burney PG, Comstock GW, Morris JS (1989) Serologic precursors of cancer: serum micronutrients and the subsequent risk of pancreatic cancer. Am J Clin Nutr 49:895–900. https://doi.org/10.1093/ajcn/49.5.895
111. Kim MJ, Kim H (2015) Anticancer effect of lycopene in gastric carcinogenesis. J Cancer Prev 20:92–96. https://doi.org/10.15430/JCP.2015.20.2.92
112. Wang X, Yang HH, Liu Y, Zhou Q, Chen ZH (2016) Lycopene consumption and risk of colorectal cancer: A meta-analysis of observational studies. Nutr Cancer 68:1083–1096. https://doi.org/10.1080/01635581.2016.1206579
113. Teodoro AJ, Oliveira FL, Martins NB, Maia Gde A, Martucci RB, Borojevic R (2012) Effect of lycopene on cell viability and cell cycle progression in human cancer cell lines. Cancer Cell Int 12:36. https://doi.org/10.1186/1475-2867-12-36
114. Huang RF, Wei YJ, Inbaraj BS, Chen BH (2015) Inhibition of colon cancer cell growth by nanoemulsion carrying gold nanoparticles and lycopene. Int J Nanomedicine 10:2823–2846. https://doi.org/10.2147/IJN.S79107
115. Tuzcu M, Aslan A, Tuzcu Z, Yabas M, Bahcecioglu IH, Ozercan IH, Kucuk O, Sahin K (2012) Tomato powder impedes the development of azoxymethane-induced colorectal cancer in rats through suppression of COX-2 expression via NF-κB and regulating Nrf2/HO-1 pathway. Mol Nutr Food Res 56:1477–1481. https://doi.org/10.1002/mnfr.20120013
116. Tang FY, Pai MH, Wang XD (2011) Consumption of lycopene inhibits the growth and progression of colon cancer in a mouse xenograft model. J Agric Food Chem 59:9011–9021
117. Kim DJ, Takasuka N, Kim JM, Sekine K, Ota T, Asamoto M, Murakoshi M, Nishino H, Nir Z, Tsuda H (1997) Chemoprevention by lycopene of mouse lung neoplasia after combined initiation treatment with DEN, MNU and DMH. Cancer Lett 120:15–22
118. Narisawa T, Hasebe M, Nomura S, Sakamoto H, Inakuma T, Ishiguro Y, Takayasu J, Nishino H (1998) Prevention of N-methylnitrosourea colon carcinogenesis in F344 rats by lycopene and tomato juice rich in lycopene. Jpn J Cancer Res 89:1003–1008
119. Erhardt JG, Meisner C, Bode JC (2003) Lycopene, beta-carotene, and colorectal adenomas. Am J Clin Nutr 78:1219–1224. https://doi.org/10.1093/ajcn/78.6.1219

120. Malila N, Virtamo J, Virtanen M, Pietinen P, Albanes D, Teppo L (2002) Dietary and serum alpha-tocopherol, beta-carotene and retinol, and risk for colorectal cancer in male smokers. Eur J Clin Nutr 56:615–621. https://doi.org/10.1038/sj.ejcn.1601366
121. Hu JF, Liu YY, Yu YK, Zhao TZ, Liu SD, Wang QQ (1991) Diet and cancer of the colon and rectum: a case-control study in China. Int J Epidemiol 20:362–367
122. Freudenheim JL, Graham S, Marshall JR, Haughey BP, Wilkinson G (1990) A case-control study of diet and rectal cancer in western New York. Am J Epidemiol 131:612–624
123. Franceschi S, Favero A, La Vecchia C, Negri E, Conti E, Montella M, Giacosa A, Nanni O, Decarli A (1997) Food groups and risk of colorectal cancer in Italy. Int J Cancer 72:56–61
124. Stahl W, Sies H (2012) Photoprotection by dietary carotenoids: concept, mechanisms, evidence and future development. Mol Nutr Food Res 56:287–295. https://doi.org/10.1002/mnfr.201100232
125. Stahl W, Sies H (2007) Carotenoids and flavonoids contribute to nutritional protection against skin damage from sunlight. Mol Biotechnol 37:26–30
126. Wright TI, Spencer JM, Flowers FP (2006) Chemoprevention of nonmelanoma skin cancer. J Am Acad Dermatol 54:933–946. https://doi.org/10.1016/j.jaad.2005.08.062
127. Ascenso A, Pedrosa T, Pinho S, Pinho F, de Oliveira JM, Cabral Marques H, Oliveira H, Simões S, Santos C (2016) The effect of lycopene preexposure on UV-B-Irradiated human keratinocytes. Oxidative Med Cell Longev 2016:8214631. https://doi.org/10.1155/2016/8214631
128. Ribaya-Mercado JD, Garmyn M, Gilchrest BA, Russell RM (1995) Skin lycopene is destroyed preferentially over beta-carotene during ultraviolet irradiation in humans. J Nutr 125:1854–1859. https://doi.org/10.1093/jn/125.7.1854
129. Stahl W, Heinrich U, Wiseman S, Eichler O, Sies H, Tronnier H (2001) Dietary tomato paste protects against ultraviolet light-induced erythema in humans. J Nutr 131:1449–1451. https://doi.org/10.1093/jn/131.5.1449
130. Stahl W, Heinrich U, Aust O, Tronnier H, Sies H (2006) Lycopene-rich products and dietary photoprotection. Photochem Photobiol Sci 5:238–242. https://doi.org/10.1039/b505312a
131. Andreassi M, Andreassi L (2003) Antioxidants in dermocosmetology: from the laboratory to clinical application. J Cosmet Dermatol 2:153–160. https://doi.org/10.1111/j.1473-2130.2004.00075.x
132. Chen P, Xu S, Qu J (2018) Lycopene protects keratinocytes against UVB radiation-induced carcinogenesis via negative regulation of FOXO3a through the mTORC2/AKT signaling pathway. J Cell Biochem 119:366–377. https://doi.org/10.1002/jcb.26189
133. Cooperstone JL, Tober KL, Riedl KM, Teegarden MD, Cichon MJ, Francis DM, Schwartz SJ, Oberyszyn TM (2017) Tomatoes protect against development of UV-induced keratinocyte carcinoma via metabolomic alterations. Sci Rep 7:5106. https://doi.org/10.1038/s41598-017-05568-7
134. Shen C, Wang S, Shan Y, Liu Z, Fan F, Tao L, Liu Y, Zhou L, Pei C, Wu H, Tian C, Ruan J, Chen W, Wang A, Zheng S, Lu Y (2014) Chemomodulatory efficacy of lycopene on antioxidant enzymes and carcinogen-induced cutaneum carcinoma in mice. Food Funct 5:1422–1431. https://doi.org/10.1039/c4fo00035h
135. Yeh SL, Huang CS, Hu ML (2005) Lycopene enhances UVA-induced DNA damage and expression of heme oxygenase-1 in cultured mouse embryo fibroblasts. Eur J Nutr 44:365–370
136. Burgess LC, Rice E, Fischer T, Seekins JR, Burgess TP, Sticka SJ, Klatt K (2008) Lycopene has limited effect on cell proliferation in only two of seven human cell lines (both cancerous and noncancerous) in an in vitro system with doses across the physiological range. Toxicol In Vitro 22:1297–1300. https://doi.org/10.1016/j.tiv.2008.03.001
137. Giovannucci E (1999) Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer Inst 91:317–331
138. Mayne ST, Cartmel B, Lin H, Zheng T, Goodwin WJ Jr (2004) Low plasma lycopene concentration is associated with increased mortality in a cohort of patients with prior oral,

pharynx or larynx cancers. J Am Coll Nutr 23:34–42. https://doi.org/10.1080/07315724.2004.10719340
139. Lodi G, Franchini R, Warnakulasuriya S, Varoni EM, Sardella A, Kerr AR, Carrassi A, MacDonald LC, Worthington HV (2006) Interventions for treating oral leukoplakia. Cochrane Database Syst Rev 7:CD001829. https://doi.org/10.1002/14651858.CD001829
140. Ngoc NB, Lv P, Zhao WE (2018) Suppressive effects of lycopene and β-carotene on the viability of the human esophageal squamous carcinoma cell line EC109. Oncol Lett 15:6727–6732. https://doi.org/10.3892/ol.2018.8175
141. Negri E, Franceschi S, Bosetti C, Levi F, Conti E, Parpinel M, La Vecchia C (2000) Selected micronutrients and oral and pharyngeal cancer. Int J Cancer 86:122–127
142. Zheng T, Boyle P, Willet WC, Hu H, Dan J, Evstifeeva TV, Niu S, MacMahon B (1993) A case-control study of oral cancer in beijing, people's republic of china. Associations with nutrient intakes, foods and food groups. Eur J Cancer B Oral Oncol 29B:45–55
143. Bhuvaneswari V, Velmurugan B, Balasenthil S, Ramachandran CR, Nagini S (2001) Chemopreventive efficacy of lycopene on 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. Fitoterapia 72:865–874. https://doi.org/10.1016/S0367-326X(01)00321-5
144. El-Rouby DH (2011) Histological and immunohistochemical evaluation of the chemopreventive role of lycopene in tongue carcinogenesis induced by 4-nitroquinoline–1-oxide. Arch Oral Biol 56:664–671. https://doi.org/10.1016/j.archoralbio.2010.12.007
145. De Stefani E, Oreggia F, Boffetta P, Deneo-Pellegrini H, Ronco A, Mendilaharsu M (2000) Tomatoes, tomato-rich foods, lycopene and cancer of the upper aerodigestive tract: a case-control in Uruguay. Oral Oncol 36:47–53. https://doi.org/10.1016/S1368-8375(99)00050-0
146. Capurso C, Vendemiale G (2017) The Mediterranean Diet Reduces the Risk and Mortality of the Prostate Cancer: A Narrative Review. Front Nutr 4:38. https://doi.org/10.3389/fnut.2017.00038
147. Krinsky NI (1998) The antioxidant and biological properties of the carotenoids. Ann N Y Acad Sci 854:443–447
148. Gong X, Marisiddaiah R, Zaripheh S, Wiener D, Rubin LP (2016) Mitochondrial β-carotene 9′,10′ oxygenase modulates prostate cancer growth via NF-κB inhibition: A lycopene-independent function. Mol Cancer Res 14:966–975. https://doi.org/10.1158/1541-7786.MCR-16-0075
149. Van Hoang D, Pham NM, Lee AH, Tran DN, Binns CW (2018) Dietary carotenoid intakes and prostate cancer risk: A case-control study from vietnam. Nutrients 10:E70. https://doi.org/10.3390/nu10010070
150. Palozza P, Sestito R, Picci N, Lanza P, Monego G, Ranelletti FO (2008) The sensitivity to beta-carotene growth-inhibitory and proapoptotic effects is regulated by caveolin-1 expression in human colon and prostate cancer cells. Carcinogenesis 29:2153–2161. https://doi.org/10.1093/carcin/bgn018
151. Yang CM, Lu YL, Chen HY, Hu ML (2012) Lycopene and the LXRα agonist T0901317 synergistically inhibit the proliferation of androgen-independent prostate cancer cells via the PPARγ-LXRα-ABCA1 pathway. J Nutr Biochem 23:1155–1162. https://doi.org/10.1016/j.jnutbio.2011.06.009
152. Stahl W, Sies H (2005) Bioactivity and protective effects of natural carotenoids. Biochim Biophys Acta 1740:101–107. https://doi.org/10.1016/j.bbadis.2004.12.006
153. Rowles JL 3rd, Ranard KM, Smith JW, An R, Erdman JW Jr (2017) Increased dietary and circulating lycopene are associated with reduced prostate cancer risk: a systematic review and meta-analysis. Prostate Cancer Prostatic Dis 20:361–377. https://doi.org/10.1038/pcan.2017.25
154. Lu QY, Hung JC, Heber D, Go VL, Reuter VE, Cordon-Cardo C, Scher HI, Marshall JR, Zhang ZF (2001) Inverse associations between plasma lycopene and other carotenoids and prostate cancer. Cancer Epidemiol Biomark Prev 10:749–756

155. Hwang ES, Bowen PE (2004) Cell cycle arrest and induction of apoptosis by lycopene in LNCaP human prostate cancer cells. J Med Food 7:284–289. https://doi.org/10.1089/jmf.2004.7.284
156. Ford NA, Elsen AC, Zuniga K, Lindshield BL, Erdman JW Jr (2011) Lycopene and apo-12-′-lycopenal reduce cell proliferation and alter cell cycle progression in human prostate cancer cells. Nutr Cancer 63:256–263. https://doi.org/10.1080/01635581.2011.523494
157. Li D, Chen L, Zhao W, Hao J, An R (2016) MicroRNA-let-7f-1 is induced by lycopene and inhibits cell proliferation and triggers apoptosis in prostate cancer. Mol Med Rep 13:2708–2714
158. Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH, Stampfer MJ (1999) Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. Cancer Res 59:1225–1230
159. Key TJ, Appleby PN, Travis RC, Albanes D, Alberg AJ, Barricarte A, Black A, Boeing H, Bueno-de-Mesquita HB, Chan JM, Chen C, Cook MB, Donovan JL, Galan P, Gilbert R, Giles GG, Giovannucci E, Goodman GE, Goodman PJ, Gunter MJ, Hamdy FC, Heliövaara M, Helzlsouer KJ, Henderson BE, Hercberg S, Hoffman-Bolton J, Hoover RN, Johansson M, Khaw KT, King IB, Knekt P, Kolonel LN, Le Marchand L, Männistö S, Martin RM, Meyer HE, Mondul AM, Moy KA, Neal DE, Neuhouser ML, Palli D, Platz EA, Pouchieu C, Rissanen H, Schenk JM, Severi G, Stampfer MJ, Tjønneland A, Touvier M, Trichopoulou A, Weinstein SJ, Ziegler RG, Zhou CK, Allen NE (2015) Endogenous Hormones Nutritional Biomarkers Prostate Cancer Collaborative Group. Carotenoids, retinol, tocopherols, and prostate cancer risk: pooled analysis of 15 studies. Am J Clin Nutr 102:1142–1157. https://doi.org/10.3945/ajcn.115.114306
160. Morgia G, Voce S, Palmieri F, Gentile M, Lapicca G, Giannantoni A, Blefari F, Carini M, Vespasiani G, Santelli G, Arnone S, Pareo RM, Russo GI (2017) Association between selenium and lycopene supplementation and incidence of prostate cancer: Results from the post-hoc analysis of the procomb trial. Phytomedicine 34:1–5. https://doi.org/10.1016/j.phymed.2017.06.008
161. Kucuk O, Sarkar F, Sakr W, Djuric Z, Khachik F, Pollak M, Bertram J, Grignon D, Banerjee M, Crissman J, Pontes E, Wood DP Jr (2001) Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. Cancer Epidemiol Biomark Prev 10:861–868
162. Gupta S (2007) Review prostate cancer chemoprevention current status and future prospect. Toxicol Appl Pharmacol 224:369–376. https://doi.org/10.1016/j.taap.2006.11.008
163. Paur I, Lilleby W, Bøhn SK, Hulander E, Klein W, Vlatkovic L, Axcrona K, Bolstad N, Bjøro T, Laake P, Taskén KA, Svindland A, Eri LM, Brennhovd B, Carlsen MH, Fosså SD, Smeland SS, Karlsen AS, Blomhoff R (2016) Tomato-based randomized controlled trial in prostate cancer patients: Effect on PSA. Clin Nutr 36(3):672–679. https://doi.org/10.1016/j.clnu.2016.06.01
164. Wang Y, Jacobs EJ, Newton CC, McCullough ML (2016) Lycopene, tomato products and prostate cancer-specific mortality among men diagnosed with nonmetastatic prostate cancer in the Cancer Prevention Study II Nutrition Cohort. Int J Cancer 138:2846–2855. https://doi.org/10.1002/ijc.30027
165. Yang CM, Yen YT, Huang CS, Hu ML (2011) Growth inhibitory efficacy of lycopene and β-carotene against androgen-independent prostate tumor cells xenografted in nude mice. Mol Nutr Food Res 55:606–612. https://doi.org/10.1002/mnfr.201000308
166. Soares ND, Machado CL, Trindade BB, Lima IC, Gimba ER, Teodoro AJ, Ch T, Borojevic R (2017) Lycopene extracts from different tomato-based food products induce apoptosis in cultured human primary prostate cancer cells and regulate TP53, Bax and Bcl-2 transcript expression. Asian Pac J Cancer Prev 18:339–345. https://doi.org/10.22034/APJCP.2017.18.2.33
167. https://www.cancer.org/cancer/kidney-cancer/about/key-statistics.html

168. Sharoni Y, Linnewiel-Hermoni K, Khanin M, Salman H, Veprik A, Danilenko M, Levy J (2012) Carotenoids and apocarotenoids in cellular signaling related to cancer: a review. Mol Nutr Food Res 56:259–269. https://doi.org/10.1002/mnfr.201100311
169. Ho WJ, Simon MS, Yildiz VO, Shikany JM, Kato I, Beebe-Dimmer JL, Cetnar JP, Bock CH (2015) Antioxidant micronutrients and the risk of renal cell carcinoma in the Women's Health Initiative cohort. Cancer 121:580–588. https://doi.org/10.1002/cncr.29091
170. Bock CH, Ruterbusch JJ, Holowatyj AN, Steck SE, Van Dyke AL, Ho WJ, Cote ML, Hofmann JN, Davis F, Graubard BI, Schwartz KL, Purdue MP (2018) Renal cell carcinoma risk associated with lower intake of micronutrients. Cancer Med 7(8):4087–4097. https://doi.org/10.1002/cam4.1639
171. Bertoia M, Albanes D, Mayne ST, Männistö S, Virtamo J, Wright ME (2010) No association between fruit, vegetables, antioxidant nutrients and risk of renal cell carcinoma. Int J Cancer 126:1504–1512. https://doi.org/10.1002/ijc.24829
172. Wang XD (2012) Lycopene metabolism and its biological significance. Am J Clin Nutr 96:1214S–1222S. https://doi.org/10.3945/ajcn.111.032359
173. Sahin K, Cross B, Sahin N, Ciccone K, Suleiman S, Osunkoya AO, Master V, Harris W, Carthon B, Mohammad R, Bilir B, Wertz K, Moreno CS, Walker CL, Kucuk O (2015) Lycopene in the prevention of renal cell cancer in the TSC2 mutant Eker rat model. Arch Biochem Biophys 572:36–39. https://doi.org/10.1016/j.abb.2015.01.006
174. Lee JE, Giovannucci E, Smith-Warner SA, Spiegelman D, Willett WC, Curhan GC (2006) Intakes of fruits, vegetables, vitamins A, C, and E, and carotenoids and risk of renal cell cancer. Cancer Epidemiol Biomark Prev 15:2445–2452. https://doi.org/10.1158/1055-9965.EPI-06-0553
175. Brock KE, Ke L, Gridley G, Chiu BC, Ershow AG, Lynch CF, Graubard BI, Cantor KP (2012) Fruit, vegetables, fibre and micronutrients and risk of US renal cell carcinoma. Br J Nutr 108:1077–1085. https://doi.org/10.1017/S0007114511006489
176. Sahin K, Yenice E, Tuzcu M, Orhan C, Mizrak C, Ozercan IH, Sahin N, Yilmaz B, Bilir B, Ozpolat B, Kucuk O (2018) Lycopene protects against spontaneous ovarian cancer formation in laying hens. J Cancer Prev 23:25–36. https://doi.org/10.15430/JCP.2018.23.1.25
177. Holzapfel NP, Shokoohmand A, Wagner F, Landgraf M, Champ S, Holzapfel BM, Clements JA, Hutmacher DW, Loessner D (2017) Lycopene reduces ovarian tumor growth and intraperitoneal metastatic load. Am J Cancer Res 7:1322–1336
178. Li X, Xu J (2014) Meta-analysis of the association between dietary lycopene intake and ovarian cancer risk in postmenopausal women. Sci Rep 4:4885. https://doi.org/10.1038/srep04885
179. Cramer DW, Kuper H, Harlow BL, Titus-Ernstoff L (2001) Carotenoids, antioxidants and ovarian cancer risk in pre- and postmenopausal women. Int J Cancer 94:128–134. https://doi.org/10.1002/ijc.1435
180. Kiani F, Knutsen S, Singh P, Ursin G, Fraser G (2006) Dietary risk factors for ovarian cancer: the Adventist Health Study (United States). Cancer Causes Control 17:137–146. https://doi.org/10.1007/s10552-005-5383-z
181. Helzlsouer KJ, Alberg AJ, Norkus EP, Morris JS, Hoffman SC, Comstock GW (1996) Prospective study of serum micronutrients and ovarian cancer. J Natl Cancer Inst 88:32–37. https://doi.org/10.1093/jnci/88.1.32
182. Jeong NH, Song ES, Lee JM, Lee KB, Kim MK, Cheon JE, Lee JK, Son SK, Lee JP, Kim JH, Hur SY, Kwon YI (2009) Plasma carotenoids, retinol and tocopherol levels and the risk of ovarian cancer. Acta Obstet Gynecol Scand 88:457–462. https://doi.org/10.1080/00016340902807215
183. Zhang XF, Huang FH, Zhang GL, Bai DP, Massimo DF, Huang YF, Gurunathan S (2017) Novel biomolecule lycopene-reduced graphene oxide-silver nanoparticle enhances apoptotic potential of trichostatin A in human ovarian cancer cells (SKOV3). Int J Nanomedicine 12:7551–7575. https://doi.org/10.2147/IJN.S144161

184. Jain A, Sharma G, Ghoshal G, Kesharwani P, Singh B, Shivhare US, Katare OP (2018) Lycopene loaded whey protein isolate nanoparticles: An innovative endeavor for enhanced bioavailability of lycopene and anti-cancer activity. Int J Pharm 546:97–105. https://doi.org/10.1016/j.ijpharm.2018.04.061
185. Uppala PT, Dissmore T, Lau BH, Andacht T, Rajaram S (2013) Selective Inhibition of cell proliferation by lycopene in MCF-7 breast cancer cells *in vitro:* a proteomic analysis. Phytother Res 27:595–601. https://doi.org/10.1002/ptr.4764
186. Al-Malki AL, Moselhy SS, Refai MY (2012) Synergistic effect of lycopene and tocopherol against oxidative stress and mammary tumorigenesis induced by 7,12-dimethyl[a] benzanthracene in female rats. Toxicol Ind Health 28:542–548. https://doi.org/10.1177/0748233711416948
187. Yang CM, Hu TY, Hu ML (2012) Antimetastatic effects and mechanisms of apo-8′-lycopenal, an enzymatic metabolite of lycopene, against human hepatocarcinoma SK-Hep-1 cells. Nutr Cancer 64:274–285. https://doi.org/10.1080/01635581.2012.643273
188. Seo JY, Masamune A, Shimosegawa T, Kim H (2009) Protective effect of lycopene on oxidativestress-induced cell death of pancreatic acinar cells. Ann N Y Acad Sci 1171:570–575. https://doi.org/10.1111/j.1749-6632.2009.04712.x
189. Oguz E, Kocarslan S, Tabur S, Sezen H, Yilmaz Z, Aksoy N (2015) Effects of lycopene alone or combined with melatonin on methotrexate-induced nephrotoxicity in rats. Asian Pac J Cancer Prev 16:6061–6066
190. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 87:1767–1776
191. Tan HL, Thomas-Ahner JM, Moran NE, Cooperstone JL, Erdman JW Jr, Young GS, Clinton SK (2017) β-Carotene 9′,10′ oxygenase modulates the anticancer activity of dietary tomato or lycopene on prostate carcinogenesis in the TRAMP model. Cancer Prev Res (Phila) 10:161–169. https://doi.org/10.1158/1940-6207.CAPR-15-0402
192. Yang CM, Lu IH, Chen HY, Hu ML (2012) Lycopene inhibits the proliferation of androgen-dependent human prostate tumor cells through activation of PPARγ-LXRα-ABCA1 pathway. J Nutr Biochem 23:8–17. https://doi.org/10.1016/j.jnutbio.2010.10.006
193. Offord EA, Gautier JC, Avanti O, Scaletta C, Runge F, Krämer K, Applegate LA (2002) Photoprotective potential of lycopene, β-carotene, vitamin E, vitamin C and carnosic acid in UVA-irradiated human skin fibroblasts. Free Radic Biol Med 32:1293–1303
194. Chiang HS, Wu WB, Fang JY, Chen DF, Chen BH, Huang CC, Chen YT, Hung CF (2007) Lycopene inhibits PDGF-BB-induced signaling and migration in human dermal fibroblasts through interaction with PDGF-BB. Life Sci 81:1509–1517. https://doi.org/10.1016/j.lfs.2007.09.018
195. Butnariu M, Giuchici C (2011) The use of some nanoemulsions based on aqueous propolis and lycopene extract in the skin's protective mechanisms against UVA radiation. J Nanobiotechnol 9:3. https://doi.org/10.1186/1477-3155-9-3
196. Ascenso A, Pinho S, Eleutério C, Praca FG, Bentley MV, Oliveira H, Santos C, Silva O, Simões S (2013) Lycopene from tomatoes: vesicular nanocarrier formulations for dermal delivery. J Agric Food Chem 61:7284–7293. https://doi.org/10.1021/jf401368w
197. Kowalczyk MC, Walaszek Z, Kowalczyk P, Kinjo T, Hanausek M, Slaga TJ (2009) Differential effects of several phytochemicals and their derivatives on murine keratinocytes in vitro and in vivo: implications for skin cancer prevention. Carcinogenesis 30:1008–1015. https://doi.org/10.1093/carcin/bgp069

Part VI

Food Polyphenols

Bound Phenolics in Foods

34

Liliana Santos-Zea, Javier Villela-Castrejón, and Janet A. Gutiérrez-Uribe

Contents

Abstract

Bound phenolic compounds are widely distributed among several plants, especially cereals. In most of the cases, covalent bonds are formed with polysaccharides, proteins, or lipids. But additionally, hydrophobic interactions

L. Santos-Zea · J. Villela-Castrejón
Tecnologico de Monterrey, School of Engineering and Science, Centro de Biotecnología-FEMSA, Monterrey, Mexico
e-mail: lilianasantos@itesm.mx; jvillela.cas@itesm.mx

J. A. Gutiérrez-Uribe (✉)
Tecnologico de Monterrey, School of Engineering and Science, Department of Bioengineering and Science, Campus Puebla, Puebla, Mexico
e-mail: jagu@itesm.mx

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_13

may affect their release from the food matrix. Many studies have reported their bioactivity after their release from foods, in most of the cases involving acid or basic hydrolysis and further extraction with organic solvents. Besides their antioxidant activity, bound phenolics have important effects on the inhibition of cancer cell growth, key enzymes involved in the metabolism of carbohydrates, as well as in the regulation of inflammatory processes. Food processing and gastrointestinal digestion affect the bound phenolic bioavailability and in consequence the potential benefits to human health. Recent studies have demonstrated that microbiota composition in the gastrointestinal tract affect the release of bound phenolics and their metabolism. Therefore future studies will help us to understand the complex interactions between bound phenolics and gastrointestinal microbiota and produce natural controlled released bioactive phenolic compounds.

Keywords
Bound phenolics · Antioxidant · Prebiotic · Extraction

1 Introduction

Phenolic compounds (PCs) are plant secondary metabolites necessary for physiology and cellular metabolism. Among the main functions in plants are the protection against insects and animals, structure, growth, and reproduction [1]. PCs come from the phenylpropanoid pathway, and the two main precursors are the amino acid phenylalanine from the shikimate pathway and malonyl-CoA from the acetate pathway [2]. The phenolic acids possess one or more aromatic rings hydroxyl-substituted. Currently, there are 8000 known phenolic structures, which are classified according to the carbon chain (Fig. 1) [3]. Furthermore, the location within the plant and the chemical structure makes possible to categorize phenolic compounds as soluble and insoluble. Soluble phenolics are composed of free simple phenols, glycosides (with single or multiple sugar moieties), and low molecular weight phenolics not bound to the other plant cell major components. Bound phenolic (BP) group is conformed by condensed tannins, phenolic acids, and other compounds of low molecular weight [4]. Particularly in cereals, the insoluble fraction account for the major part of phenolic acids [5].

Phenolic compounds have gained a lot of attention, since these molecules have proved their antioxidant, antimutagenic, antiviral, and antibacterial activities [6–10]. Epidemiological studies have linked the consumption of foods and beverages high in phenolic acids with a reduced risk of development of several diseases [3]. Since bound phenolics are poorly absorbed in the small intestine, their fermentation products generated by the microorganisms in the large intestine generate positive effects on microbiota and inhibit the growth of pathogens.

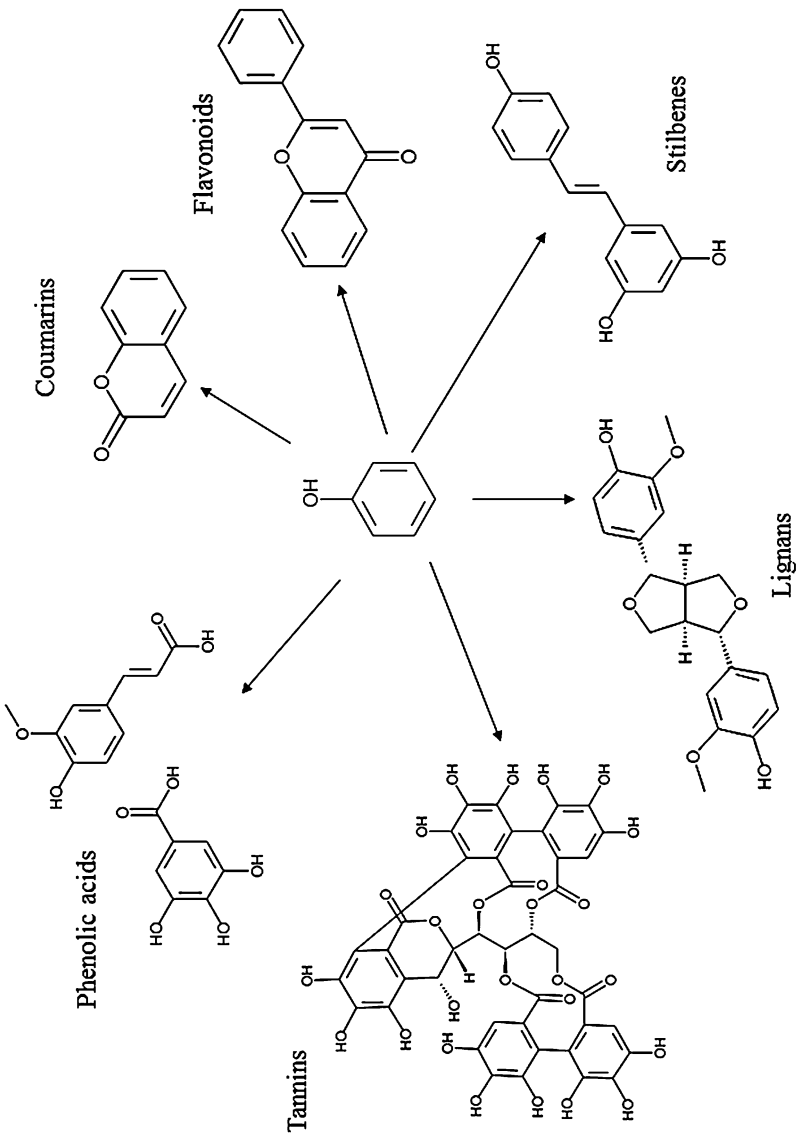


Fig. 1 Diversity of phenolic compound structure

2 Bound Phenolics in Nature

Bound phenolic compounds are widely distributed among several plants, such as cereal grains (>62% of the total phenolic are bound), whereas fruits and vegetables have most of their phytochemicals in the free or soluble form (<24% of the total phenolic are bound) [11]. Phenolics, proteins, and polysaccharides are separated in different compartments in the cell, normally they do not interact until an external stimuli triggers the intracellular contact and then several subsequent reactions can occur and force the interaction (adsorption, oxidation, solubilization, and migration) [12].

PCs are linked to the cell wall structure macromolecules, such as arabinoxylans, pectin, cellulose, lignin, or proteins through ester, ether, and C-C bonds [13]. PCs can interact through hydrogen bonding, covalent bonding, hydrophobic interactions, and ionic bonding with other macromolecules [14]. Some phenolic acids are more abundant in the bound fraction, such as in the case of whole grain oats, where ferulic and p-coumaric acids were found 10- to 100-fold higher in concentration with respect to the free phenolic fraction. However, the profile was less diverse in the bound fraction than in the free phenolics [15].

In the past 20 years, there has been an important increment in research in the field of phenolic interactions with other macromolecules, but there is still limited knowledge about the changes that occur during plant development, postharvest treatments, or interactions produced during food processing and that may affect the release of free phenolics.

2.1 Conjugates with Polysaccharides

Phenolic compounds, mainly phenylpropanoids and simple phenolic acids, can be bound to carbohydrates. Hydrogen bonding and hydrophobic interactions are the driving mechanisms for this type of complexes [14]. The non-covalent complexation between cyclodextrin and phenolic acids is one of the most studied interactions. This complexation is driven by the size of cyclodextrin and its polarity [12].

2.2 Conjugates with Proteins

The molecular weight, affinity of the phenolic acid for water, structural flexibility, and the hydroxyl groups are the main characteristics that dictate the binding of phenolic acids with proteins [16]. In addition, this type of reaction is favored under neutral and basic pH conditions. One of the most studied interactions is the complex with the bovine serum albumin (BSA) [17]. Irreversible interactions involve the oxidation of phenolic compounds and the formation of o-quinones or o-semiquinones or the cleavage of proanthocyanidin interflavanic bonds.

Hydrolysable tannins are conformed by simple phenolic acids (gallotannins) or hexahydroxydiphenic acids (ellagitannins) esterified to polyols (most commonly glucose). Condensed tannins or proanthocyanidins are polymers and oligomers of

flavan-3-ol unit that are distinguished by their degree of polymerization and type of linkage [12]. Tannins can interact with proteins due to hydrophobic interactions between the aromatic ring of the tannin and the hydrophobic region of the protein and the hydrogen bonds. The tannin-protein complexes depend on the size, length, pH, and flexibility of tannins. These molecules are able to precipitate proteins from an aqueous solution. Interaction with PCs can increase the thermal stability of proteins [14, 18, 19]. Protein aggregation is affected by the average size of the condensed tannins rather than the hydroxylation pattern. In special, the procyanidins and *cis*-flavan-3-ol units contributed most to the tannin interactions on the BSA surface [20].

3 Bound Phenolic Extraction and Analysis

For the precise identification and quantification of phenolic compounds, they need to be correctly extracted. Several extraction processes underestimate the total phenolic compounds due to recovery inefficiency [21].

3.1 Traditional Methods

The most common method is the solid-liquid extraction; it involves the extraction of fresh or freeze-dried materials. But a critical step to extract bound phenolic compounds is their release from the insoluble food matrix. Acid or alkaline hydrolysis has been extensively used for this purpose. Alkaline hydrolysis has been proved to be more effective but time, temperature, and base concentration need to be optimized for each crop.

Once the compounds have been released from the matrix and the pH adjusted, extraction with solvents is required to recover bound phenolics. The most used solvents are water, methanol, ethanol, acetone, or several mixes. The parameters in this process are extraction time, temperature, solvent type, solvent to sample ratio, and number of repeated extractions. These parameters must be optimized in order to obtain a substantial recovery of PCs. The main disadvantages of solid-liquid extraction include the need to remove contaminants or other unwanted compounds from the extract, the use of hazardous organic reagents, and the time required for extraction [21].

Traditional food processing releases bound phenolics. Boiling is still the most common cooking method, and it has a negative impact on the total phenolics content of food since bound phenolics are released. After their release, they may form new irreversible covalent bonds with proteins due to the hydrothermal energy [22].

3.2 Emerging Technologies

Several physical methods have been recently used to release bound phenolics from the matrix. They may be used in combination with alkaline hydrolysis. Microwaves, ultrasound, far infrared radiation, pulsed electric field, and pressurized liquids are

examples of emerging technologies for the release of bound phenolics [11]. All these methods increase the extraction yield and reduce the hydrolysis time significantly.

4 Bioactive Potential of Bound Phenolics

Over the years, diverse biological activities from dietary and medicinal plants have been attributed to phenolic compounds, in both free and bound forms. Antioxidant capacity has been the most widely studied effect of phenolics, but effects, such as antiproliferative and apoptotic effect on cancer cells, inhibition of carbohydrate and lipid metabolism enzymes, and anti-inflammatory and vasodilator effects, among others, have been recognized (Table 1). It is important to point out that in these studies, bound phenolics are initially subjected to hydrolysis, as documented in the previous section, to release the phenolic moiety from the carbohydrate or protein moiety and therefore determine their biological effects.

With respect to in vivo potential, bound phenolics are essentially considered non-bioavailable, as they reach the colon still covalently linked. In general, it has been determined that only about 5–10% of insoluble bound phenolics are absorbed in the small intestine and the majority reaches the colon intact [13]. These compounds can be released by pH conditions in the stomach, processed by ß-glycosidase in the intestinal border brush cells, transported as glycosides by sugar transporters, or enzymatically released by fermentation of the colonic microbiota [11, 13]. Additionally, during and after absorption, phenolics undergo conjugation by intestinal and liver enzymes, such as methylation, sulfation, glucuronidation, increasing hydrophilicity, and therefore excretion [23].

4.1 Antioxidant

Antioxidant capacity of bound phenolics, particularly in the case of grains, has been well studied [24]. In most cases only the in vitro or chemical capacity as antioxidants has been analyzed by diverse methods, such as total oxygen scavenging capacity (TOSC), ferric reducing antioxidant power (FRAP), or oxygen radical antioxidant capacity (ORAC) (Table 1). To determine the impact that phenolic compounds can have in a real biological system, models such as LDL cholesterol oxidation, supercoiled DNA fragmentation, and cellular antioxidant activity assays have become more widely used in recent years [46].

4.1.1 Antioxidant Capacity by Chemical Methods

With respect to chemical antioxidant capacity of bound phenolics, Adom and Liu (2002) determined that in the case of whole grains, such as corn, wheat, and oats, this fraction corresponds to over 60% of the total antioxidant capacity evaluated by the TOSC assay, and this activity was highly correlated to total phenolics and ferulic acid content in the grains [24]. In the case of dehulled highland barley, antioxidant capacity evaluated by ORAC showed that around 50–60% of total antioxidant

Table 1 Potential bioactivity of bound phenolic compounds

Source	Phenolics	Bioactivity	Dose/concentration	Reference
Corn, wheat, oats, rice	Ferulic acid and other phenolic acids	Chemical antioxidant capacity evaluated by total oxygen scavenging capacity (TOSC)	50–150 μmol vit C eq/g grain	[24]
Dehulled highland barley	Catechin, phenolic acids	Cellular antioxidant capacity on HepG2 cells	11.3–19.2 μmol QE/mL	[25]
Model system of quercetin-BSA	Quercetin	TEAC antioxidant capacity of BSA-bound quercetin was increased hydrolyzing the protein	2.0–4.6 mM as BSA-quercetin, 3.0–4.0 as peptide-quercetin	[26]
Rice soluble fiber	Ferulic acid bound to arabinoxylans	Antioxidant capacity evaluated by inhibition of ß-carotene oxidation (IC_{50})	1.14–1.24 mg	[27]
White, red, and tannin sorghum bran, corn fiber	Phenolic acids bound to arabinoxylans	Chemical antioxidant capacity evaluated by ORAC	20–100 mM Trolox equivalents (TE)/g	[28]
Cinnamon, oregano, and clove	Phenolic acids, cinnamaldehyde, coumarin, eugenol	Chemical antioxidant capacity evaluated by FRAP and DPPH methods	2.6–34.6 mmol $FeSO_4$/100 g and 2.3–17.7 mmol TE/100 g	[29]
Ginger	Cinnamic and *p*-coumaric acids	Inhibition of lipid peroxidation (IC_{50}), inhibition of DNA fragmentation	5.2 μg GAE/g_{liver}, 2–4 μg GAE	[30]
Blackberry, black raspberry, and blueberry meal seeds	Phenolic acids, catechins, flavonoids, and anthocyanins	Chemical antioxidant capacity, LDL cholesterol oxidation inhibition (48–60% at 22 h), supercoiled DNA strand breakage inhibition (80–98%)	5–68 μmol TE/g, 0.5 mg/mL, 0.10 mg/mL	[6]
Chlorogenic acid-BSA	Model system	Binding of chlorogenic acid to BSA inhibits LDL oxidation in a dose-response way	1–10 μM chlorogenic acid +4% BSA	[31]
Blueberries	Phenolic acids, flavonoids, and anthocyanins	Cellular antioxidant capacity on HepG2 cells	13.5–63.5 μmol QE/100 g	[32]
Litchi pulp	Phenolic acids, (−)-epicathechin, 4-methylcatechol, rutin, quercetin	Cellular antioxidant activity on HepG2 cells, ORAC antioxidant capacity	7.4 ± 1.5 μmol QE/100 g, 286.1 ± 1.5 μmol TE/100 g	[33]
Cancer treatment or prevention				

(*continued*)

Table 1 (continued)

Source	Phenolics	Bioactivity	Dose/concentration	Reference
Maize lime-cooking wastewater (nejayote)	Feruloyl putrescines, ferulic acid	Induction of phase II enzyme quinone reductase	1.28–3.07 μg/mL	[34]
Dehulled highland barley	Catechin, phenolic acids	Antiproliferative activity on hepatic cancer HepG2 cells (IC_{50})	66.4–159.4 mg/mL	[25]
Cactus cladodes (*Opuntia ficus-indica*)	Isorhamnetin glycosides	Apoptotic activity on colon cancer HT-29 (IC_{50}) cells, mediated by caspase 3/7	4.9–34.8 μg/mL	[35]
Tea catechins encapsulated in milk	Epigallocatechin gallate bound to casein micelles	Antiproliferative activity on colon cancer HT-29 cells (IC_{50})	10–20 μg/mL	[36]
Foxtail millet (*Setaria italica*) bran	Phenolics	Apoptotic activity on colon cancer HCT-116 cells, mediated by ROS pathway, and inhibition of NF-κB pathway	0.28–1.43 mg/mL. 0.57–0.88 mg/mL, 0.5–0.7 mg/mL	[37]
Carbohydrate and lipid metabolism				
Citrus fruits	Phenolic acids, hesperidin	Inhibition of α-glucosidase (49.3–80.7%)	Not reported	[38]
Foxtail and little millet	*p*-coumaric, caffeic and ferulic acids, quercetin	Inhibition of α-amylase (IC_{50}) and α-glucosidase (IC_{50})	38.3–54.3 FAE μg/mL, 31.3–35.5 FAE μg/mL	[39]
Whole grain oats and products	Phenolic acids	Modulation of maltose hydrolysis (49–61%)	34–85 GAE μg/mL and 8.5–17 GAE μg/mL	[15]
		Inhibition of intestinal glucose transport (31–67%)		
Shaddock (*Citrus maxima*) peels	Not reported	Inhibition of α-amylase (12.9–64-2%) and α-glucosidase (45.9–95.9%)	80–320 μg/mL	[40]
Soybeans	Not reported	Inhibition of α-amylase (IC_{50}) and α-glucosidase (IC_{50})	320.5 and 458.7 μg/mL	[41]
Black quinoa seeds	Phenolic acids and flavonoids	Mild inhibition of α-glucosidase (IC_{50}) and pancreatic lipase (IC_{50})	37.6–55.6 mg/mL and 9.0–10.5 mg/mL	[42]
Grapefruit peel	Resveratrol, caffeic acid, ellagic acid, and other phenolic acids	HMG-CoA reductase (IC_{50}) inhibition	115.3 μg/mL	[43]
Inflammation and cardiovascular health				
				[44]

(*continued*)

Table 1 (continued)

Source	Phenolics	Bioactivity	Dose/concentration	Reference
Cactus cladodes (*Opuntia ficus-indica*)	Isorhamnetin glycosides	In vivo decrease of cell infiltration (51.8%); inhibition of: NO• (77.2–81.4%), COX-2 (76.3–77.7%), TNF-α (85.2%), and IL-6 (53.0%)	5 mg/kg (rat animal model)	
Antihypertensive and antiulcerative				
Grapefruit peel	Resveratrol, caffeic acid, ellagic acid, and other phenolic acids	Angiotensin converting enzyme (ACE) inhibition (IC_{50})	137.4 μg/L	[43]
Raw and cooked pigmented and nonpigmented rice	Not identified	ACE inhibition (40%) by pigmented rice and (20%) by nonpigmented rice extracts	8 mg FAE/mL. 4 mg FAE/mL	[45]
Ginger	Cinnamic and *p*-coumaric acids	Parietal cell proton pump inhibition (IC_{50}), *Helicobacter pylori* growth inhibition (MIC)	1.5 μg/mL, 38 μg/mL	[30]

capacity corresponded to the bound phenolic fraction [25]. The proportion of bound phenolics with respect to total content may vary among different cultivars or varieties of the same species. In different colored bran rice, it was seen that bound phenolic DPPH radical scavenging capacity represented from 7% to 41% of the total antioxidant capacity of rice, while the ORAC activity varied from 26% to 75% of the total capacity coming from the bound fraction [48]. These results indicated that, in the case of whole grains, evaluating the effect of only free phenolics would underestimate the total antioxidant potential of these particular matrices.

Feruloyl arabinoxylans form an interesting group of bound phenolic compounds with antioxidant potential. The antioxidant capacity of arabinoxylans obtained from rice was found to be a synergy between the phenolic and carbohydrate moieties. Lower IC_{50} values were reported (1.14–1.24 mg) with respect to the predicted amount (54.8–56.9 mg), calculated as the equivalent concentration of ferulic acid bound to the fiber [27]. The authors of this work concluded that ferulic acid is also able to exert antioxidant capacity without needing to be released. Similar results were observed for arabinoxylans extracted from sorghum and maize bran, in which antioxidant capacity in the extracts was attributed to ferulic acid and other bound phenolics to this cell wall component [28]. Since arabinoxylans can be used as ingredients in gluten-free bread formulation, antioxidant capacity of the bound phenolics to the carbohydrates can give added value to such food products [49].

With respect to other non-grain sources, the contribution of bound phenolics to antioxidant capacity is generally lower than 50%, depending on the source. In the case of spices such as cinnamon, oregano, and clove, bound phenolics represented between 3% and 17% of the total antioxidant capacity evaluated by the FRAP method and 3–28% by the DPPH method [29]. In the case of litchi pulp, variation in total antioxidant capacity and the proportion represented by bound phenolics was variable among different cultivars, from 10% to 49% of the antioxidant capacity measured by FRAP assay, and EC_{50} values in DPPH method demonstrated that bound phenolics were three to ten times less effective than free phenolics [50]. Similar results were obtained for litchi pulp when using the ORAC method, where bound antioxidant capacity was reported as 286.1 μmol TE/100 g, while free ORAC values reached up to 3406.8 μmol TE/100 g, representing 7.7% of the total antioxidant capacity [33]. Therefore, in general terms, contribution of bound phenolics to antioxidant capacity of herbs and fruits is lower when compared to cereals and other grains. However, in fruit seeds, a higher amount of antioxidants can be found in the bound fraction. For example, 35–70% of total antioxidant capacity from blackberry, black raspberry, and blueberry seed meals were accounted after hydrolyzing bound phenolics [6].

Binding of PCs to proteins, such as bovine serum albumin (BSA), can affect their antioxidant potential. Such was the case with a quercetin-BSA complex, which had a lower Trolox equivalent antioxidant capacity (TEAC) (2.0–4.6 mM), at concentrations from 7.9 to 17.5 μg of quercetin/mg of protein, with respect to the same amounts of free quercetin (4.4–6.0 mM). Furthermore, a higher antioxidant capacity (3.0–4.0 mM) of the peptide containing 12.2 μg of quercetin/mg of protein obtained after trypsin hydrolysis with respect to the same amount of free quercetin (5.0 mM) [26].

4.1.2 Antioxidant Capacity in Biological Systems

So far, the antioxidant capacity has been discussed in terms of chemical assays; however, the real protective potential of a compound requires evaluation in more complex systems. Antioxidant activity of ginger (*Zingiber officinale*) extracts was analyzed by two methods to determine its protective effect on biomolecules. Results showed that cinnamic acid and *p*-coumaric-rich extract inhibited lipid peroxidation (48–60%) at 5.2 μg gallic acid equivalent/g rat liver homogenate (μg GAE/g_{liver}) and protected against DNA oxidative fragmentation (80–98% inhibition) when adding 2–4 GAE μg [30]. Blackberry, black raspberry, and blueberry seed meal extracts at 0.5 mg/mL inhibited LDL cholesterol oxidation from 46% to 60% after 22 h incubation, and DNA strand breakage was inhibited at levels up to 98% with addition of 0.10 mg/mL of extract [6]. Cellular antioxidant activity is a measurement that takes into account the internalization of the compounds into the cell, therefore, making a more realistic approach to the actual antioxidant activity of a given compound or extract in a living system [51]. This assay has been used to evaluate the antioxidant potential of sources, such as blueberry extracts, where CAA values were found in the range of 13.5–63.5 μmol quercetin equivalents (QE) per 100 g fresh weight of berries, from which 8% to 20% of the total phenolic content

corresponded to the bound fraction [32]. In the case of litchi pulp, bound phenolic fraction exerted a lower antioxidant capacity (7.4 ± 1.5 μmol QE/100 g) than its corresponding free fraction, which reached up to 56.7 μmol QE/100 g, where bound fraction represented 11% of the CAA [33].

As described with respect to chemical antioxidant evaluation, PCs bound to proteins have also exhibited activity in other models. One example is the inhibition of LDL cholesterol oxidation when incubated in the presence of 4% BSA solutions with different doses of chlorogenic acid. It was shown that up to 80% delay in oxidation initiation occurred when 10 μM chlorogenic acid was used in the system, after removing the non-bound phenolic acid from the solution [31]. This study shows promising activity for these complexes, particularly when considering that protein-binding can confer protection during the digestion process but allowing release and increase of bioavailability, as well as stability during storage [16, 52].

In general, these studies have been conducted after hydrolyzing the bound phenolic fraction from the matrix, by any of the methods discussed in Sects. 3.1 and 3.2. This approach allows us to determine whether bound phenolics should be taken into account when evaluating the antioxidant capacity of a given food source. However, when considering bioavailability, bound phenolics are usually not released and absorbed during gastrointestinal digestion [23]. Food processing such as fermentation, malting, extrusion, or cooking allows the release of this fraction, increasing its uptake and making it available to target organs [11].

Therefore, most of the classical assays underestimate the true antioxidant capacity of foods since most of the compounds responsible of this activity are bound to insoluble matrices. Most of the research on the total antioxidant capacity of foods has ignored the interactions that may occur due to the coexistence of multiple antioxidants. In fact, the multiple extraction procedures used as a preliminary step for classical antioxidant determination hinder the generation of a standardized database. "QUENCHER" (Quick, Easy, New, CHEap, Reproducible) is a simple and direct assay for antioxidant capacity measurement that is extraction-independent and may enable interlaboratory comparison [47]. With this method, the functional groups of the soluble and insoluble bound antioxidants in solid samples make contact with free radicals via the liquid-liquid type of reaction and solid-liquid type of reaction, respectively [47].

4.2 Cancer

Antioxidant mechanisms are proposed as one of the primary ways in which PCs exert their bioactive potential [3]. However, there is evidence of additional anticancer mechanisms for preventive and prophylactic effect of PCs against cancer (Table 1).

One of the ways in which BPs can contribute to chemoprevention of cancer is inducing phase II enzymes, such as NADPH-dependent quinone reductase (QR), which work in vivo to inactivate free radicals and electrophiles [53]. Acosta-Estrada et al. (2015) determined that a bound phenolic extract obtained from maize lime-cooking wastewater solids was able to induce QR activity at a concentration of

76 μg/mL and presented a chemopreventive index of 4.58 (cytotoxicity requires a 4.58-fold higher concentration). In this study, QR induction was attributed to coumaroyl-feruloyl and di-feruloyl putrescines that were able to induce QR at 1.28 μg/mL and 3.07 mg/mL, respectively [34].

Antiproliferative effect of BPs released from different sources has been assayed on diverse cell lines. Catechins and phenolic acids from dehulled highland barley showed antiproliferative potential on hepatic cancer HepG2 cells (66.4 μ$\leq$ IC_{50} $\leq$ 159.4 mg/mL), observing variation among four barley varieties [25]. In this study, a lower bioactivity occurred with bound phenolics with respect to free phenolics. Glycosylated flavonoids released by alkaline hydrolysis from *Opuntia ficus-indica* cactus pads and subsequently purified presented a high antiproliferative effect. Alkaline crude extracts were effective on HT-29 (IC_{50}=4.9–9.1 μg/mL) and Caco-2 (IC_{50}=8.2–16.7 μg/mL) colon cancer cell lines. Purified isorhamnetin diglycosides presented IC_{50} values from 8.6 to 15.2 μg/mL on HT-29 and 25.8–36.7 μg/mL Caco-2 [35]. The authors determined that glycosylation pattern of the isorhamnetin derivatives had an important impact, since diglycosides were more effective than triglycosides [35].

Haratifar et al. (2014) demonstrated that PCs capacity to bind to protein could allow encapsulation of compounds, such as epigallocatechin gallate, in casein micelles, while preserving antiproliferative activity on HT-29 cells (16 $\leq$ IC_{50} $\leq$ 20 μg/mL) [36]. This PC-protein complex could be an alternative for delivery of PCs as a food ingredient or dietary supplement, such as fortified breads, dairy products, and processed meats [16, 52].

Besides the evaluation of the antiproliferative effect, some mechanisms related to the anticancer effect of BPs have been explored. In the case of *Opuntia ficus-indica* alkaline extracts, an increase in caspase 3/7 was observed by flow cytometry in HT-29 (33%) and Caco-2 (28–43%) cells treated with the extracts when compared to non-treated cells. Moreover, apoptosis was observed in 32–53% of HT-29 cells treated with isorhamnetin diglycosides and 59% when crude extract was used [35]. On the other hand, bound phenols from foxtail millet induced apoptosis by generation of reactive oxygen species (ROS) in HCT-116 colon cancer cells at concentrations from 0.57 to 0.88 mg/mL. Additionally, apoptotic effect was shown by a decrease in Bcl-2 protein and increase in BAX protein, along with inhibition of the NF-κB pathway [37]. Foxtail millet bran contains BPs such as catechin, myricetin, daidzein, luteolin, quercetin, apigenin, naringenin, and kaempferol, likely accountable for the bioactive effect [39].

4.3 Carbohydrate and Lipid Metabolism

Phenolic compounds, such as phenolic acids and flavonoids, are known inhibitors of starch-hydrolyzing enzymes α-amylase and α-glucosidase, capable of delaying carbohydrate digestion [54, 55] or even absorption by glucose transporters [15]. Bound phenolics also have potential to interfere with the activity of

the fat-digesting enzymes known as lipases, due to PCs affinity with proteins, diminishing the rate of fat absorption [56].

The BP fraction of several citrus fruits showed α-glucosidase inhibitory activity [38]. The main compounds found in these extracts were ferulic acid, *p*-coumaric acid, vanillic acid, and the flavonoid hesperidin. It can be noted that the authors reported in the same study that these extracts activated α-amylase and angiotensin converting enzyme (ACE) instead of inhibiting them [38]. Other authors report a strong inhibitory potential, as observed with BP extracts from foxtail (IC_{50}=35.21–35.26 μg ferulic acid equivalents per mL) (μg FAE/mL) and little millet (IC_{50}=38.27–46.9 μg FAE/mL); in both cases the extracts were obtained from hull and bran [39]. Pradeep and Sreerama (2017) determined that *p*-coumaric, caffeic, and ferulic acids were the major component of the extracts. Additionally, α-glucosidase inhibition was observed for BPs obtained from whole grain oats, with a 49.6–61.3% decrease in maltose hydrolysis at 34–85 μg gallic acid equivalents per gram (GAE μg/g). In this case, ferulic acid represented from 52% to 89.3% of bound phenolics from whole grain oats [15]. The effect of black quinoa seed BPs was weaker, with IC_{50} in the range between 37.6 and 55.6 mg/mL, and the main components of the extract were phenolic acids and flavonoids [42].

With respect to α-amylase, inhibition by PCs has also been reported. Foxtail and little millet showed IC_{50} values of 41.7 and 38.3 FAE μg/mL, respectively, in the hull fraction and 54.3 and 46.9 FAE μg/mL in the bran fraction [39]. Bound phenolics from the citrus shaddock (*Citrus maxima*) fruit peel were able to inhibit α-amylase activity up to 64.2% in a dose-dependent manner, at concentrations from 80 to 320 μg/mL [40]. Soybean BP extracts were found to inhibit 50% of α-amylase activity at 320.5 μg/mL [41]. In general, phenolics are stronger inhibitors of α-glucosidase than of α-amylase, such as in the case of BPs extracted from little millet and shaddock peels, where significant differences were observed [39]. This difference in BPs effect on both enzymes could be important when considering their use as a therapeutic agent for hyperglycemia, since inhibiting α-glucosidase preferentially can prevent side effects from excess inhibition of α-amylase [40, 41]. However, it should be taken into account that in some cases α-amylase was activated by treatment with BPs, as happened with whole grain oat phenolics and citrus fruits [15, 38].

4.4 Interactions with Microbiota

Bound phenolics help to maintain a "healthy" microbiota. For example, cocoa bound flavanols increased the number of *Lactobacilli* and *Bifidobacteria* [57]. Many bound phenolics resist enzymatic digestion (oral, gastric, and small and large intestine phases) and exert their antioxidant activity in the large intestine [58]. But more importantly, their release is slow and continuous [59]. The slow release of bound phenolic compounds could be related to the complexity of the matrix and the diversity of microorganisms required to breakdown the chemical interactions [60].

5 Conclusions

For many years, bound phenolics were considered as poor contributors to improve human health due to their strong interactions with the food matrix and low bioavailability. Particularly in cereals, bound phenolics are major contributors to total phenolic content and antioxidant activity. Most of the studies on bound phenolic compounds involve their extraction after acid or base hydrolysis, and therefore a lot about their chemical structure is known. But when gastrointestinal microbiota is taken into consideration as an important element in the digestibility of dietary fiber, the release of bound phenolics is more complex as well as their potential bioactivity. Microbiota heterogeneity is important to have a vast enzymatic tool to release bound phenolics continuously and slowly to maintain a constant dose of circulating bioactive compounds.

Acknowledgments We appreciate the support from NutriOmics Chair from Tecnológico de Monterrey.

References

1. Tomás-Barberán FA, Espín JC (2001) Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J Sci Food Agric 81:853–876. https://doi.org/10.1002/jsfa.885
2. Mandal SM, Chakraborty D, Dey S (2010) Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behav 5:359–368. https://doi.org/10.4161/psb.5.4.10871
3. Tsao R, Rong (2010) Chemistry and biochemistry of dietary polyphenols. Nutrients 2:1231–1246. https://doi.org/10.3390/nu2121231
4. Reis Giada MdeL (2013) Food phenolic compounds: main classes, sources and their antioxidant power. In: Oxidative stress and chronic degenerative diseases- a role for antioxidants. pp 87–112
5. Goufo P, Trindade H (2014) Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ -oryzanol, and phytic acid. Food Sci Nutr 2:75–104. https://doi.org/10.1002/fsn3.86
6. Ayoub M, De Camargo AC, Shahidi F (2016) Antioxidants and bioactivities of free, esterified and insoluble-bound phenolics from berry seed meals. Food Chem 197:221–232. https://doi.org/10.1016/j.foodchem.2015.10.107
7. Girgin N, El SN (2015) Effects of cooking on in vitro sinigrin bioaccessibility, total phenols, antioxidant and antimutagenic activity of cauliflower (*Brassica oleraceae* L. var. Botrytis). J Food Compos Anal 37:119–127. https://doi.org/10.1016/J.JFCA.2014.04.013
8. Karimi A, Mohammadi-Kamalabadi M, Rafieian-Kopaei M et al (2016) Determination of antioxidant activity, phenolic contents and antiviral potential of methanol extract of *Euphorbia spinidens* Bornm (Euphorbiaceae). Trop J Pharm Res 15:759. https://doi.org/10.4314/tjpr.v15i4.13
9. Luo J, Zhang P, Li S, Shah NP (2016) Antioxidant, antibacterial, and antiproliferative activities of free and bound phenolics from peel and flesh of Fuji apple. J Food Sci 81:M1735–M1742. https://doi.org/10.1111/1750-3841.13353
10. Mushtaq M, Sultana B, Anwar F, Batool S (2015) Antimutagenic and antioxidant potential of aqueous and acidified methanol extracts from *Citrus limonum* fruit residues. J Chil Chem Soc 60:2979–2983. https://doi.org/10.4067/S0717-97072015000200025
11. Acosta-Estrada BA, Gutiérrez-Uribe JA, Serna-Saldívar SO (2014) Bound phenolics in foods, a review. Food Chem 152:46–55. https://doi.org/10.1016/j.foodchem.2013.11.093

12. Le Bourvellec C, Renard CMGC (2012) Interactions between polyphenols and macromolecules: quantification methods and mechanisms. Crit Rev Food Sci Nutr 52:213–248. https://doi.org/10.1080/10408398.2010.499808
13. Shahidi F, Yeo JD (2016) Insoluble-bound phenolics in food. Molecules 21. https://doi.org/10.3390/molecules21091216
14. Alu'datt MH, Rababah T, Alhamad MN et al (2017) Occurrence, types, properties and interactions of phenolic compounds with other food constituents in oil-bearing plants. Crit Rev Food Sci Nutr:1–10. https://doi.org/10.1080/10408398.2017.1391169
15. Li M, Koecher K, Hansen L, Ferruzzi MG (2017) Phenolics from whole grain oat products as modifiers of starch digestion and intestinal glucose transport. J Agric Food Chem 65:6831–6839. https://doi.org/10.1021/acs.jafc.7b02171
16. Buitimea-Cantúa NE, Gutiérrez-Uribe JA, Serna-Saldívar SO (2017) Phenolic–protein interactions: Effects on food properties and health benefits. J Med Food 0:jmf.2017.0057. https://doi.org/10.1089/jmf.2017.0057
17. He T, Liang Q, Luo T, Wang Y, Luo G (2010) Study on interactions of phenolic acid-like drug candidates with bovine serum albumin by capillary electrophoresis and fluorescence spectroscopy. J Solut Chem 39:1653–1664. https://doi.org/10.1007/s10953-010-9608-8
18. Li S, Huang K, Zhong M, Guo J, Wang WZ, Zhu R (2010) Comparative studies on the interaction of caffeic acid, chlorogenic acid and ferulic acid with bovine serum albumin. Spectrochim Acta Part A Mol Biomol Spectrosc 77:680–686. https://doi.org/10.1016/J.SAA.2010.04.026
19. Skrt M, Benedik E, Podlipnik Č, Ulrih NP (2012) Interactions of different polyphenols with bovine serum albumin using fluorescence quenching and molecular docking. Food Chem 135:2418–2424. https://doi.org/10.1016/J.FOODCHEM.2012.06.114
20. Ropiak HM, Lachmann P, Ramsay A, Green RJ, Mueller-Harvey I (2017) Identification of structural features of condensed tannins that affect protein aggregation. PLoS One 12:e0170768. https://doi.org/10.1371/journal.pone.0170768
21. Cong-Cong X, Bing W, Yi-Qiong P, Jian-Sheng T, Zhang T (2017) Advances in extraction and analysis of phenolic compounds from plant materials. Chin J Nat Med 15:721–731. https://doi.org/10.1016/S1875-5364(17)30103-6
22. Yeo J, Shahidi F (2017) Effect of hydrothermal processing on changes of insoluble-bound phenolics of lentils. J Funct Foods 38:716–722. https://doi.org/10.1016/J.JFF.2016.12.010
23. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–747. https://doi.org/10.1038/nature05488
24. Adom KK, Liu RH (2002) Antioxidant activity of grains. J Agric Food Chem 50:6182–6187. https://doi.org/10.1021/jf0205099
25. Zhu Y, Li T, Fu X, Abbasi AM, Zheng B, Liu RH (2015) Phenolics content, antioxidant and antiproliferative activities of dehulled highland barley (*Hordeum vulgare* L.) J Funct Foods 19:439–450. https://doi.org/10.1016/j.jff.2015.09.053
26. Rohn S, Rawel HM, Kroll J (2004) Antioxidant activity of protein-bound quercetin. J Agric Food Chem 52:4725–4729. https://doi.org/10.1021/JF0496797
27. Rao RSP, Muralikrishna G (2006) Water soluble feruloyl arabinoxylans from rice and ragi: changes upon malting and their consequence on antioxidant activity. Phytochemistry 67:91–99. https://doi.org/10.1016/J.PHYTOCHEM.2005.09.036
28. Ayala-Soto FE, Serna-Saldívar SO, Welti-Chanes J, Gutierrez-Uribe JA (2015) Phenolic compounds, antioxidant capacity and gelling properties of glucoarabinoxylans from three types of sorghum brans. J Cereal Sci 65:277–284. https://doi.org/10.1016/j.jcs.2015.08.004
29. Chan CL, Gan RY, Corke H (2016) The phenolic composition and antioxidant capacity of soluble and bound extracts in selected dietary spices and medicinal herbs. Int J Food Sci Technol 51:565–573. https://doi.org/10.1111/ijfs.13024
30. Siddaraju MN, Dharmesh SM (2007) Inhibition of gastric H+,K+-ATPase and *Helicobacter pylori* growth by phenolic antioxidants of *Zingiber officinale*. Mol Nutr Food Res 51:324–332. https://doi.org/10.1002/mnfr.200600202

31. Gordon MH, Wishart K (2010) Effects of chlorogenic acid and bovine serum albumin on the oxidative stability of low density lipoproteins in vitro. J Agric Food Chem 58:5828–5833. https://doi.org/10.1021/jf100106e
32. Wang H, Guo X, Hu X, Li T, Fu X, Liu RH (2017) Comparison of phytochemical profiles, antioxidant and cellular antioxidant activities of different varieties of blueberry (*Vaccinium spp.*) Food Chem 217:773–781. https://doi.org/10.1016/j.foodchem.2016.09.002
33. Su D, Zhang R, Hou F, Zhang M, Guo J, Huang F, Deng Y, Wei Z (2014) Comparison of the free and bound phenolic profiles and cellular antioxidant activities of litchi pulp extracts from different solvents. BMC Complement Altern Med 14:9. https://doi.org/10.1186/1472-6882-14-9
34. Acosta-Estrada BA, Serna-Saldívar SO, Gutiérrez-Uribe JA (2015) Chemopreventive effects of feruloyl putrescines from wastewater (Nejayote) of lime-cooked white maize (*Zea mays*). J Cereal Sci 64:23–28. https://doi.org/10.1016/j.jcs.2015.04.012
35. Antunes-Ricardo M, Moreno-García BE, Gutiérrez-Uribe JA, Aráiz-Hernández D, Alvarez MM, Serna-Saldivar SO (2014) Induction of apoptosis in colon cancer cells treated with isorhamnetin glycosides from *Opuntia ficus-indica* pads. Plant Foods Hum Nutr 69:331–336. https://doi.org/10.1007/s11130-014-0438-5
36. Haratifar S, Meckling KA, Corredig M (2014) Antiproliferative activity of tea catechins associated with casein micelles, using HT29 colon cancer cells. J Dairy Sci 97:672–678. https://doi.org/10.3168/jds.2013-7263
37. Shi J, Shan S, Li Z, Li H, Li X, Li Z (2015) Bound polyphenol from foxtail millet bran induces apoptosis in HCT-116 cell through ROS generation. J Funct Foods 17:958–968. https://doi.org/10.1016/J.JFF.2015.06.049
38. Alu'datt MH, Rababah T, Alhamad MN, Al-Mahasneh MA, Ereifej K, Al-Karaki G, Al-Duais M, Andrade JE, Tranchant CC, Kubow S, Ghozlan KA (2017) Profiles of free and bound phenolics extracted from *Citrus* fruits and their roles in biological systems: content, and antioxidant, anti-diabetic and anti-hypertensive properties. Food Funct 8:3187–3197. https://doi.org/10.1039/C7FO00212B
39. Pradeep PM, Sreerama YN (2017) Soluble and bound phenolics of two different millet genera and their milled fractions: comparative evaluation of antioxidant properties and inhibitory effects on starch hydrolysing enzyme activities. J Funct Foods 35:682–693. https://doi.org/10.1016/J.JFF.2017.06.033
40. Oboh G, Ademosun AO (2011) Shaddock peels (*Citrus maxima*) phenolic extracts inhibit α-amylase, α-glucosidase and angiotensin I-converting enzyme activities: a nutraceutical approach to diabetes management. Diabetes Metab Syndr Clin Res Rev 5:148–152. https://doi.org/10.1016/j.dsx.2012.02.008
41. Ademiluyi AO, Oboh G (2013) Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (alpha-amylase and alpha-glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. Exp Toxicol Pathol 65:305–309. https://doi.org/10.1016/j.etp.2011.09.005
42. Tang Y, Zhang B, Li X, Chen PX, Zhang H, Liu R, Tsao R (2016) Bound phenolics of quinoa seeds released by acid, alkaline, and enzymatic treatments and their antioxidant and α-glucosidase and pancreatic lipase inhibitory effects. J Agric Food Chem 64:1712–1719. https://doi.org/10.1021/acs.jafc.5b05761
43. Ademosun AO, Oboh G, Passamonti S, Tramer F, Ziberna L, Boligon AA, Athayde ML (2015) Phenolics from grapefruit peels inhibit HMG-CoA reductase and angiotensin-I converting enzyme and show antioxidative properties in endothelial EA.Hy 926 cells. Food Sci Human Wellness 4:80–85. https://doi.org/10.1016/j.fshw.2015.05.002
44. Antunes-Ricardo M, Gutiérrez-Uribe JA, López-Pacheco F, Alvarez MM, Serna-Saldívar SO (2015) In vivo anti-inflammatory effects of isorhamnetin glycosides isolated from *Opuntia ficus-indica* (L.) mill cladodes. Ind Crop Prod 76:803–808. https://doi.org/10.1016/j.indcrop.2015.05.089
45. Massaretto IL, Alves MF, de Mira NV, Carmona AK, Marquez UM (2011) Phenolic compounds in raw and cooked rice (*Oryza sativa* L.) and their inhibitory effect on the activity of angiotensin I-converting enzyme. J Cereal Sci 54:236–240. https://doi.org/10.1016/j.jcs.2011.06.006

46. Shahidi F, Zhong Y (2015) Measurement of antioxidant activity. J Funct Foods 18:757–781. https://doi.org/10.1016/j.jff.2015.01.047
47. Cömert ED, Gökmen V (2017) Antioxidants bound to an insoluble food matrix: their analysis, regeneration behavior, and physiological importance. Compr Rev Food Sci Food Saf 16:382–399. https://doi.org/10.1111/1541-4337.12263
48. Min B, Gu L, McClung AM, Bergman CJ, Chen MH (2012) Free and bound total phenolic concentrations, antioxidant capacities, and profiles of proanthocyanidins and anthocyanins in whole grain rice (*Oryza sativa* L.) of different bran colours. Food Chem 133:715–722. https://doi.org/10.1016/j.foodchem.2012.01.079
49. Ayala-Soto FE, Serna-Saldívar SO, Welti-Chanes J (2017) Effect of arabinoxylans and laccase on batter rheology and quality of yeast-leavened gluten-free bread. J Cereal Sci 73:10–17
50. Zhang R, Zeng Q, Deng Y, Zhang M, Wei Z, Zhang Y, Tang X (2013) Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China. Food Chem 136:1169–1176. https://doi.org/10.1016/j.foodchem.2012.09.085
51. Wolfe KL, Liu RH (2007) Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. J Agric Food Chem 55:8896–8907. https://doi.org/10.1021/jf0715166
52. Wegrzyn TF, Farr JM, Hunter DC, Au J, Wohlers MW, Skinner MA, Stanley RA (2008) Stability of antioxidants in an apple polyphenol–milk model system. Food Chem 109:310–318. https://doi.org/10.1016/J.FOODCHEM.2007.12.034
53. Cuendet M, Oteham CP, Moon RC, Pezzuto JM (2006) Quinone reductase induction as a biomarker for chancer chemoprevention. J Nat Prod 69:460–463
54. de Sales PM, de Souza PM, Simeoni LA, Magalhães PO, Silveira D (2012) α-amylase inhibitors: a review of raw material and isolated compounds from plant source. J Pharm Pharm Sci 15:141–183. https://doi.org/10.18433/J35S3K
55. Kumar V, Prakash O, Kumar S, Narwal S (2011) α-glucosidase inhibitors from plants: a natural approach to treat diabetes. Pharmacogn Rev 5:19. https://doi.org/10.4103/0973-7847.79096
56. Birari RB, Bhutani KK (2007) Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov Today 12. https://doi.org/10.1016/j.drudis.2007.07.024
57. Fogliano V, Corollaro ML, Vitaglione P, Napolitano A, Ferracane R, Travaglia F, Arlorio M, Costabile A, Klinder A, Gibson G (2011) In vitro bioaccessibility and gut biotransformation of polyphenols present in the water-insoluble cocoa fraction. Mol Nutr Food Res 55:S44–S55. https://doi.org/10.1002/mnfr.201000360
58. Papillo VA, Vitaglione P, Graziani G, Gokmen V, Fogliano V (2014) Release of antioxidant capacity from five plant foods during a multistep enzymatic digestion protocol. J Agric Food Chem 62:4119–4126. https://doi.org/10.1021/jf500695a
59. Vitaglione P, Napolitano A, Fogliano V (2008) Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. Trends Food Sci Technol 19:451–463. https://doi.org/10.1016/j.tifs.2008.02.005
60. Williams BA, Grant LJ, Gidley MJ, Mikkelsen D (2017) Gut fermentation of dietary fibres: Physico-chemistry of plant cell walls and implications for health. Int J Mol Sci 18:2203

Tea, Coffee and Health Benefits

35

Sumio Hayakawa, Yumiko Oishi, Hiroki Tanabe, Mamoru Isemura, and Yasuo Suzuki

Contents

S. Hayakawa (✉) · Y. Oishi
Department of Cellular and Molecular Medicine, Medical Research Institute, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan
e-mail: hayakawa.dcmm@mri.tmd.ac.jp; yuooishi.dcmm@mri.tmd.ac.jp

H. Tanabe
Department of Nutritional Sciences, Faculty of Health and Welfare Science, Nayoro City University, Nayoro-City, Hokkaido, Japan
e-mail: htanabe@nayoro.ac.jp

M. Isemura
Tea Science Research Center, University of Shizuoka, Suruga-ku, Shizuoka, Japan
e-mail: isemura@u-shizuoka-ken.ac.jp

Y. Suzuki
Department of Nutrition Management, Faculty of Health Science, Hyogo University, Kakogawa, Hyogo, Japan
e-mail: suz-cbio@hyogo-dai.ac.jp

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_14

Abstract

A number of epidemiological studies and clinical trials have reported the beneficial effects of both green tea and coffee on human health, including anticancer, anti-obesity, antidiabetic, antihypertensive, and hepatoprotective effects. Furthermore, these findings in humans are supported by cell-based and animal experiments. These effects have been attributed to epigallocatechin gallate (EGCG) in green tea and chlorogenic acid (CGA) in coffee, which have been proposed to function via various mechanisms of action, the most important of which appears to implicate reactive oxygen species (ROS). Both EGCG and CGA can exert conflicting dual actions as an antioxidant and a prooxidant. Their antioxidative action can scavenge ROS, leading to downregulation of nuclear factor-κB to produce various favorable effects such as anti-inflammatory effects and cancer cell apoptosis. The prooxidant actions, however, can promote the generation of ROS leading to the activation of 5'AMP-dependent protein kinase, which modulates various enzymes and factors with beneficial roles. At present, it remains unclear how EGCG and CGA can be directed to act as either a prooxidant or an antioxidant, although their cellular concentrations, the presence of metal cations such as Cu^{+} and Fe^{++}, and the redox state of the cells appear to be important factors. Notably, several human studies did not report the beneficial health effects of green tea and coffee. The inconsistent results may have been caused by various confounding factors including smoking, intestinal microbiota, and genetic factors. This chapter examines the current information on these properties of green tea and coffee with the aim of improving the understanding of a way to enjoy healthy longevity.

Keywords

Green tea · Coffee · Polyphenol · Catechin · EGCG · Chlorogenic acid · Human health · ROS · NF-κB

Abbreviations

ACC	Acetyl-CoA carboxylase
ACE	Angiotensin-converting enzyme
ACF	Aberrant crypt foci
ALT	Alanine aminotransferase
AMPK	5′-AMP-activated protein kinase
ANI	α-Naphthylisothiocyanate
AOM	Azoxymethane
AST	Aspartate aminotransferase

BBN	*N*-Butyl-*N*-(4-hydroxybutyl)-nitrosamine
BMI	Body mass index
C/EBP	CCAAT/enhancer-binding protein
CGA	Chlorogenic acid
CLL	Chronic lymphocytic leukemia
COX	Cyclooxygenase
CPP	Coffee polyphenols
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
EC	(−)-Epicatechin
EGCG	(−)-Epigallocatechin-3-gallate
ERK	Extracellular signal-regulated kinase
FASN	Fatty acid synthase
G6Pase	Glucose-6-phosphatase
GCE	Green coffee extract
GLUT	Glucose transporter
GST	Glutathione S-transferase
GTC	Green tea catechin
GTE	Green tea extract
GTP	Green tea polyphenol
HbA1c	Hemoglobin A1c
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDL	High-density lipoprotein
HFD	High-fat diet
HNF	Hepatocyte nuclear factor
HO	Heme oxygenase
HR	Hazard ratio
HuR	Human antigen R
IFN	Interferon
IGF	Insulin-like growth factor
IL	Interleukin
IRS	Insulin receptor substrate
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
LXR	Liver X receptor
MAPK	Mitogen-activated protein kinase
MetS	Metabolic syndrome
MMP	Matrix metalloproteinase
mTOR	Mechanistic target of rapamycin kinase
NAFLD	Nonalcoholic fatty liver disease
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
NOS	Nitric oxide synthase

Nrf2	Nuclear factor, erythroid 2 like 2
OR	Odds ratio
PCa	Prostate cancer
PEPCK	Phosphoenolpyruvate carboxykinase
PKC	Protein kinase C
PPAR	Peroxisome proliferator-activated receptor
PPE	Polyphenon E
QHD	Qushi Huayu Decoction
ROS	Reactive oxygen species
RR	Relative risk
RXR	Retinoid X receptor
SBP	Systolic blood pressure
SHR	Spontaneously hypertensive rats
SREBP	Sterol-responsive element-binding protein
STAT	Signal transducer and activator of transcription
STZ	Streptozotocin
T2DM	Type 2 diabetes mellitus
TNF	Tumor necrosis factor
Treg	Regulatory T
VEGF	Vascular endothelial growth factor

1 Introduction

Green tea is produced by the processing of tea leaves from the plant *Camellia sinensis* (Theaceae) and is popularly consumed worldwide, particularly in Japan and China. Green tea has been shown to have beneficial effects on human health such as anticancer, anti-obesity, antidiabetic, anti-cardiovascular, anti-infectious, and hepatoprotective effects [1–6]. Most of these biological effects are thought to be ascribable to polyphenol catechins, specifically (−)-epigallocatechin-3-gallate (EGCG) (Fig. 1), which is the major catechin. A single 200 mL cup of typically brewed green tea supplies 240–320 mg of catechins, of which EGCG accounts for 60%–65%, together with much lower quantities of other polyphenols including quercetin, myricetin, and kaempferol [7]. Green tea is a rich source of caffeine (Fig. 1), which has strong physiological effects on bodily systems such as the central nervous, respiratory, cardiovascular, urinary, and gastrointestinal systems [8].

Black tea is also produced from *C. sinensis* through enzymic processing (sometimes called fermentation) by intrinsic enzymes and microorganisms during which catechins are polymerized to yield catechin derivatives such as theaflavin (Fig. 1) and theasinensins [9]. It has been shown to have physiological effects similar to those of green tea, albeit with a lesser efficacy than green tea in most cases.

Coffee is also consumed worldwide and, like green tea, exerts various health-related effects. It contains about 2,000 chemicals, including caffeine, and the major polyphenols are chlorogenic acid (CGA) or 5-caffeoylquinic acid (Fig. 1) and its derivatives, which amount to about 3 g per 100 g of roasted coffee powder [8].

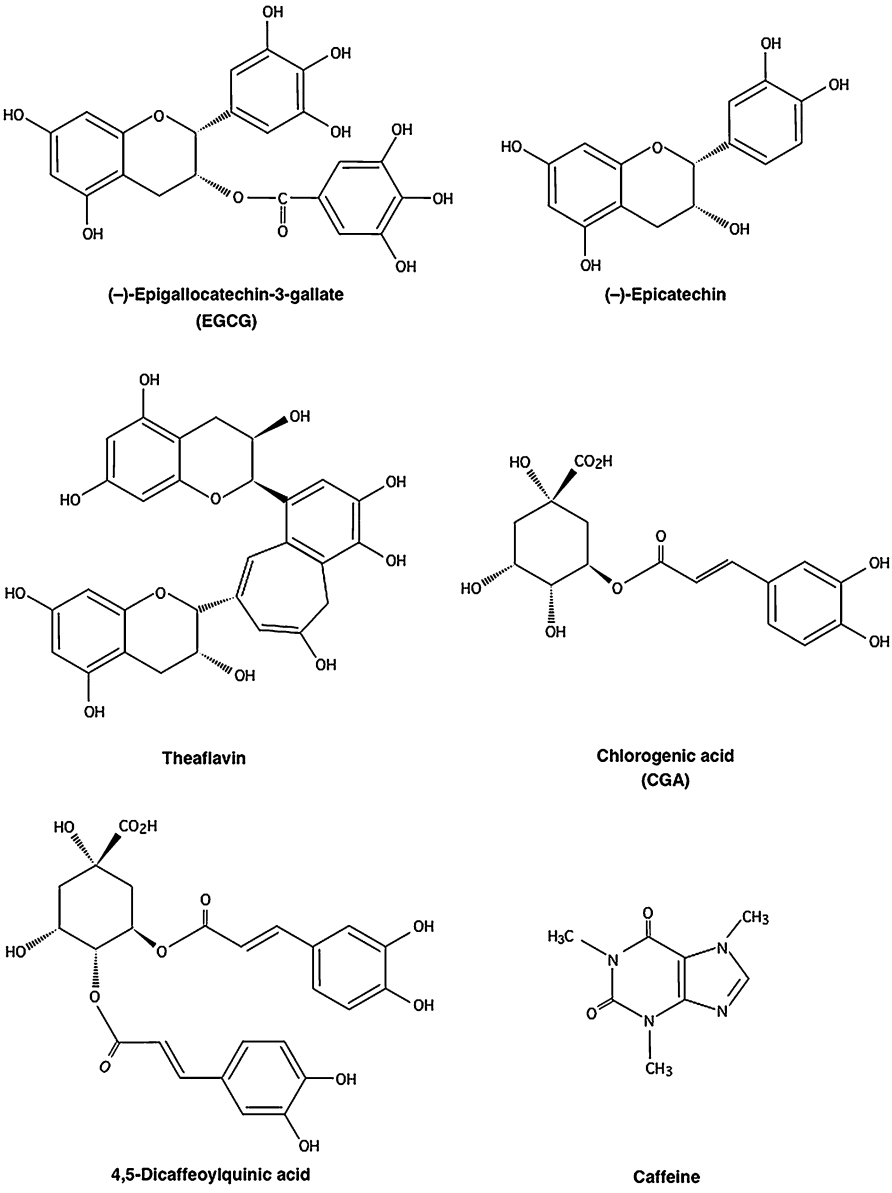


Fig. 1 Chemical structures of compounds pertinent to this chapter

A single serving of coffee provides 20–675 mg of CGAs [10]. It should be noted that a recent analysis using ultrahigh-performance liquid chromatography showed that green tea leaves collected from public markets in Brazil contained 1.1 g of CGAs per 100 g of dried leaves [11].

In this review, we discuss recent evidence that supports the beneficial effects of tea and coffee consumption in relation to the mechanistic aspects of catechins and CGAs by focusing on selected diseases in which we have studied the action of green tea. Caffeine, a highly bioactive constituent contained in both tea and coffee, has been comprehensively reviewed by Temple et al. [12] and is discussed only briefly here. For the sake of readability, 95% confidence interval values and statistical *p*-values, which an original datum contains in statistical evaluation, are not presented here unless otherwise described.

2 Effects on Cancer

2.1 Effects of Tea on Cancer

2.1.1 Human Epidemiological Studies

A number of human epidemiological studies have shown that green tea exerts beneficial effects against various cancers [1–6]. A review article by Yang et al. reported an inverse association between green/black tea consumption and cancer risk for various types of cancer including bladder, breast, colon, gastric, kidney, lung, ovarian, pancreatic, and prostate cancers in 51 of 127 case-control studies and 19 of 90 cohort studies which were carried out from 1965 to 2008 [1]. Yuan and co-workers reviewed the results of 13 studies in which green tea consumption was associated with a significant risk reduction for breast, colorectal, gastric, esophagus, liver, lung, oral cavity, and prostate cancers in 9 studies [13, 14].

Recent studies also demonstrated the beneficial effects of tea, with some examples as follows. The European Prospective Investigation into Cancer and Nutrition study, involving 486,799 men/women for a median follow-up of 11 years, found that increased tea intake was associated with a 59% reduction in the risk of developing hepatocellular carcinoma (HCC) [15]. The analysis of 87 datasets from 57 studies, which included a total of 49,812 subjects, showed that high tea consumption was associated with a reduced risk of oral cancer with a risk ratio (RR) of 0.72, although it had no significant effect on the risk of bladder, breast, colon, gastric, liver, lung, rectal, ovarian, pancreatic, and prostate cancers or gliomas. In a subgroup analysis of individuals in Western countries, the consumption of tea was associated with a reduced risk of bladder cancer, although the consumption of black tea was associated with an increased risk of breast cancer [16].

The results of a hospital-based, case-control study including 160 cases and 320 controls in China showed that the regular consumption of larger amounts of green tea ($\geq$35 g/week) was associated with a lower risk of stomach cancer, with odds ratios (ORs) of 0.72 and 0.53, respectively. Among regular tea drinkers, lower temperature and longer interval between tea being poured and drunk also reduced the risk, suggesting that green tea was inversely associated with risk of stomach cancer [17].

In a study to determine the prostate cancer (PCa) risk associated with green tea and EGCG intake among Hong Kong Chinese men, data from 32 cases and 50 controls showed that habitual green tea drinking had an adjusted OR of 0.60. An

inverse association was also found between intake of EGCG and PCa risk [18]. Similarly, data from the Japan Public Health Center-Based Prospective Study showed that green tea intake may decrease the risk of advanced PCa [19].

A case-control study in Vietnamese men showed that after adjustment for confounding factors, increased tea consumption was associated with a reduced risk of PCa [20]. The adjusted ORs were 0.52 and 0.30 for participants drinking 100–500 mL/day and >500 mL/day, respectively, relative to those drinking <100 mL/day. Significant inverse dose-response relationships were also observed for years of drinking and number of cups consumed daily, showing that habitual tea consumption was associated with a reduced risk of PCa. A meta-analysis of seven epidemiological studies and three randomized controlled clinical trials indicated that green tea consumption reduced the incidence of PCa with a linear dose-response effect and significantly reduced the risk of PCa risk at more than 7 cups/day [21].

A meta-analysis of eight studies comprising 18 independent reports on biliary tract cancer showed that tea intake reduced the risk of cancer by about 34% compared with a no-intake group. This inverse relationship was statistically significant in women but not in men [22]. Chen et al. conducted a meta-analysis to evaluate relationships between tea intake and the risk of biliary tract cancer in 29 qualified studies. The summary OR of developing colorectal cancer for the highest versus the lowest tea consumption was 0.93. A stratified analysis revealed that tea, especially green tea, had a protective effect in female and rectal cancer patients. The dose-response analysis showed that there was a significant inverse association between an increment of 1 cup/day of tea consumption and colorectal cancer risk (OR, 0.68) in women [23].

A meta-analysis performed in April 2016 in a total of 18 (11 case-control and 7 cohort) studies, comprising data for 701,857 female subjects including 8,683 ovarian cancer cases, showed that tea consumption had a significant protective effect against ovarian cancer (relative risk [RR], 0.86). The relationship was confirmed after adjusting for family history of cancer (RR, 0.85), menopause status (RR, 0.85), education (RR, 0.82), body mass index (BMI) (RR, 0.85), and smoking (RR, 0.83) [24].

2.1.2 Clinical Studies

One of the most significant studies may be that of an Italian research group which showed that green tea catechins (GTCs) were safe and highly effective for the treatment of premalignant lesions prior to the development of PCa. Only 1 tumor was detected among a group of 30 men with precancerous lesions who received daily oral administration of 600 mg GTCs, as compared with 9 detected tumors among 30 placebo-treated male patients after 1 year [25]. A later systematic review of 15 studies with 11 reports on the effect of green tea consumption on PCa prevention and 4 reports on the effect of green tea on treatment revealed that green tea appeared to be an effective chemopreventive agent for PCa, particularly in patients with high-grade prostate intraepithelial neoplasia, although evidence of efficacy in the treatment of PCa is currently lacking [26].

To investigate whether erythrocyte oxidative stress was associated with PCa and whether daily consumption of green tea improved the oxidative phenotype, Lassed et al. performed a study on 70 Algerian PCa patients and 120 age-matched healthy subjects. The results at baseline showed reduced glutathione levels and catalase activity and a high level of malondialdehyde in erythrocytes from PCa patients. The consumption of 2–3 cups of green tea per day for 6 months significantly increased glutathione concentration and catalase activity and decreased malondialdehyde concentration. Green tea also significantly decreased oxidative stress in these patients, indicating that regular consumption of green tea for a long period may prevent the development of PCa or at least delay its progression [27].

In a clinical trial in ten patients with stage 0 chronic lymphocytic leukemia (CLL) and ten healthy subjects administered oral green tea extract (GTE) therapy for 6 months, eight out of ten patients showed a reduction in lymphocytosis and absolute number of circulating regulatory T (Treg) cells. Only one nonresponding patient had disease progression at 5 months after the end of GTE administration and chemotherapy. These findings suggest that green tea can control lymphocytosis and prevent disease progression [28].

In a clinical trial in 124 subjects who were recruited and randomly assigned to low-dose GTCs (500 mg), high-dose GTCs (1,000 mg), or placebo for 3 months, urinary fumonisin B_1, a carcinogen, was significantly decreased after 1 month in the high-dose group compared with the placebo group, with reduction rates of 18.95% in the low-dose group and 33.62% in the high-dose group. After a 3-month intervention, urinary levels of fumonisin B_1 were reduced to 40.18% in the low-dose group and 52.6% in the high-dose group compared with both the placebo group and baseline levels. These findings suggest that supplementation with GTCs may represent a useful chemopreventive strategy for reducing co-exposure to aflatoxin B_1 and fumonisin B_1 [29].

In a randomized placebo-controlled trial, 99 women received either Polyphenon E (PPE), a green tea polyphenol formulation primarily consisting of EGCG, or placebo once a day for 4 months. A complete response, defined as negative for high-risk human papilloma virus and normal histopathology, was noted in 17.1% and 14.6% of women in the PPE and placebo arms, respectively, showing a preferable effect of PPE [30].

In a phase II pharmacodynamic prevention trial of PPE, patients with bladder tumors were randomized to receive PPE containing either 800 or 1,200 mg of EGCG or placebo for 14–28 days prior to transurethral resection of the bladder tumor or cystectomy. EGCG levels in plasma and urine increased significantly, and the expression of proliferating cell nuclear antigen and clusterin was downregulated in the bladder tissues. Despite the limitations of this pilot study, the authors pointed out that the observed pharmacodynamics and desirable biological activity warranted further clinical studies of PPE in bladder cancer prevention [31].

In a randomized clinical trial to evaluate GTE for the prevention of metachronous colorectal polyps, 143 patients who underwent the endoscopic removal of colorectal adenomas were divided into a supplementation group (0.9 g GTE/day for 12 months) and a control group without supplementation. Follow-up colonoscopy after conducted

12 months found that the incidence of metachronous adenomas was 42.3% in the control group and 23.6% in the GTE group. The number of relapsed adenomas also decreased in the GTE group compared with that in the control group [32].

Although several studies have demonstrated the anticancer effects of green tea, as described above, conflicting results have also been reported [1, 4]. For example, Je and Park identified five eligible cohort studies comprising 231,870 female participants and 1,831 cases of endometrial cancer [33]. The pooled RR of the three studies conducted in the United States, in which black tea was consumed by most people, was 1.00. These findings do not support an association between tea consumption and endometrial carcinogenesis risk. Furthermore, a meta-analysis of 25 case-control studies (15,643 patients and 30,795 controls) and 7 prospective cohort studies (1,807 cases and 443,076 participants) showed that tea consumption was not significantly associated with bladder cancer risk [34].

In a double-blind randomized controlled trial, subjects with primary multifocal high-grade prostatic intraepithelial neoplasia and/or atypical small acinar proliferation received 35 mg lycopene, 55 μg selenium, and 600 mg GTCs, or placebo, per day for 6 months. The results indicated that the administration of high doses of lycopene, GTCs, and selenium in men was associated with a higher incidence of PCa, suggesting that the use of these supplements should be avoided [35].

In a comprehensive review article, Yang and Wang concluded that the results of human studies on GTCs, mostly from small randomized clinical trials, have been inconsistent [6]. The authors highlighted the following examples. An earlier randomized clinical trial on oral cancer prevention in China showed that a mixed tea product (3 g/day) caused a significant decrease in cancer growth, but a later phase II randomized clinical trial in the United States showed that GTE (500, 750, or 1,000 mg/m^2, twice daily) for 12 weeks resulted in only potentially beneficial effects, which were not significant in reducing oral premalignant lesions. Furthermore, in spite of seemingly promising results reported in the Italian intervention study mentioned previously, a subsequent trial in Florida with a similar design showed that catechin supplementation for 6–12 months did not cause a reduction in the number of PCa cases compared with placebo [6].

Thus, further studies are needed to determine the chemopreventive effects of green tea, but its potentially beneficial effects are supported by Yang and Wang in a phase 2 trial in patients with early CLL where oral doses of PPE (2,000 mg twice/day) caused a sustained decline in absolute lymphocyte count and/or lymphadenopathy in the majority of patients [6]. Furthermore, in a study to assess salivary antioxidant alterations in smokers, participants who consumed 2 cups of green tea per day (2 g of green tea dissolved in 150 mL hot water per cup) had increased levels of salivary antioxidants, suggesting that green tea may reduce the rate of oral cancer, given the likely association between oxidative stress and oral cancer [36].

This optimistic expectation may also be supported by a highly encouraging case report of an EGCG-based ointment (PPE/sinecatechins), approved by the US Food and Drug Administration, which was successfully used to treat anogenital warts. Rob et al. found that after application of the ointment for 10 weeks in an 11-year-old child, the warts disappeared completely without recurrence during a 12-week follow-up [37].

2.1.3 Laboratory Studies and Mechanism of Action

A large number of animal and cell-based experiments have indicated the anticancer effects of green tea [1–6, 38, 39]. For black tea and oolong tea, however, fewer studies are available. Hibasami et al. reported for the first time that catechins, including EGCG, induced apoptosis or programmed cell death in cancer cells [40]. Our research group also observed similar apoptosis-inducing actions of EGCG and has proposed that the binding of EGCG to the cell surface Fas protein is involved in its anticancer activity [41]. Tachibana et al. found that the 67 kDa laminin receptor on the cell surface is an EGCG receptor and mediates various types of EGCG activity, including its anticancer activity [42]. The role of the protein-binding capability of EGCG in its mechanism of action has been reviewed elsewhere by Yang et al. as well as our research group [1, 2].

The number of animal and cell-based experiments showing anticancer effects of EGCG and GTCs continues to increase, as described next. C3H/He mice (8 weeks old; $n = 46$) were treated with 0.05% *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) solution for 14–24 weeks. Mice in the BBN + GTP group ($n = 47$) were additionally treated with 0.5% GTP solution over the same period. Cytoplasmic human antigen R (HuR) expression in cancer cells increased at 14 and 24 weeks in the BBN group compared with that in the control group and was associated with increased invasion of tumor cells in muscle. However, these effects were not observed in the BBN + GTP group. GTP was independently associated with cyclooxygenase (COX)-2 and heme oxygenase (HO)-1 expression, while cytoplasmic HuR expression was associated with COX-2 and vascular endothelial growth factor (VEGF)-A levels. Expression of COX-2 and HO-1 was associated with cell proliferation while that of VEGF-A and HO-1 was associated with angiogenesis. Therefore, GTP potentially suppresses tumor cell proliferation and angiogenesis both directly and indirectly via HuR-related pathways in bladder cancer [43].

When the cytotoxic effects of GTPs were examined in human cell lines (MCF-7, A549, Hela, PC3, and HepG2 cells), GTPs were found to inhibit cell growth, particularly in MCF-7 cells. Mechanistic studies showed that the main modes of cell death induced by GTCs were cell cycle arrest at G1/M and G2/M and apoptosis. GTP also caused a reduction in mitochondrial membrane potential, increased the generation of reactive oxygen species (ROS), induced DNA fragmentation, and activated caspase 3 and caspase 9 [44].

The cancer-preventive activity of PPE was demonstrated in an animal model of colorectal cancer induced by azoxymethane (AOM). Dietary PPE increased the plasma and colonic levels of tea polyphenols and decreased tumor multiplicity and size. It also decreased β-catenin nuclear expression, induced apoptosis, and increased the expression of retinoid X receptor (RXR)-α, β, and γ in adenocarcinomas. These results demonstrate the inhibitory effects of orally administered PPE on colon carcinogenesis [45].

Posadino et al. investigated the impact of PPE on PCa cells. PPE treatment at 30 and 100 μg/mL significantly decreased cell viability and proliferation. PPE-induced cell death was associated with mitochondrial dysfunction and the downregulation of Akt activation. Cell exposure to the ROS scavenger *N*-acetylcysteine prevented

PPE-induced ROS increase, Akt activation impairment, and cell death, indicating the causative role of ROS. In cells over-expressing Akt, PPE failed to cause ROS increase, Akt activation impairment, and cell death. Thus, PPE induced apoptotic cell death through a prooxidant rather than an antioxidant mechanism [46].

When estrogen receptor α-positive breast cancer T47D cells were treated with 0–80 μM EGCG, cell viability was decreased, and EGCG at 80 μM increased the gene expression of *PTEN*, caspase 3, and caspase 9 but decreased that of *Akt*. Furthermore, EGCG increased the Bax/Bcl-2 ratio of gene and protein expression and decreased the gene expression of *hTERT*. These findings suggest that EGCG may be a useful adjuvant therapeutic agent for the treatment of breast cancer [47]. Chen et al. found that EGCG inhibited the spheroid formation of colorectal cancer cells as well as the expression of colorectal cancer stem cell markers, suppressed cell proliferation, and induced apoptosis. EGCG downregulated the activation of the Wnt/β-catenin pathway, supporting its potential as an anticancer agent targeting colorectal cancer stem cells through the suppression of this pathway [48]. Aberrant expression of β-catenin is associated with the progression of various cancers, including head and neck cancer. Shin et al. found that EGCG induced apoptosis in KB and FaDu cells via the suppression of β-catenin signaling and promotion of ubiquitin-mediated 26S proteasomal degradation. These effects of EGCG were confirmed in a syngeneic mouse model [49].

Harati et al. found that EGCG suppressed the proliferation and viability of liposarcoma, synovial sarcoma, and fibrosarcoma cells [50]. Cornwall et al. showed that EGCG at concentrations ranging from 25 to 100 μg/mL induced apoptosis in CLL B-cells but did not affect healthy control B-cells. They also showed that, in contrast to healthy controls, T-cells from CLL patients underwent apoptosis in the presence of EGCG. Thus, EGCG differentially induces apoptosis in CLL B- and T-cells but not in healthy B- and T-cells [51].

In an attempt to identify its anticancer activities against cholangiocarcinoma cells, Kwak et al. found that EGCG inhibited the growth of HuCC-T1 cells but not of human embryonic kidney 293 T cells, indicating that EGCG induced apoptosis in cancer cells without adverse effects in normal cells. EGCG inhibited the expression of mutant p53 and induced apoptotic molecular signals such as Bax/Bcl-2, caspases, and cytochrome c. EGCG also inhibited the activity of matrix metalloproteinase (MMP)-2/9, invasion, and migration. In an animal tumor xenograft model using HuCC-T1 cells, EGCG inhibited tumor growth and suppressed carcinogenic molecular signals such as Notch1, MMP-2/9, and proliferating cell nuclear antigen [52].

Similarly, Luo et al. showed that treatment of bladder cancer SW780 cells with EGCG resulted in the significant inhibition of cell proliferation by induction of apoptosis, without obvious toxicity to normal bladder epithelium SV-HUC-1 cells. EGCG also inhibited SW780 cell migration and invasion at 25–100 μM. EGCG induced apoptosis in SW780 cells by the activation of caspases 8, 9, and 3; Bax; Bcl-2; and PARP. Animal studies demonstrated that EGCG decreased tumor volume and weight in mice bearing SW780 tumors and downregulated the expression of nuclear factor-kappa B (NF-κB) and MMP-9 at both the protein and mRNA level in tumor and SW780 cells [53]. As exemplified by this finding and the studies described

above, EGCG exerts stronger apoptosis-inducing effects on cancerous cells than on normal cells. Our research group has also provided evidence that differentiated HL-60 cells are notably less susceptible to apoptosis than undifferentiated cells [54].

Interestingly, Ward et al. reported that different diets may have different effects on the action of GTE. They hypothesized that GTE would have different effects on colon carcinogenesis, body composition, and lipid metabolism in mice fed a basal diet formulated to promote health and growth (AIN93G) compared with total Western diet, which emulates the typical American diet. Mice were fed either AIN93G or the total Western diet for 18 weeks with or without GTE. The quantity of a precancerous marker, aberrant crypt foci (ACF), was nearly three times greater in AOM-treated mice fed the total Western diet than in those fed AIN93G. The consumption of GTE suppressed ACF development only in mice fed the total Western diet. Similarly, supplementation with GTE suppressed weight gain and fasted glucose level only in mice fed the total Western diet, while GTE suppressed fat mass gain in mice fed either diet, suggesting that diet is an important factor for the efficacy of GTCs [55].

In their review of animal and cell experiments, Yang and Wang extensively discussed the molecular mechanisms by which GTCs exert anticancer actions. For example, in tumorigenesis of the small intestine in ApcMin/+ mice, EGCG action was associated with increased levels of E-cadherin on the plasma membrane and decreased levels of nuclear β-catenin, c-Myc, phospho-Akt, and phospho-ERK1/2 in tumors [6]. In a model of AOM-induced precancerous lesions, the inhibitory activity of PPE was associated with decreased levels of nuclear β-catenin and cyclin D1 and increased levels of RXR-α. In male C57BL/KsJ-db/db mice, the inhibition of AOM-induced ACF formation by EGCG was associated with the suppression of insulin-like growth factor 1 (IGF1) signaling. EGCG also increased the levels of IGF1 receptor (IGF1R), phospho-IGF1R, phospho-GSK3, and β-catenin in colonic mucosa. Yang and Wang further described that oral administration of 0.5% PPE or 0.044% caffeine in drinking water to tumor-bearing A/J mice inhibited the progression of lung adenomas to adenocarcinomas by enhancing apoptosis and decreasing the levels of c-Jun and phospho-ERK1/2 in adenocarcinomas.

In a study which examined the cytotoxicity of green, black, and purple tea infusions, green tea inhibited breast cancer 4TI cell proliferation to the greatest extent with an IC_{50}: 13.12 μg/mL. Results also revealed the differential expression of apoptosis-related genes. Caspases 8, 9, 3, and 6 and *8AP2*, *Aifm1*, *Aifm2*, and *Apopt1* genes were significantly upregulated, indicating the process of apoptosis was initiated and executed [56].

In a PCa model, the antitumor action of GTC was associated with the modulation of IGF1 and IGFBP3 levels with reduced levels of phosphatidylinositol 3-kinase (PI3K), phospho-Akt, and phospho-ERK1/2. Furthermore, GTC significantly decreased the levels of angiogenic and metastatic markers such as VEGF-A, MMP-2, and MMP-9 [6].

Tumor metastases are responsible for approximately 90% of all cancer-related deaths [57]. In these events, cancer cells released from the tumor invade surrounding tissues, enter the blood vessels, and extravasate to spread to new organs through

several steps including attachment to and subsequent degradation of the endothelial basement membranes [2]. In 1992, Taniguchi et al. reported that GTCs rich in EGCG inhibited the metastasis of melanoma B16-F10 and BL6 cells in both experimental and spontaneous metastasis systems [58]. Our research group has also observed similar effects of green tea in an animal model of metastasis [59].

In 2001, GTEs were demonstrated to inhibit metastasis in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, and a later study showed that PPE was an effective chemopreventive agent in preventing metastasis of PCa in this mouse model [60, 61]. A likely molecular basis for the inhibition of metastasis by green tea and GTCs is their ability to inhibit the enzymatic activity and gene expression of MMPs [2]. The results of our research group suggest that EGCG inhibits MMP activity by directly binding to MMPs [62].

Theaflavins have been shown to inhibit the growth and metastasis of HCC in an orthotopic model and a lung metastasis model. Theaflavins induced apoptosis by activating the caspase pathway, suppressing the phosphorylation of constitutive and inducible signal transducer and activator of transcription 3 (STAT3), and down-regulating downstream proteins regulated by STAT3, including antiapoptotic proteins (Bcl-2 and survivin) and invasion-related proteins MMP-2 and MMP-9 [63].

Angiogenesis is required for many physiological processes, including embryogenesis and postnatal growth, but pathological angiogenesis is a hallmark of several diseases including cancer and subsequent metastasis. Rashidi et al. reviewed the ability of green tea constituents to suppress angiogenesis signaling, summarized the mechanism by which EGCG might act on the VEGF family of proteins, and highlighted the microRNAs affected by green tea that are involved in anti-angiogenesis [64].

2.2 Effects of Coffee on Cancer

2.2.1 Epidemiological Studies

Some early epidemiological studies of the health effects of coffee showed that coffee consumption was associated with an increase in cancer risk [65]. For example, Yu et al. reported that daily coffee consumption was a risk factor for renal cell carcinoma in women [66]. However, several later studies have demonstrated the beneficial effects of coffee on cancer. A comprehensive review by Wierzejska showed that coffee consumption reduced cancer risk in 1/4 studies on bladder cancer, 10/17 studies on breast cancer, 6/9 studies on colorectal cancer, 5/5 studies on liver cancer, 2/4 studies on pancreatic cancer, and 3/4 studies on PCa, while 3/3 studies on lung cancer showed an increased risk [65]. Since then, a growing number of studies have sought to further examine these effects, as reviewed here.

In a study of 64,603 participants with a follow-up period of up to 15 years for breast cancer, consumption of $\geq$4 cups/day of boiled coffee was associated with a reduced risk of cancer (hazard ratio (HR): 0.52) as compared with $<$1 cup/day, although no association was found for all cancer sites combined or for prostate and colorectal cancers. An increased risk of premenopausal breast cancer and a reduced

risk of postmenopausal breast cancer were found for both total coffee (HR, 1.69 for premenopausal breast cancer; HR, 0.60 for postmenopausal breast cancer) and filtered coffee (HR, 1.76 for premenopausal breast cancer; HR, 0.52 for postmenopausal breast cancer). Boiled coffee was associated with an increased risk of respiratory tract cancer (HR, 1.81), a finding limited to men. The main results for less common cancer types included reduced risk for renal cell cancer with total coffee (HR, 0.30) and increased risk for pancreatic cancer for boiled coffee (HR, 2.51). These findings demonstrate that coffee has beneficial effects in certain types of cancer and that the effect may be dependent on the brewing method [67].

A population-based prospective cohort study of >215,000 men and women in Hawaii and California with an 18-year follow-up period showed that high levels of coffee consumption were associated with reduced risk of incident HCC and chronic liver disease mortality. Compared with non-coffee drinkers, those who drank 2–3 cups/day had a 38% reduction in risk for HCC (RR, 0.62), while those who drank ≥4 cups/day had a 41% reduction in HCC risk (RR, 0.59). Compared with non-coffee drinkers, participants who consumed 2–3 cups of coffee/day had a 46% reduction in risk of death from chronic liver disease (RR, 0.54), and those who drank ≥4 cups/day had a 71% reduction (RR, 0.29). These findings suggested that coffee consumption reduces the risk of HCC and chronic liver disease in multiethnic US populations [68].

A study of 738 middle-aged Japanese patients with adenoma and 697 controls showed that high coffee consumption was associated with a reduced risk of adenoma. A multivariate-adjusted OR for the highest versus lowest quartile of coffee intake was 0.67, suggesting a protective effect of coffee drinking on colon adenoma, a precursor of colon cancer [69].

Results from a Danish case-control study from 1995 through 1999 showed that both coffee (OR, 0.90 per cup/day) and total caffeine consumption from coffee and tea combined (OR, 0.93 per 100 mg/day) decreased the risk of ovarian cancer [70].

The results of the European Prospective Investigation into Cancer and Nutrition study in a total of 335,060 women from 1992 to 2000 indicated that higher caffeinated coffee intake may be associated with a lower risk of postmenopausal breast cancer, with a null association in the case of decaffeinated coffee [71].

A prospective study of breast cancer in the Swedish Women's Lifestyle and Health study of 42,099 female participants suggested that coffee consumption and caffeine intake were inversely associated with the overall risk of breast cancer and of estrogen receptor-positive/prolactin-negative breast cancer [72].

In a case-control study, during and 6 months after adjuvant chemotherapy, 953 patients with stage III colon cancer prospectively answered questionnaires which included the dietary intake of caffeinated coffee, decaffeinated coffee, and non-herbal tea. The results showed that patients who consumed ≥4 cups/day of total coffee had an adjusted HR of 0.58 for colon cancer recurrence or mortality, compared with nondrinkers. The daily consumption of ≥4 cups of caffeinated coffee resulted in reduced cancer recurrence or mortality risk (HR, 0.48), while decaffeinated coffee was not associated with cancer outcome [73].

In a prospective cohort study in 307 patients over 4 years, the risk of colorectal tumor recurrence was significantly lower (OR, 0.21) in patients who consumed >3 cups of coffee/day compared with those who did not consume coffee. In a sub-analysis of tumor location, OR of colorectal tumor recurrence in the proximal colon showed a tendency toward reduction as coffee consumption increased; however, increased coffee consumption significantly increased colorectal tumor recurrence in the distal colon [74].

A meta-analysis with a total of 1,534,039 participants from 13 published studies showed that the RR of total coffee consumption and endometrial cancer was 0.80. A stronger inverse association between coffee intake and cancer incidence was found in patients who had never received hormone therapy (RR, 0.60) and subjects with a BMI $\geq$25 kg/m^2 (RR, 0.57). The overall RR for caffeinated and decaffeinated coffee was 0.66 and 0.77, respectively. Endometrial cancer risk decreased by 5% for every 1 cup of daily coffee intake, 7% for every 1 cup of daily caffeinated coffee intake, 4% for every 1 cup of daily decaffeinated coffee intake, and 4% for every 100 mg of daily caffeine intake. These findings suggest that coffee and caffeine may reduce the incidence of endometrial cancer and that these effects may be modified by BMI and history of hormone therapy [75].

A systematic review and meta-analysis of nine observational studies with a total of 927,173 study participants showed that the pooled RR for melanoma among regular coffee drinkers was 0.75 compared with controls. The pooled RR for melanoma among decaffeinated coffee drinkers was, however, not statistically significant [76].

A systematic review and meta-analysis of prospective cohort studies including 12 studies on HCC (3,414 cases) and 6 studies on chronic liver disease (1,463 cases) found that the summary RRs for HCC were 0.66 for regular, 0.78 for low, and 0.50 for high coffee consumption, respectively. The summary RRs for chronic liver disease were 0.62 for regular, 0.72 for low, and 0.35 for high consumption and 0.74 for an increment of 1 cup/day. These findings indicate an inverse relation between coffee consumption and the risk of HCC and chronic liver disease [77].

In a meta-analysis of observational studies published until February 2016, the intake of caffeinated coffee was inversely associated with nonmelanoma skin cancer risk (summary RR, 0.82 for those in the highest versus lowest category of intake), as was the intake of caffeine (summary RR, 0.86). In a subgroup analysis, these associations were limited to the basal cell cancer histotype. There was no association between decaffeinated coffee intake and summary RR, suggesting that caffeine may contribute to the risk reduction [78].

A meta-analysis of 9 cohort and 13 case-control studies involving 7,631 cases and 1,019,693 controls reported a summary RR for gastric cancer of 0.94 for the highest category of coffee consumption compared with the lowest category and 0.93 for coffee drinkers compared with nondrinkers. The pooled RRs for the population consuming <1 cup/day, 1–2 cups/day, and 3–4 cups/day compared with that of nondrinkers were 0.95, 0.92, and 0.88, respectively, indicating that an increase in coffee consumption was associated with a decreased risk of gastric cancer [79].

Another meta-analysis of the cohort and case-control studies reported a summary RR for nonmelanoma skin cancer of 0.96 for 1 cup of coffee, 0.92 for 1–2 cups, 0.89 for 2–3 cups, and 0.81 for >3 cups/day, respectively. The results suggest that caffeinated coffee might have dose-dependent chemopreventive effects against basal cell carcinoma [80].

The results of a study of 18 cohorts, involving 2,272,642 participants and 2,905 cases, and of 8 case-control studies, involving 1,825 cases and 4,652 controls, showed that increased consumption of caffeinated coffee and, to a lesser extent, decaffeinated coffee was associated with reduced risk of HCC, including in patients with preexisting liver disease. An extra intake of 2 cups/day of coffee was associated with a 35% reduction in the risk of HCC [81].

However, several studies have failed to demonstrate the beneficial effects of coffee, as exemplified by the following studies. A cohort study with 560,356 participants in the UK Million Women Study found no significant association between endometrial cancer risk and consumption of coffee [82]. In a systematic meta-analysis of 2,803 cases and 503,234 controls in ten independent studies, Chen et al. found that coffee consumption was significantly associated with the increased risk of laryngeal carcinoma (RR:1.47) [83].

A meta-analysis of 9 prospective cohort studies involving 1,250,825 participants and 3,027 gastric cancer cases demonstrated that coffee consumption was not associated with overall gastric cancer risk and that it may even be a risk factor for gastric cardia cancer [84].

A meta-analysis of 17 studies (5 cohort and 12 case-control studies) involving 12,276 cases and 102,516 controls showed that coffee intake was associated with an increased risk of lung cancer. Particularly over the past 5 years, studies have consistently indicated that lung cancer risk is significantly increased by 47% in the population with the highest category intake of coffee compared to that with the lowest category intake [85].

In a large population-based case-control study in Italy, no association was observed between regular coffee consumption and any type of leukemia [86]. A meta-analysis of 12 case-control studies, comprising a total of 3,649 cases and 5,705 controls, showed that high maternal coffee consumption was associated with increased risk of acute lymphoblastic leukemia (OR, 1.43) and acute myeloid leukemia (OR, 2.52) in children. The finding indicates a detrimental association between maternal coffee consumption and childhood leukemia risk [87].

A multicentric case-control study on 690 bladder cancer cases and 665 hospital controls conducted in Italy between 2003 and 2014 showed that decaffeinated coffee, tea, cola, and energy drinks were not related to bladder cancer risk [88]. A meta-analysis of 13 prospective cohort studies with 20 independent reports involving 3,368 patients with gastric cancer and 1,372,811 participants during a follow-up period ranging from 4.3 to 8 years did not support the hypothesis that coffee consumption was associated with the reduced risk of gastric cancer and even indicated an increased risk of gastric cancer for participants in the United States [89].

The results of a population-based prospective cohort study in Japan on 89,555 people aged 45–74 years showed no clear association between coffee consumption

and biliary tract, gallbladder, or extrahepatic bile duct cancer [90]. Furthermore, based on the meta-analysis of five cohort studies and nine case-control studies, Akter et al. concluded that the evidence was insufficient to support that coffee drinking increased or decreased the risk of colorectal cancer [91].

These conflicting results may have been caused by several confounding factors, including the methods of quantifying coffee consumption, coffee temperature, cigarette smoking, alcohol consumption, and differences in genetic and environmental factors such as race, sex, age, intestinal microbiota, and lifestyle as in the case of tea consumption [1, 2, 92].

2.2.2 Clinical Studies

A total of 31 men and 33 women were randomly assigned to two groups with two intervention periods of 2 weeks separated by a washout period of 8 weeks, and they consumed 1,000 mL of cafetière (French press) coffee daily or no coffee [93]. The results showed that unfiltered coffee significantly increased the glutathione content in the colorectal mucosa by 8% and in plasma by 15%. Unfiltered coffee did not influence the colorectal mucosal proliferation rate, but appeared to cause an increase in detoxification capacity and antimutagenic properties in the colorectal mucosa by increasing the glutathione concentration. Thus, the findings suggest a possible reduction of colon cancer risk by coffee consumption.

A controlled intervention trial with a crossover design in which 38 participants consumed 800 mL coffee or water daily over 5 days demonstrated that the proportion of DNA migration attributable to the formation of oxidized purines was decreased by 12.3% after coffee intake. However, other biochemical parameters including the total antioxidant levels in plasma, glutathione concentrations in blood, and superoxide dismutase and glutathione peroxidase activity in lymphocytes were not markedly altered. These results indicate that coffee consumption prevents the endogenous formation of oxidative DNA damage in humans [94].

A clinical trial in which ten participants consumed 1 L of unfiltered coffee/day over 5 days showed a weak induction of glutathione S-transferase (GST) and a threefold increase in the induction of placental-type GST in blood, whereas the level of GST-α was not altered [95]. Although serum cholesterol levels were increased without statistical significance, other clinical parameters (creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase), which are markers for organ damage, were not altered. In a similar trial with seven participants who consumed 1 L coffee/day over 3 days, a significant threefold induction of placental-type GST was observed. The effects were identical between filtered and unfiltered coffee, indicating that caffeine concentration was not responsible for this result. In a further trial, the consumption of unfiltered coffee (1 L/day for 5 days) resulted in a 45% reduction effect. These findings show that coffee induces placental-type GST, which may confer protection against chemical carcinogenesis [95].

However, a placebo-controlled intervention trial on 160 healthy human subjects who consumed 3 or 5 cups of coffee per day for 8 weeks showed that blood pressure, oxidation of DNA and lipids, blood glucose level, insulin, cholesterol, triglycerides,

and inflammatory markers were unchanged, although a slight elevation of serum creatinine level and a significant elevation of serum γ-glutamyl transaminase level were observed in the 5 cups/day group. These findings indicated that there was no detectable effect, either beneficial or harmful, of coffee consumption on human health [96]. Given the conflicting results of these clinical studies, further studies are warranted to understand the relationship between coffee and cancer.

2.2.3 Laboratory Studies and Mechanism of Action

Numerous cell-based and animal experiments have demonstrated the anticancer effects of CGAs, the most common polyphenols found in coffee.

A cell-based experiment found that CGA inhibited the viability of colon cancer HCT116 and HT29 cells. CGA induced ROS production and cell cycle arrest at the S phase and suppressed the activation of ERK. These events may lead to a reduction in viability of cancer cells and suggest that CGA is a potential treatment for colorectal cancer. Deka et al. found that CGA killed MDAMB-231 and MCF-7 breast cancer cells with an IC_{50} of about 76 and 53 μg/mL, respectively. CGA bound to protein kinase C (PKC) with a dissociation constant of about 29 μM and caused the translocation of PKC from the cytosol to the plasma membrane, leading to cell cycle arrest at the G1 phase. CGA induced apoptosis through a mitochondrial pathway which involves a reduction in mitochondrial potential and the release of cytochrome c into the cytosol [97].

Salomone et al. reviewed the effects of coffee and its components in experimental models of liver cancer. Coffee exerted beneficial effects, including a reduction in preneoplastic lesions in an aflatoxin-induced liver cancer model, a reduction in tumor growth and metastasis in hepatoma-bearing rats, and a reduction in the incidence of liver tumors in an aminopyrine-sodium nitrite-induced cancer model. Regarding the mechanism of action of coffee and CGA, the authors highlighted the antioxidant effects associated with nuclear factor, erythroid 2 like 2 (Nrf2) signaling, the activation of which leads to (1) the induction of enzymes involved in xenobiotic detoxification processes and cellular antioxidant defenses; (2) the induction of gene expression of hepatic and intestinal NAD(P)H/quinone oxidoreductase 1, GST class α1, intestinal uridine-5′-diphosphoglucose-glucuronosyl transferase 1A6, and the glutamate cysteine ligase catalytic subunit; (3) the induction of the transcription of several UDP-glucuronosyltransferases in hepatoma cells; and (4) an increase in hepatic superoxide dismutase, catalase, and glutathione peroxidase activity [98].

When G422 glioma cells were injected subcutaneously into the right flank of ICR mice in a xenograft model experiment and the mice were intraperitoneally administered either CGA or vehicle daily for 2 weeks, CGA inhibited glioblastoma growth. Moreover, CGA increased the population of CD11c-positive M1 macrophages and decreased the distribution of CD206-positive M2 macrophages in tumor tissue via the promotion of STAT1 activation and inhibition of STAT6 activation, respectively, suggesting its therapeutic potential for the reduction of glioma growth [99].

CGA was also shown to have therapeutic effects in breast cancer, brain tumors, lung cancer, colon cancer, and chronic myelogenous leukemia. Suggested mechanisms of action of CGA include (1) the induction of *GSK-3 β* and *APC* genes; (2) the

inhibition of the *β-catenin* gene; (3) the inhibition of activator protein-1, NF-κB, and MAPKs; and (4) the induction of phase 2 detoxifying enzyme activity [100].

The bark of *Odina wodier* is one of the Indian tribes for treating inflammatory disorders, and its major constituent is CGA. Ojha et al. demonstrated that both the methanol extract of bark and CGA exerted significant anti-inflammatory activity, inhibiting the expression of tumor necrosis factor (TNF-α), interleukin (IL)-1β, IL-6, and IL-12. The expression of murine TLR4, NF-κBp65, MyD88, iNOS, and COX-2 molecules was also reduced in CGA-treated groups. These findings suggest that CGA can inhibit inflammation by downregulating the TLR4/MyD88/NF-κB signaling pathway. Through these activities, CGA may be beneficial for the prevention of chronic inflammatory diseases including cardiovascular diseases, diabetes, and cancer [101].

In contrast, Choi et al. found that neither caffeine, caffeic acid, nor CGA showed any cytotoxicity against colon adenocarcinoma HT-29 cells, while a coffee diterpene, kahweol, did [102].

Cancer metastasis may be prevented by coffee polyphenols. Weng and Yen reviewed several studies to show anti-invasive and anti-metastasis activities of dietary phenolic compounds including CGA and caffeic acid in various cancer cells such as hepatoma Hep3B and SKHep1 cells, glioma U-87 cells, prostate cancer PC3 cells, fibrosarcoma HT1080 cells, and colon adenocarcinoma CT26 cells [103]. The authors concluded that the daily consumption of natural dietary components that are rich in phenolics could be beneficial for the prevention of cancer metastasis.

2.3 Simultaneous Evaluation of the Anticancer Effects of Tea and Coffee

Several epidemiological studies have simultaneously evaluated the anticancer effects of tea and coffee. These results are briefly summarized in Table 1. In the European Prospective Investigation into Cancer and Nutrition study in 486,799 subjects, the results from a median follow-up of 11 years showed that increased coffee and tea intake was associated with lower HCC risk. Coffee and tea consumers in the highest quintile had a lower HCC risk by 72% and 59%, respectively, compared with the lowest quintile [15].

The results of a Danish case-control study indicated that both coffee and total caffeine consumption from coffee and tea combined decreased the risk of ovarian cancer, while no relationship was observed between tea consumption and ovarian cancer risk [70].

In a middle-aged Japanese population, Budhathoki et al. found that high coffee consumption was associated with a reduced risk of colorectal adenoma, with a multivariate-adjusted OR of 0.67 for the highest versus the lowest quartile of coffee intake, indicating a protective effect of coffee drinking against colon adenoma, a precursor of colon cancer. Green tea intake was not found to be associated with colorectal adenoma risk [69].

Table 1 Examples of the simultaneous evaluation of the effects of tea and coffee on human cancer risk[a]

Cancer type	Tea	Coffee	Reference
Liver cancer	—	↓	[106]
Hepatocellular carcinoma	↓	↓	[15]
Colorectal adenoma	—	↓	[69]
Colorectal tumors	—	↓	[74]
Laryngeal carcinoma	—	↑	[83]
Breast cancer	—	↓	[71]
Breast cancer	↑	↓	[72]
Ovarian cancer	—	↓	[70]
Endometrial cancer	—	—	[82]
Endometrial cancer	—	↓	[104]
Bladder cancer	—	—	[88]
Biliary tract cancer	↓	—	[90]
Nonmelanoma skin cancer	—	↓	[78]
Brain tumor	—	↓	[105]
Leukemia	↓	—	[86]
All cancers combined	↓	—	[104]

[a]Cancer risk is reduced (↓), increased (↑), or not affected/evaluated (—)

A systematic meta-analysis of 2,803 cases and 503,234 controls in ten independent studies, including case-control and cohort studies, showed that tea drinking was not associated with laryngeal carcinoma. However, coffee consumption was positively associated with laryngeal carcinoma (RR, 1.47) [83].

A cohort study with 560,356 participants in the UK Million Women Study found no significant association between endometrial cancer risk and the consumption of either tea or coffee [82].

The prospective study of breast cancer in the Swedish Women's Lifestyle and Health study among 42,099 female participants suggested that coffee consumption and caffeine intake reduced the risk of both overall and estrogen receptor-positive/prolactin-negative breast cancer, while tea consumption increased the risk [72].

A multicenter case-control study on 690 bladder cancer cases and 665 hospital controls conducted in Italy between 2003 and 2014 showed that consumption of decaffeinated coffee, tea, cola, and energy drinks was not related to bladder cancer risk [88].

In a meta-analysis of observational studies reported up to February 2016, intake of both caffeinated coffee and caffeine was inversely associated with nonmelanoma skin cancer risk (summary RR, 0.82). In a subgroup analysis, these associations were limited to the basal cell cancer histotype. There was no association between intake of decaffeinated coffee and green tea and nonmelanoma skin cancer risk [78].

A meta-analysis of 12 case-control studies, comprising a total of 3,649 cases and 5,705 controls, showed that high maternal coffee consumption was associated with increased risk of acute lymphoblastic leukemia (OR, 1.43) and acute myeloid leukemia (OR, 2.52). On the contrary, low-to-moderate tea consumption was inversely associated with overall leukemia (OR, 0.85), although the trend was not significant. These findings

indicate the detrimental association between maternal coffee consumption and childhood leukemia risk. In contrast, an inverse association was found with tea, implying that other micronutrients contained in this beverage could potentially counterbalance the deleterious effects of caffeine [87].

The results of a population-based prospective cohort study in Japan on 89,555 people aged 45–74 years showed no clear association between coffee consumption and biliary tract, gallbladder, or extrahepatic bile duct cancer. However, the findings suggested that high green tea consumption might lower the risk of biliary tract cancer [90].

In a large population-based case-control study in Italy, no association was observed between regular coffee consumption and any type of leukemia. A small protective effect of tea intake was found among myeloid malignancies, which was more evident among acute myeloid leukemia (OR, 0.68) [86].

In a study of 97,334 eligible individuals, 10,399 developed cancers including 145 head and neck, 99 esophageal, 136 stomach, 1137 lung, 1703 breast, 257 endometrial, 162 ovarian, 3037 prostate, 318 kidney, 398 bladder, 103 glioma, and 106 thyroid cancers. Coffee intake was not associated with the risk of all cancers combined, whereas tea drinking was associated with an overall decreased risk of cancer (RR, 0.95 for 1 cup-increment/day versus <1 cup/day). For endometrial cancer, a decreased risk was observed for coffee intake (RR, 0.69) of ≥ 2 cups/day [104].

A Japanese cohort study with 106,324 subjects (50,438 men and 55,886 women) found a significant inverse association between coffee consumption and brain tumor risk in both total subjects (≥ 3 cups/day; HR: 0.47) and in women (≥ 3 cups/day; HR: 0.24) [105]. No association was observed between green tea and brain tumor risk. A prospective cohort study with 18,815 subjects aged 40–69 years showed that coffee consumption may reduce the risk of liver cancer regardless of hepatitis C virus (HCV) or hepatitis B virus (HBV) infection status, whereas green tea may not [106].

Thus, there are conflicting results related to the effects of both tea and coffee in a variety of human cancers. These differences may have arisen from several confounding factors, including the method of quantifying tea consumption, tea temperature, cigarette smoking, alcohol consumption, and differences in genetic and environmental factors such as race, sex, age, and lifestyle [1, 2, 6, 92]. In addition, caffeine consumption is an important factor to be adjusted for. Intestinal microbiota and genetic polymorphisms may also have influenced the effects of coffee in these studies [107]. The differences in results between human and animal experiments may have been due to different doses of tea and coffee [3].

3 Effects on Metabolic Syndrome and Related Disorders

3.1 Effects of Green Tea on Metabolic Syndrome

3.1.1 Epidemiological Studies

Metabolic syndrome (MetS) is diagnosed based on variables related to the five components of obesity, blood triglycerides, high-density lipoprotein (HDL) cholesterol, systemic hypertension, and fasting glucose. Any agent that exerts beneficial

effects on these components may potentially prevent MetS [108]. Several human studies have suggested that tea is one such agent.

Grosso et al. conducted a cross-sectional survey among 1,889 inhabitants in Sicily, southern Italy, and found that tea consumption was inversely associated with MetS (OR, 0.51) after adjusting for all covariates. Although no direct association between caffeine intake and MetS or its components was observed, tea and coffee were significantly related to reduced OR of MetS. Similarly, results from a cross-sectional population-based survey including 8,821 adults of the Polish arm of the Health, Alcohol and Psychosocial Factors in Eastern Europe cohort study showed that a high consumption of tea was inversely related to MetS, and the analysis stratified by gender revealed a significant association for men but not for women. After adjusting for potential confounding factors, tea consumption was inversely associated with MetS (OR, 0.79) [109].

In a comprehensive review, Yang et al. provided several examples of reports, including two epidemiological studies to demonstrate the mitigating effects of tea on MetS [7]. One example is the study by Vernarelli and Lambert in 6,472 US adults. Hot tea consumption was inversely associated with obesity, mean waist circumference, and BMI and also increased HDL cholesterol and reduced blood triglyceride levels in women [7, 92]. It should be noted that these associations were not observed with iced tea consumption.

In contrast, some studies did not show a beneficial effect of tea on MetS [7, 92]. For example, a cross-sectional study by Tsubono and Tsugane found no association between green tea intake and serum lipid levels [110]. An epidemiological study on 1,902 Japanese men and women showed no correlation between green tea intake and MetS, since green tea consumption did not influence blood pressure, abdominal circumference, fasting plasma glucose, or lipid levels [111]. The results of a cross-sectional study that enrolled 554 adults in Tokushima, Japan, showed that green tea consumption was not associated with the prevalence of MetS. Thus, epidemiological studies have provided conflicting results, which may have resulted from various factors as discussed above. Further studies are therefore required to determine whether there is an association between tea consumption and MetS [112].

3.1.2 Clinical Studies

Legeay et al. reviewed six human intervention studies and found that EGCG was associated with decreased BMI (three cases), body weight (four cases), low-density lipoprotein (LDL) cholesterol (five cases), blood pressure (three cases), triglycerides (two cases), and blood glucose (two cases) [113]. In one of these studies, a randomized, double-blind trial in 115 women with central obesity, significant weight loss, from 76.8 kg to 75.7 kg, was observed as well as decreased BMI and waist circumference after 12 weeks of high-dose EGCG treatment, with a consistent trend of reduced total cholesterol and decreased LDL plasma levels [114].

Amiot et al. conducted a systematic review of dietary polyphenols on subjects with MetS and summarized the effects of green tea and pu-erh tea extracts in ten studies [115]. These studies showed significant improvements in BMI (eight cases), weight circumference (seven cases), blood pressure (one case), LDL cholesterol

(five cases), triglycerides (four cases), and blood glucose (two cases) in subjects with MetS. One example showed that in older adults with MetS, the consumption of 3 cups of green tea per day for 60 days was effective in inducing weight loss and reducing both BMI and waist circumference [116].

3.1.3 Laboratory Studies and Mechanism of Action

A number of experiments using animal models and cultured cells have demonstrated the beneficial effects of tea consumption on MetS and its components and underlying molecular mechanism. For example, a study to evaluate the effect of GTE on drug-induced weight gain and metabolic abnormalities in rats found that GTE exerted protective effects against obesity, partially due to its lowering effect on leptin [117]. In this study, GTE significantly decreased body weight gain and average food and water intake, improved the lipid profile and fasting blood glucose levels, and decreased hyperleptinemia and hypertension in this animal model.

A study using a rat model of benign prostatic hyperplasia accompanied with MetS induced by a high-fat diet (HFD) combined with testosterone injection demonstrated that orally administered EGCG decreased the levels of glucose, total cholesterol, triglycerides, insulin-like growth factors, and inflammatory cytokines, normalized the activities of antioxidant enzymes, and increased the prostatic expression of insulin-like growth factor-binding protein-3 and peroxisome proliferator-activated receptors (PPARs) [118].

Yang et al. have described two major mechanisms of action for tea: one is the action of tea constituents in the gastrointestinal tract in decreasing the digestion and absorption of macronutrients or by altering the gut microbiota, and the other is that produced by tea constituents following systemic absorption, namely, the inhibition of anabolism and stimulation of catabolism in liver, muscle, adipose, and other tissues [7]. GTCs may decrease the digestion and absorption of nutrients through the inhibition of pancreatic lipase, phospholipases, and lipid transporters to reduce body weight gain. Furthermore, green tea consumption can increase the proportion of favorable intestinal bacteria such as *Bifidobacterium* species [7].

Yang et al. have proposed the "AMPK hypothesis," in which the activation of 5'AMP-activated protein kinase (AMPK) is the main mechanism by which EGCG and other catechins influence energy metabolism to alleviate MetS [7]. AMPK activated by phosphorylation can decrease the expression of enzymes involved in gluconeogenesis, such as phosphoenolpyruvate carboxykinase (PEPCK); lipogenesis, such as fatty acid synthase (FASN); adipogenesis; and protein synthesis, such as homolog of target of rapamycin (mTOR), and increase the expression of those involved in lipolysis, such as acyl-CoA dehydrogenase (ACAD). Activated AMPK can also modulate the expression of transcription factors such as HNF, SREBP, PPAR-α, and PPAR-γ [7]. Through the activation of AMPK, GTCs can activate the ERK1/2-PPAR-γ-adiponectin pathway, leading to a reduction in fat deposits in HFD-fed rats [119].

Thus, the AMPK hypothesis can explain many of the beneficial actions of tea/GTCs on MetS and other diseases (Fig. 2). However, it is not clear how GTCs activate AMPK. One possible explanation is the GTC-mediated generation of ROS,

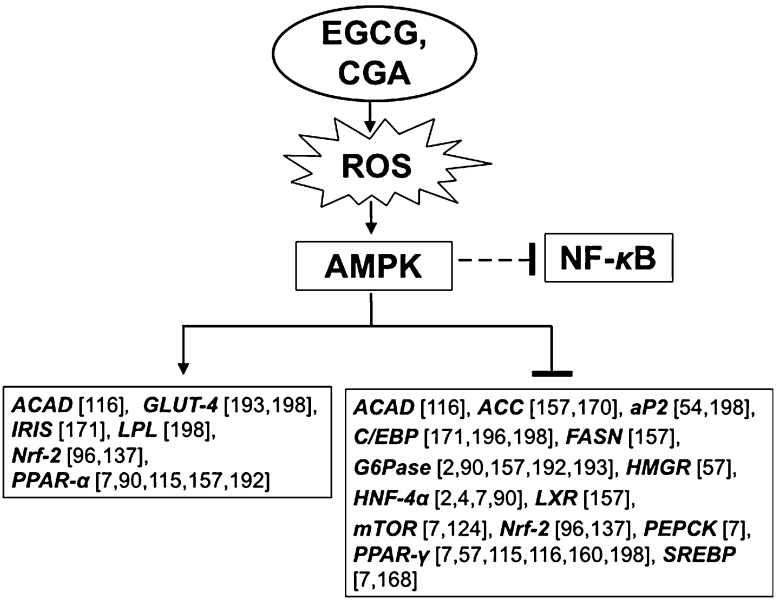


Fig. 2 EGCG and CGA as a prooxidant can modulate gene expressions via promoting ROS production. Related genes to those described in the text are shown. AMPK may possibly suppress NF-κB activity [224, 225], leading to modulation shown in Fig. 2.

which may activate AMPK (Fig. 2) [120]. On the other hand, the scavenging ROS activity of GTCs is well established (Fig. 3) [4, 107]. The factor which directs GTCs to act as either an antioxidant or a prooxidant agent thus remains to be determined.

3.2 Effects of Green Tea on Obesity

3.2.1 Epidemiological Studies

A limited number of epidemiological studies on obesity are available to date. Some of these studies have indicated a beneficial effect of tea on obesity [107]. For example, Vernarelli and Lambert found an inverse association between hot tea consumption and obesity as a component of MetS, as described above [121].

3.2.2 Clinical Studies

Several clinical studies have demonstrated a beneficial effect of tea on obesity [92, 115]. For example, in a meta-analysis of 11 studies which met the inclusion criteria, Hursel et al. found that GTCs significantly decreased body weight and also significantly maintained body weight after a period of weight loss [122].

A clinical trial in which 240 Japanese subjects with visceral fat-type obesity ingested green tea containing 583 mg of catechins (catechin group) or 96 mg of catechins (control) per day for 12 weeks found that decreases in body weight, BMI,

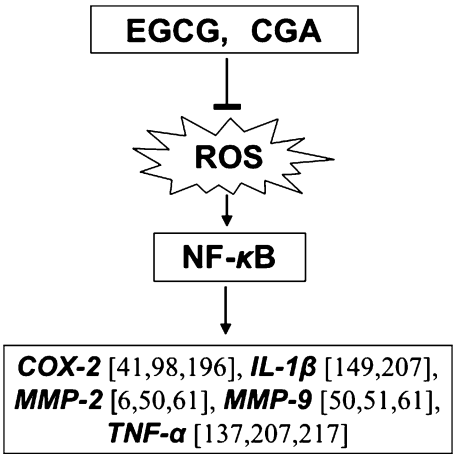


Fig. 3 EGCG and CGA as an antioxidant can modulate gene expressions via elimination of ROS. Related genes to those described in the text are shown.

body fat ratio, body fat mass, waist circumference, hip circumference, visceral fat area, and subcutaneous fat area were greater in the catechin group than in the control group [123].

As mentioned previously, Yang et al. provided several examples of studies that indicated the preventive effect of tea on MetS through the mitigation of obesity [7]. Similarly, a systematic review by Amiot et al. reported the beneficial effects of green tea on BMI [115]. In a literature search, Ferreira et al. found that six out of eight randomized intervention studies showed a beneficial effect of green tea on obesity, such as a reduction in body weight and body fat, while two studies did not [124].

Green tea may also be useful in the treatment of obesity, as suggested by Sulibursk a et al. [125]. In their randomized, double-blind, placebo-controlled study, 46 obese patients were randomly assigned to receive either 379 mg of green tea extract or placebo daily for 3 months. They found that 3 months of GTE supplementation decreased BMI, waist circumference, and the levels of total cholesterol, LDL cholesterol, and triglycerides.

By contrast, a randomized controlled trial showed that two kinds of Japanese brand green tea did not affect body weight, although they did show an LDL cholesterol-lowering effect [126].

3.2.3 Laboratory Studies

The molecular mechanisms for the anti-obesity effects of green tea have been presented in many studies, as described in above section. A comprehensive article published by Huang et al. in 2014 reviewed the anti-obesity effects of green tea in human and laboratory studies and summarized the mechanisms of action of GTC. These included (1) interference with energy absorption and metabolism to inhibit the proliferation of preadipocytes and induce apoptosis in the preadipocyte and matured adipocyte; (2) inhibition of preadipocyte differentiation and the adipogenesis of

maturing adipocytes; (3) inhibition of the activity of gastrointestinal digestive enzymes, luminal emulsification, and micellar solubilization of lipids; (4) interference with the uptake and intracellular processing of lipids and secretion of chylomicrons in enterocytes; (5) enhancement of fecal excretion; (6) downregulation of hepatic gene expression of lipogenic enzymes and related transcription factors; (7) upregulation of hepatic mRNA levels of β-oxidation genes; (8) stimulation of fatty acid oxidation and glucose uptake in skeletal muscle; (9) stimulation of the gene expression of lipolysis and fatty acid oxidation-related genes in adipose tissue; and (10) suppression of glucose intake and gene expression of lipogenesis-related genes in adipose tissue [127]. Many of these actions may be explained by the "AMPK hypothesis" of Yang et al. [7, 107] (see Fig. 2).

A recent cell-based experiment also provided one possible mechanism. In adipocytes differentiated from $C_3H_{10}T1/2$ cells and immortalized preadipocytes in vitro, EGCG reduced the triglyceride content. While EGCG did not affect protein kinase A signaling or brown adipocyte marker expression in adipocytes, it did increase autophagy and reduce mitochondrial membrane potential and intracellular ATP levels. Although mTOR signaling was not upregulated by EGCG treatment, AMPK phosphorylation was induced and lipophagy was activated. These results indicated that EGCG upregulated autophagic lipolysis in adipocytes, supporting the therapeutic potential of EGCG as a caloric restriction mimetic to prevent obesity and obesity-related metabolic diseases [128]. AMPK activation by EGCG was also demonstrated in the brown adipose tissue of diet-induced obese mice [129].

For black tea, detailed information is available in the review article by Pan et al. [130].

3.3 Effects of Green Tea on Hypertension

3.3.1 Epidemiological Studies

As compared with clinical studies, less information is available for epidemiological cohort studies on the antihypertensive effect of tea/green tea. In a cross-sectional epidemiological study of Singaporean Chinese residents aged ≥40 years, consumption of green tea at least 150 mL per week was associated inversely with hypertension risk (OR, 0.63). Drinking combination of green tea and British tea was associated with higher reduction in the risk of hypertension (OR, 0.58), suggesting that consumption of tea lowers the risk of hypertension [131]. By contrast, a study of the population-based prospective cohort that recruited 63,257 Chinese aged 45–74 years and residing in Singapore found that daily drinkers of green tea/black tea had slight increase in the hypertension risk, but these risk estimates were attenuated and became nonsignificant after adjustment for caffeine [132].

3.3.2 Clinical Studies

Several studies have demonstrated the beneficial effects of tea on blood pressure. For example, a meta-analysis of 10 studies (834 participants) published from 1946 to September 27, 2013, showed statistically significant reductions in systolic blood

pressure (SBP, mean differences −2.36 mmHg) and diastolic blood pressure (DBP, mean differences −1.77 mmHg) with tea consumption in individuals within the prehypertensive and hypertensive blood pressure ranges [133]. Similarly, a meta-analysis of 14 randomized controlled trials in 971 participants found that green tea or GTE supplementation caused a small but significant reduction in blood pressure [134].

A crossover, randomized, double-blind, placebo-controlled clinical trial in 20 middle-aged women found that GTE supplementation for 4 weeks resulted in a significant decrease in SBP compared with placebo, but not in DBP [135].

In contrast to reports describing the favorable effect of green tea on hypertension, several studies did not show such an effect. For example, Suliburska et al. found that daily supplementation of 379 mg of GTE for 3 months had no effect on blood pressure in a randomized, double-blind, placebo-controlled study in 46 obese patients, although the supplementation decreased BMI, waist circumference, and the levels of total cholesterol, LDL cholesterol, and triglycerides [125].

3.3.3 Laboratory Studies

Several laboratory studies have shown the favorable effects of green tea on hypertension and cardiovascular disease (CVD). For example, Yi et al. found that EGCG delayed the progression of hypertension in spontaneously hypertensive rats (SHR). SHR have higher mean arterial pressure, plasma pro-inflammatory cytokines, and circulating norepinephrine levels compared with normotensive control rats and also manifest increased NF-κB activity and higher levels of the subunit of NAD(P)H oxidase, ROS, and pro-inflammatory cytokines and lower levels of IL-10 in the hypothalamic paraventricular nucleus. The bilateral hypothalamic paraventricular nucleus infusion of EGCG (20 μg/hour) for 4 weeks improved these parameters in SHR, and the involvement of ROS and NF-κB activity in the mechanism of action of EGCG was suggested [136] (see Fig. 3).

In another experiment in rats fed a high-NaCl diet, supplementation with GTE was shown to reduce blood pressure, concentration of TNF-α, and antioxidant status compared with a control group that did not receive GTE [137].

Kluknavsky et al. found that subchronic (−)-epicatechin (EC) (Fig. 1) significantly prevented the development of hypertension, increased the total antioxidant capacity of blood, and decreased blood nitrotyrosine concentration in young SHR. In the aorta, EC significantly increased nitric oxide (NO) synthase (NOS) activity and elevated NO-dependent vasorelaxation [138]. These findings suggest that a pathway involving ROS and NF-κB is associated with the antihypertensive activity of EGCG.

In addition, the direct action of EGCG on angiotensin I-converting enzyme (ACE) may also mediate its antihypertensive effect. Takagaki and Nanjo demonstrated that EGCG and its metabolites produced by intestinal bacteria showed inhibitory activity against ACE and that a single oral intake of metabolites decreased SBP in SHR [139]. A molecular docking mechanism may explain the potential of EGCG as a new class of ACE inhibitors. Further chemical modification via fragment modification guided by structure and ligand-based computational methodologies may lead to the discovery of better inhibitors as clinical candidates [140].

3.4 Effects of Green Tea on Diabetes

3.4.1 Epidemiological Studies

Many epidemiological studies have reported the antidiabetic effects of tea and GTE [92]. For example, Panagiotakos et al. reported that long-term tea intake reduced levels of fasting blood glucose and was associated with a lower prevalence of diabetes. In a cohort of 937 older adults living on Mediterranean islands, the consumption of 1–2 cups/day of green tea and/or black tea was associated with 70% lower odds of developing type 2 diabetes mellitus (T2DM), irrespective of age, sex, body mass, smoking, physical activity status, dietary habits, and other clinical characteristics [141]. In a literature review of the antidiabetic effects of tea, Fu et al. identified six, ten, and one epidemiological studies published from 2006 to 2016 showing the beneficial effects of green tea, black tea, and oolong tea, respectively [142].

However, several epidemiological studies failed to demonstrate antidiabetic effects [92, 142]. For example, Pham et al. found a rather positive association between green tea consumption and insulin resistance in 1,440 participants aged 18–69 years [143]. Thus, further studies are necessary to confirm the antidiabetic effects of tea in human subjects.

3.4.2 Clinical Studies

A number of intervention studies have reported the antidiabetic effect of green tea [92]. For example, a randomized controlled trial conducted in 66 Japanese T2DM patients found that daily ingestion of GTE containing 544 mg catechins for 2 months caused significant reductions in hemoglobin A1c (HbA1c) levels and DBP [144]. Similarly, a 2-month intervention study in 60 patients with mild hyperglycemia showed that the daily ingestion of GTE decreased the HbA1c level, although other biomarkers were unaffected [145].

In a randomized, double-blind, placebo-controlled trial performed in 92 Taiwanese subjects, the ingestion of 500 mg GTE three times a day for 16 weeks ameliorated the expression levels of an insulin resistance marker and the secretion of glucagon-like peptide-1 in T2DM patients [146]. In a double-blind randomized intervention study in nondiabetic overweight or obese male subjects in the United Kingdom, Brown et al. found that twice-daily ingestion of 400 mg EGCG for 8 weeks resulted in reduced DBP, although no significant effects on glucose tolerance, insulin sensitivity, or insulin secretion were observed [147].

In a randomized, double-blind study in 42 diabetic subjects with a urinary albumin-creatinine ratio >30 mg/g, patients were randomly assigned to two groups to receive either GTP containing 800 mg of EGCG (17 patients with T2DM and 4 with type 1 diabetes) or placebo (21 patients with T2DM) for 12 weeks. The results indicated that GTP reduced the urinary albumin-creatinine ratio by 41%, while the placebo group had a 2% increase, suggesting that GTP may reduce the risk of diabetic nephropathy [148].

In contrast, several intervention studies found no beneficial effects of green tea on diabetes. For example, a double-blind, placebo-controlled, randomized multiple-dose (0, 350, or 750 mg catechins and theaflavins for 3 months) study

conducted in the United States showed no effect on the level of HbA1c in patients with a medical history of diabetes of more than 6 months [149]. A crossover randomized controlled trial in southern Sweden showed that no glucose or insulin-lowering effects were demonstrated by the consumption of 300 mL of green tea or water [150].

Furthermore, a meta-analysis of randomized controlled trials found that the consumption of green tea did not decrease the levels of fasting plasma glucose, fasting serum insulin, hemoglobin HbA1c, or the insulin resistance index in populations at risk of T2DM [151]. Thus, clinical trials in humans on the effects of green tea in diabetes have demonstrated conflicting results. The discrepancy may be explained as discussed in the previous section.

3.4.3 Laboratory Studies and Mechanism of Action

Multiple studies using cultured cells and laboratory animals have demonstrated the antidiabetic effects of green tea and GTCs [92, 152].The underlined mechanisms include (1) inhibition of α-amylase and α-glucosidase activity, (2) inhibition of glucose absorption in the small intestine, (3) protection of pancreatic β-cells, (4) improvement of insulin sensitivity in peripheral organs, and (5) inhibition of glucose production from noncarbohydrates such as amino acids in the liver, known as gluconeogenesis.

Inhibition of α-amylase and α-glucosidase results in reduced glucose production leading to the prevention and suppression of diabetes by impeding the rise in blood sugar levels [92, 152]. Similarly, the inhibition of glucose absorption in the small intestine suppresses the increase in blood sugar levels [92, 152]. Improvements in insulin sensitivity would result in the rapid suppression of blood glucose levels by promoting glucose uptake by peripheral tissues. Green tea ingredients such as EGCG have exhibited insulin-like activity in terms of increased glucose uptake [153]. EGCG may also protect insulin-secreting pancreatic β-cells from injury since EGCG protected IL-1β and interferon (IFN)-γ-mediated cytotoxicity in an insulinoma cell line, presumably through the inhibition of NF-κB activation [154] (see Fig. 3).

Several studies have reported inhibitory effects of EGCG on gluconeogenesis. EGCG exhibited insulin-like activity by suppressing the gene expression of gluconeogenic enzymes, glucose-6-phosphatase (*G6Pase*), and phosphoenolpyruvate carboxykinase [155]. One of the proposed mechanisms is the suppression of transcription factor, hepatocyte nuclear factor-4α (HNF4α) expression by EGCG, leading to a decrease in expression of these gluconeogenic enzymes and resulting in diminished glucose production [2, 92]. The suppressive action of EGCG on HNF4α expression may be explained by its prooxidative activity to generate ROS, leading to the activation of AMPK which is known to inhibit HNF4α expression [2, 4] (see Fig. 2). Collins et al. postulated that ROS was involved in the activation of AMPK by EGCG and the suppression of hepatic gluconeogenesis [120].

In addition, several other mechanisms have been proposed for the antidiabetic activity of tea. These include (1) improvement of endothelial dysfunction, (2) modulation of cytokine expression, and (3) amelioration of insulin resistance,

as reviewed by Fu et al. [142]. These authors pointed out the involvement of GTCs in the modulation of gene expression under its antidiabetic effects [142]. GTE may increase the mRNA levels of glucose transporter family proteins. EGCG can also attenuate the formation of advanced glycation end products; activate Nrf2, which regulates the expression of antioxidant proteins protecting against oxidative damage; and inhibit the expression of the AGE receptor. Green tea decreases the expression of receptor activator of NF-κB and pro-inflammatory cytokine TNF-α in a rat model of diabetes and increases anti-inflammatory cytokine IL-10. EGCG may also suppress the expression of genes related to inflammation such as *IL-1β*, *TNF-α*, *IL-6*, *CD11s*, *CD18*, and *MCP-1* in adipose tissues in another animal model [142] (see Fig. 3).

3.5 Effects of Coffee on Metabolic Syndrome and Related Disorders

3.5.1 Epidemiological Studies

Hino et al. conducted a population-based health check in 1999 and found that all components of MetS (blood pressure, waist circumference, fasting plasma glucose, and lipid profiles) except for HDL cholesterol were inversely related to the consumption of coffee but not of green tea after adjusting for confounding factors [111].

Using the PubMed, Embase, Scopus, and Science Direct databases, Yesil et al. found that four out of six human studies showed an inverse association between coffee consumption and the risk of MetS, although two studies showed no such association in a cohort consisting of young persons with a low prevalence of MetS [156].

In addition, the following studies reported beneficial effects of coffee. From May 2009 to December 2010, a cross-sectional survey was conducted on 1,889 inhabitants living in Sicily, southern Italy. As a result, coffee consumption (OR, 0.43) was found to be associated inversely with MetS after adjusting for all covariates. No association was observed between caffeine intake and MetS. Triglycerides were inversely associated with the consumption of espresso coffee. Thus, coffee consumption was demonstrated to have a reduced OR for MetS [108].

Results from a cross-sectional population-based survey including 8,821 adults showed that high coffee drinkers had lower BMI, waist circumference, SBP and DBP, and triglycerides and higher HDL cholesterol than those drinking < 1 cup/day. After adjusting for potential confounding factors, higher coffee consumption was demonstrated to be inversely associated with MetS (OR, 0.75) [109].

A Mendelian randomization study with 93,179 individuals from two large general population cohorts found that in a cross-sectional analysis, there was a lower risk of MetS with higher coffee intake. Compared with individuals with no coffee intake, ORs for MetS were 0.91, 0.89, 0.88, 0.83, 0.84, and 0.89 for 0.1–1, 1.1–2, 2.1–3, 3.1–4, 4.1–5, and >5 cups/day, respectively [157].

Shang et al. conducted a meta-analysis of 11 studies published between January 1999 and May 2015 with a total of 159,805 participants to determine the association between coffee intake and MetS risk. The aggregated result for the highest versus

lowest category of coffee consumption was 0.872, suggesting that coffee consumption was associated with a reduced risk of MetS [158]. A cross-sectional study of a random sample of 5,146 participants aged ≥20 years found that moderate drinkers had 17% lower odds of developing MetS compared with nondrinkers. Tea consumption was related to some components, but not to MetS in general [159].

Although these findings appear to show beneficial effects of coffee on MetS, caution should be taken as coffee, particularly instant coffee mix, may have a potentially harmful effect arising from the excessive intake of sugar and powdered creamer [160].

3.5.2 Clinical Studies

Several clinical studies on the effect of coffee and CGAs have been reported. Patti et al. evaluated the impact of a natural supplement, Kepar, which contains several plant extracts such as curcuma longa, silymarin, guggul, CGAs, and inulin, in 78 patients with MetS. Although Kepar exerted beneficial effects on body weight, BMI, and waist circumference, fasting glucose and total cholesterol levels, further studies are required to verify whether CGAs indeed contribute to these effects [161].

Santana-Gálvez et al. reviewed several clinical trials to evaluate the effects of CGA on the prevention and treatment of obesity and hypertension, which are the component disorders of MetS. These studies are discussed in the Sects. 3.6.2 and 3.7.2 [162].

3.5.3 Laboratory Studies and Mechanism of Action

Many studies have evaluated the effect of CGA on MetS or associated disorders, including obesity, dyslipidemia, diabetes, and hypertension. Santana-Galvez et al. reviewed the literature and found the beneficial effects of CGA on MetS and its components [162].

Similarly, Yesil and Yilmaz identified three animal studies on the effects of coffee on MetS and five on the risk of fatty liver infiltration. All showed a protective effect of coffee against the development of MetS and nonalcoholic fatty liver disease (NAFLD) [156].

In a study investigating the effects of Colombian coffee extract in an animal model of MetS, rats were fed a corn starch-rich diet, whereas two other groups were given a high-carbohydrate, HFD with 25% fructose in drinking water for 16 weeks. The high-carbohydrate, HFD group showed the symptoms of MetS leading to cardiovascular remodeling and NAFLD. Colombian coffee extract supplementation attenuated the impaired glucose tolerance, hypertension, cardiovascular remodeling, and NAFLD without affecting abdominal obesity and dyslipidemia. This study suggests that Colombian coffee extract can attenuate diet-induced changes in the structure and function of the heart and the liver without changing abdominal fat deposition [163].

Tsumura-Suzuki obese diabetic mice are a newly developed MetS model which spontaneously exhibits obesity, diabetes, hyperlipidemia, and nonalcoholic steatohepatitis with liver nodules. When animals were divided into two coffee intake groups (with and without caffeine), coffee intake did not affect obesity and

hyperlipidemia. However, coffee intake caused various degrees of improvement in pancreatic β-cell damage and steatohepatitis with liver carcinogenesis. Most of the effects were likely caused by a synergistic effect of caffeine with other components such as polyphenols, and the anti-fibrotic effects of coffee appeared to be attributable to the polyphenols rather than the caffeine [164].

Ma et al. conducted two sets of experiments. In one experiment, 6-week-old C57BL/6 mice were fed regular chow or HFD for 15 weeks with twice-weekly intraperitoneal injection of CGA (100 mg/kg) or vehicle. In another experiment, obese mice (average weight of 50 g) received intraperitoneal injection of CGA (100 mg/kg, twice a week) or vehicle for 6 weeks. CGA significantly inhibited the development of diet-induced obesity but did not affect body weight in the obese mice. CGA treatment also curbed HFD-induced hepatic steatosis and insulin resistance; suppressed the hepatic expression of *PPAR-γ*, *Cd36*, *Fabp4*, and *Mgat1* genes; and attenuated inflammation in the liver and white adipose tissue accompanied by a decrease in mRNA levels of macrophage marker genes including *F4/80*, *Cd68*, *Cd11b*, *Cd11c*, and *TNF-α*, *Mcp-1*, and *Ccr-2* encoding inflammatory proteins. These results suggest that CGA is a potent compound for preventing diet-induced obesity and obesity-related MetS [165].

Santana-Galvez et al. proposed a possible mechanism of action for CGA, whereby it may act as an antioxidant to reduce ROS, which stimulate inflammation leading to fat accumulation, weight gain, and insulin resistance (see Fig. 3). The antioxidative activity of CGA may also stimulate NO production, leading to an improvement in endothelial function and blood pressure. CGA may also improve lipid metabolism by downregulating transcriptional factors such as LXRα and PPAR and the gene expression of enzymes such as FASN, acetyl-CoA carboxylase (ACC), and HMGCR and by upregulating PPAR-α, adiponectin, and AMPK phosphorylation [162] (see Fig. 2).

Although these studies support a beneficial effect of CGA in MetS, others failed to show such effects. For example, in a controlled dietary intervention of over 12 weeks in which male C57BL/6 mice were divided into three groups: (i) normal diet, (ii) HFD, and (iii) HFD plus CGA (1 g/kg of diet), Mubarak et al. found that CGA supplementation in mice fed HFD did not reduce body weight compared with mice fed HFD alone. CGA caused increased insulin resistance compared with mice fed HFD and led to reduced phosphorylation of AMPK and ACC-β, a downstream target of AMPK in the liver. The livers of mice fed an HFD supplemented with CGA had a higher lipid content and more steatosis relative to mice fed an HFD, indicating impaired fatty acid oxidation. This study suggests that CGA supplementation in HFD does not protect against the characteristics of MetS in diet-induced obese mice [166].

3.6 Effects of Coffee on Obesity

3.6.1 Epidemiological Studies

Few studies have been published on the effect of coffee on obesity. The result of cross-sectional analyses in a Mendelian randomization study showed that higher

coffee intake of up to 4 cups/day was associated with a lower risk of obesity. Compared with individuals with no coffee intake, ORs were 0.82, 0.86, 0.86, 0.83, 0.95, and 1.02 for 0.1–1, 1.1–2, 2.1–3, 3.1–4, 4.1–5, and >5 cups/day, respectively [157]. A study on 137 patients with NAFLD and 108 controls found that coffee consumption was inversely associated with insulin resistance and obesity [167].

3.6.2 Clinical Studies

Several clinical studies have reported the effects of coffee or CGAs on obesity. In a meta-analysis of randomized clinical trials, Onakpoya et al. found a significant reduction (−2.47 kg) in body weight in a green coffee extract-treated group compared with placebo. The magnitude of the effect was moderate, and there was significant heterogeneity among the studies. The authors summarized that the results from these trials were promising but that the studies were of poor methodological quality. More rigorous trials are thus required to assess the usefulness of green coffee extract as a weight-loss tool [168].

In a randomized controlled trial, overweight men with a mild-to-moderate elevation of fasting plasma glucose were randomly allocated to a 16-week intervention of the consumption of 5 cups of caffeinated ($n = 17$) or decaffeinated ($n = 15$) instant coffee per day or no coffee ($n = 13$). The results indicated that waist circumference decreased by 1.5 cm in the caffeinated coffee group, increased by 1.3 cm in the decaffeinated coffee group and decreased by 0.6 cm in the non-coffee group. Body weight at 16 weeks showed a similar pattern; the corresponding changes from baseline were −1.1 kg, 0.5 kg, and −0.6 kg, respectively. The authors proposed that the decreases in the caffeinated coffee group were attributable to caffeine, which increases thermogenesis and fat oxidation [169].

In a review article, Santana-Gálvez et al. listed two clinical studies showing the effect of CGA on obesity [162]. One study was a randomized, double-blind, 12-week study in 30 overweight people. The result indicated that the average loss in body weight among subjects who consumed CGA-enriched coffee was 5.4 kg, while that in the normal instant coffee groups was 1.7 kg, suggesting a beneficial effect of CGA on body weight reduction [170]. Another study was a placebo-controlled, double-blind, crossover intervention study in 18 healthy male subjects in which those who consumed 185 mL of a test beverage with or without CGAs (329 mg) per day for 4 weeks showed no effects on body weight, BMI, or body fat, although a significantly higher postprandial energy expenditure was observed in the CGA group compared with the control group [171]. Thus, further studies are required to determine the effects of coffee and CGAs on obesity.

3.6.3 Laboratory Studies and Mechanism of Action

A number of laboratory studies have provided evidence for the anti-obesity effect of coffee and its mechanism of action. Hsu et al. found that the addition of coffee phenols to culture medium decreased the cell growth of 3T3-L1 preadipocytes. The IC_{50} values of CGA, gallic acid, *o*-coumaric acid, and *m*-coumaric acid on the preadipocytes were 72.3, 43.3, 48.2, and 49.2 μM, respectively. A relationship analysis indicated that there was a linear correlation between the influence of

phenolic acids on cell growth and their antioxidant activity. The treatment of preadipocytes with CGA, *o*-coumaric acid, and *m*-coumaric acid caused cell cycle arrest in the G1 phase. These results suggest that coffee phenols have anti-obesity effects [172].

When C57BL/6 J mice were fed a control diet, HFD, or HFD supplemented with 0.5–1.0% coffee polyphenols (CPP) for 2–15 weeks, CPP supplementation reduced body weight gain, abdominal and liver fat accumulation, and infiltration of macrophages into adipose tissues. The mRNA levels of sterol regulatory element-binding protein (SREBP)-1c, ACC-1 and -2, stearoyl-CoA desaturase-1, and pyruvate dehydrogenase kinase-4 in the liver were also significantly lower in CPP-fed mice than in HFD and control mice (see Fig. 2). Similarly, CPP suppressed the expression of these molecules in Hepa 1-6 cells, concomitant with an increase in microRNA-122. Structure-activity relationship studies of nine quinic acid derivatives isolated from CPP suggested that CGA and di-caffeoylquinic acids were active substances in the beneficial effects of CPP in these cells. Furthermore, CPP and 5-CQA decreased the nuclear active form of SREBP-1, ACC activity, and cellular malonyl-CoA levels. These findings indicate that CPP enhances energy metabolism and reduces lipogenesis by downregulating SREBP-1c and related molecules, leading to the suppression of body fat accumulation [173].

When a commercially available supplement composed of cocoa, coffee, green tea, and garcinia which contains 196 mg/g of total polyphenols and 4.0 mg/g of EGCG was given to high-energy diet (HED)-induced obese rats, a reduction was observed in the levels of free fatty acids, triglycerides, total cholesterol, LDL-C, and LDL-C/HDL-C, AST, ALT, and ketone bodies in serum as well as hepatic triglycerides and total cholesterol content, while the levels of HDL-C in serum and lipase activity in fat tissues increased compared with the HED group. These results suggest that the supplement stimulated lipid metabolism in HED-induced obese rats through fat mobilization from adipose tissue [174].

When hyperlipidemia was induced in Wistar rats using HFD, the animals given CGA complex from green coffee bean, CGA7 (50, 100, and 150 mg/kg body weight), showed decreased triglycerides and free fatty acid levels in plasma and the liver compared with the control group. CGA7 administration led to the activation of AMPK and a subsequent increase in the levels of carnitine palmitoyltransferase 1 and a decrease in ACC acetyl-CoA carboxylase (ACC) activity (see Fig. 2). These results suggest that CGA7 complex may be suitable as an active ingredient in nutrition for obesity management [175].

Maki et al. found that coffee intake significantly suppressed HFD-induced metabolic changes such as increased body weight and the accumulation of adipose tissue and the upregulation of glucose, free fatty acid, total cholesterol, and insulin levels in the blood. In the early phase of adipogenesis, 3T3-L1 cells treated with coffee extract displayed delayed cell cycle entry into the G2/M phase, termed as mitotic clonal expansion. Coffee extract also inhibited the activation of CCAAT/enhancer-binding protein β (C/EBP-β) by preventing its phosphorylation by ERK and suppressed adipogenesis-related events such as mitotic clonal expansion and C/EBP-β activation through the downregulation of insulin receptor substrate 1 (IRS1). The stability of the IRS1 protein was markedly

decreased by treatment with coffee extract, due to proteasomal degradation. These results showed an anti-adipogenic function for coffee intake and identified IRS1 as a novel target for coffee extract in adipogenesis [176].

Although these results indicate the favorable effects of coffee and/or its extract in obesity, several other studies do not support these findings. For example, Cheong et al. carried out a study in which C57BL6 mice were randomly divided into the following experimental groups: (i) normal diet, (ii) HFD, or (iii) HFD supplemented with 0.5% w/w GCE rich in CGA. The results showed that groups (ii) and (iii) displayed MetS symptoms more profoundly than group (i) and that GCE did not attenuate HFD-induced obesity, glucose intolerance, insulin resistance, or systemic oxidative stress [177].

3.7 Effects of Coffee on Hypertension

3.7.1 Epidemiological Studies

A meta-analysis of 7 cohorts including 205,349 individuals showed a 9% significant decreased risk of hypertension per 7 cups of coffee per day in a nonlinear analysis, while in a linear dose-response association, there was a 1% decreased risk of hypertension for each additional cup of coffee per day. The analysis also suggested smoking as an important confounder [178].

In contrast, a large prospective study during 112,935 person-years of follow-up in 5,566 cases of incident hypertension showed that neither caffeinated coffee nor caffeine intake was associated with mean SBP or DBP but that decaffeinated coffee intake was associated with a small but clinically irrelevant decrease in mean DBP. Decaffeinated coffee intake was not associated with mean SBP. Thus, no anti-hypertension effects were demonstrated, but caffeinated coffee, decaffeinated coffee, and caffeine appeared not to be risk factors for hypertension in postmenopausal women [179].

A cross-sectional study conducted in 2012 among 1,164 individuals aged $\geq$63 years showed that among the 715 hypertensive participants, those consuming $\geq$3 cups of coffee per day showed higher 24-hour SBP and DBP than non-coffee drinkers. Compared with non-coffee drinkers, ORs for uncontrolled BP among those consuming 1, 2, and $\geq$3 cups of coffee/day were 1.95, 1.41, and 2.55, respectively [180].

3.7.2 Clinical Studies

Santana-Gálvez et al. reviewed human studies on the effects of CGA on blood pressure and found that CGA or coffee extracts reduced SBP and DBP in three out of four human intervention studies [162]. In a similar approach, Tajik et al. found that five out of six human intervention studies showed favorable effects on blood pressure [181]. One example is the report by Revuelta-Iniesta and Al-Dujaili, who conducted a randomized pilot crossover study in healthy subjects. The results indicated that green coffee consumption reduced SBP, arterial elasticity, BMI, and abdominal fat [182].

3.7.3 Laboratory Studies and Mechanism of Action

Several studies have demonstrated the beneficial effects of CGA on blood pressure and suggested the underlying molecular mechanisms. Suzuki et al. demonstrated that a single ingestion of CGA (30–600 mg/kg) reduced blood pressure in SHR, an effect that was blocked by the administration of an NOS inhibitor, *N(G)*-nitro-L-arginine methyl ester. When SHR were fed diets containing 0.5% CGA for 8 weeks (approximately 300 mg/kg per day), the development of hypertension was inhibited. The authors proposed, as the underlying mechanism, that dietary CGA reduces oxidative stress and improves NO bioavailability by inhibiting the excessive production of ROS in the vasculature, leading to the attenuation of endothelial dysfunction, vascular hypertrophy, and hypertension in this animal model [183].

Zhao et al. summarized that CGA may exert an antihypertension effect through (1) inhibition of NAD(P)H oxidase expression and activity, leading to reduction in free radical production; (2) direct free radical scavenging; (3) stimulation of NO production by the endothelial-dependent pathway; and (4) inhibition of ACE in the plasma and possibly also in the organs and tissues. The anti-inflammatory effects of CGA may also contribute to the effect [184].

3.8 Effects of Coffee on Diabetes

3.8.1 Epidemiological Studies

Ding et al. conducted a systematic review and meta-analysis of 28 prospective studies with 1,109,272 participants and 45,335 cases of T2DM for coffee consumption and disease risk. The results showed that compared with no or rare coffee consumption, RR for diabetes was 0.92, 0.85, 0.79, 0.75, 0.71, and 0.67 for 1–6 cups/day, respectively. Thus, the decreases in RRs for every 1 cup/day were 9% for caffeinated coffee consumption and 6% for decaffeinated coffee consumption, indicating that the consumption of both types of coffee is inversely associated with the risk of T2DM [185].

Based on three large cohort studies of men and women in the United States, Bhupathiraju et al. found that coffee consumption was associated with a lower risk of T2DM. During 1,663,319 person-years of follow-up, participants who increased their coffee consumption by >1 cup/day over a 4-year period had an 11% lower risk of T2DM in the subsequent 4 years compared with those who made no changes in consumption. Participants who decreased their coffee intake by >1 cup/day had a 17% higher risk for T2DM [186].

In another study in 90,317 US adults, where 8,718 deaths occurred during 805,644 person-years of follow-up from 1998 through 2009, coffee drinkers had a lower HR for several diseases, including diabetes, compared with nondrinkers [187].

Nordestgaard et al. conducted a Mendelian randomization study among 93,179 individuals from two large general population cohorts. The results indicated that higher coffee intake was associated with a lower risk of obesity, MetS, and T2DM. However, per-allele meta-analyzed ORs for T2DM were in the range of 0.98–1.01,

indicating that there was no genetic evidence to support corresponding causal relationships [157].

In 2001–2002, a random sample of 1,514 men and 1,528 women was selected to participate in a study in the Athens metropolitan area in Greece. This 10-year follow-up study showed that individuals who consumed ≥250 mL of coffee had 54% lower odds of developing diabetes compared with abstainers, indicating the significance of long-term habitual coffee drinking in the onset of diabetes. The inverse association between habitual coffee drinking and diabetes was suggested to be mediated by serum amyloid-A levels [188].

A cross-sectional study in a large Brazilian cohort of 12,586 middle-aged and older individuals provided evidence of a protective effect of coffee on the risk of adult-onset diabetes. The results showed 23% and 26% lower odds of diabetes among those consuming coffee 2–3 and >3 times per day, respectively, compared with those reporting no or almost no consumption of coffee. An inverse association was also found for 2-hour post-load glucose but not for fasting glucose concentrations, suggesting the action of coffee on postprandial glucose homeostasis [189].

A population-based cohort study over a follow-up period of 4 years was conducted to examine the association between habitual coffee intake and the risk of T2DM and to determine whether this association varied by genetic polymorphisms related to T2DM. The results on 4,077 Korean adults aged 40–69 years with a normal glucose level at baseline showed an inverse association between coffee intake and the combined risk of T2DM and prediabetes. This inverse association was found among individuals with the GT/TT of IGF2BP2 rs4402960, GG/GC of CDKAL1 rs7754840, or CC of KCNJ11 rs5215 polymorphisms, which are known to be related to T2DM in East Asians [190].

The European Prospective Investigation into Cancer and Nutrition-Potsdam study involving 27,548 middle-aged participants found that coffee consumption was inversely associated with diacyl-phosphatidylcholine and liver injury markers in both sexes and positively associated with certain acyl-alkyl-phosphatidylcholines in women. Coffee consumption showed an inverse relationship with C-reactive protein in women and with triglycerides and phenylalanine in men. These findings may partly explain the inverse association between long-term coffee consumption and T2DM risk [191].

A population-based cohort study on first-trimester coffee and tea intake and risk of gestational diabetes among 71,239 nondiabetic women with singleton pregnancies showed that moderate first-trimester coffee and tea intake was not associated with the risk of gestational diabetes and possibly may have a protective effect [192].

A recent review article based on review papers from in vivo, ex vivo, and in vitro experimental studies in animals and human tissues as well as epidemiological studies reported a reduced risk of developing T2DM in regular coffee drinkers of 3–4 cups a day. The effects were proposed as attributable to CGAs and caffeine [193].

In a cross-sectional epidemiological study aimed to investigate the mechanism of the association of coffee with liver injury, caffeinated coffee showed a significant inverse association with ALT, AST, and NAFLD liver fat score but not with fetuin-A, another liver injury marker. However, there was no significant association

between decaffeinated coffee intake and markers of liver injury. These results indicate a beneficial impact of caffeinated coffee on liver morphology and/or function and suggest that this relationship may mediate the inverse association of coffee with risk of T2DM [194].

By reviewing the literature, Chrysant concluded that coffee consumption has either neutral or beneficial effects on blood pressure, CVD, heart failure, cardiac arrhythmias, and diabetes, and that the established concept that coffee consumption is a risk factor for hypertension, heart disease, or diabetes is no longer warranted, although some caution should be exercised in vulnerable populations [195].

3.8.2 Clinical Studies

Santana-Gálvez et al. reviewed clinical trials to evaluate the effects of CGA on MetS-related diseases and found that five studies showed beneficial effects on diabetes [162]. One such study was a randomized crossover trial for the effects of 12 g decaffeinated coffee, 1 g CGA, and placebo (1 g mannitol) on glucose and insulin concentrations during a 2-hour oral glucose tolerance test in 15 overweight men. The results indicated that CGA ingestion significantly reduced blood glucose and insulin concentrations [196].

3.8.3 Laboratory Studies and Mechanism of Action

Evidence has been accumulating to support the beneficial effects of CGA on diabetes in cell-based and animal experiments. A comprehensive review by Meng et al. indicated that CGA stimulated glucose uptake in murine adipocytes and L6 muscle cells and inhibited G6Pase in hepatic cells [197]. It also reported that more than ten animal experiments found favorable effects of CGA such as improvement in blood glucose levels, glucose tolerance, and insulin resistance and inhibition of α-glucosidase, together with AMPK activation and the upregulation of hepatic PPAR-α [197] (see Fig. 2).

Similarly, a recent review of five animal studies found that CGA had beneficial effects in diabetes through a reduction in the levels of blood glucose and HbA1c, prevention of the development of sugar cataracts, acceleration of wound healing, and inhibition of hepatic G6Pase levels [162].

In an experiment in which female db/db mice were administered 80 mg/kg/day CGA by lavage for 12 weeks, the percentage of body fat and the fasting plasma HbA1c level decreased compared with that in the control group; transforming growth factor-β1 protein expression and aldose reductase activity in the kidney also decreased, while the adiponectin level in visceral adipose increased. CGA significantly upregulated phospho-AMPK in the liver and skeletal muscle and downregulated G6Pase in the liver, while upregulating GLUT4 (see Fig. 2). Thus, CGA lowered the levels of fasting plasma glucose and HbA1c during late diabetes and improved kidney fibrosis through the modulation of adiponectin receptor signaling pathways in db/db mice [198].

Several other investigations also demonstrated the beneficial effects of CGA in diabetes. For example, in a study aimed to investigate whether short-term treatment with plant polyphenols, including CGA, could improve endothelial dysfunction related to diabetes, streptozotocin-induced diabetic mice received CGA (0.03 mmol/kg/day) by injection for 5 days. This treatment improved the NO components of relaxation without the normalization of acetylcholine-stimulated NO production. In addition, CGA treatment suppressed the acetylcholine-stimulated level of thromboxane B2 in aortas from streptozotocin-induced diabetic mice. Thus, the treatment caused basal NO production and a prompt improvement in endothelial function in diabetic mice, which may involve the normalization of thromboxane B2 levels but not NO production under acetylcholine stimulation [199].

In an animal experiment to evaluate the effects of CGA on diabetic auditory pathway impairment, CGA was shown to prevent the progression of auditory pathway dysfunction caused by diabetes. The study also found that CGA may aid in the recovery from outer hair cell and otic hair cell damage. Thus, CGA appears to have beneficial effects in the management of diabetic sensorineural auditory dysfunction [200].

There is a possibility that CGA can protect kidney function against oxidative stress in diabetic nephropathy. Ye et al. showed that CGA decreased the levels of blood glucose, blood urea nitrogen, and serum creatinine in a rat model of diabetic nephropathy. CGA increased the activity of superoxide dismutase, glutathione peroxidase, and catalase and decreased the level of lipid peroxidation. Immunohistochemical analysis showed that CGA downregulated COX-2 protein expression in renal tissues. In addition, CGA blocked the expression of activating transcription factor-6 and C/EBP homology protein as well as the phosphorylation of eukaryotic initiation factor 2α and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase. These findings suggest that CGA attenuates oxidative stress in diabetic nephropathy, leading to modulation of the endoplasmic reticulum signaling pathway [201].

In alloxan-induced diabetic rats, oral administration of aqueous infusions of *Artemisia herba-alba Asso* and *Ajuga iva Schreber*, used in folk medicine, was shown to reduce blood glucose levels. Since *A. herba-alba* infusion contains CGA as the main compound, CGA may contribute to this effect [202].

On the other hand, some studies did not show the beneficial effects of CGA in diabetes. For example, in a study using 3T3-L1 cells, coffee was shown to reduce the accumulation of lipids during adipocytic differentiation of these cells, which may explain the antidiabetic action of coffee. Coffee also inhibited expression of PPAR-γ, a transcription factor which upregulates the differentiation of adipocytes. However, CGA showed no effect on *PPAR-γ* gene expression, suggesting that CGA does not contribute to the antidiabetic activity of coffee [203].

In a study aimed to determine whether bioactive substances in coffee increase insulin secretion from β-cells and improve insulin sensitivity in skeletal muscle cells, cafestol and caffeic acid but not CGA were found to increase insulin secretion, both acutely and chronically [204].

3.9 Comparison of the Effects of Tea and Coffee in Simultaneous Studies on Metabolic Syndrome and Related Disorders

A cross-sectional epidemiological study among Singaporean Chinese residents aged ≥40 years reported that drinking at least 150 mL green tea per week was associated with lower hypertension risk (OR, 0.63). Drinking a combination of green tea and British tea was associated with a higher reduction in the risk of hypertension (OR, 0.58). In contrast, consumption of coffee (OR, 1.44–1.46) was found to be a potential risk factor for hypertension [131].

A population-based prospective cohort study among 63,257 Singapore Chinese aged 45–74 years found that, compared to those who drank 1 cup of coffee/day, the HRs were 0.87 for <1 cup/week drinkers and 0.93 for ≥3 cups/day drinkers. Compared to <1 cup/week drinkers, daily drinkers of black or green tea had a slight nonsignificant increase in risk, after adjustment for caffeine. Compared with the lowest caffeine intake (<50 mg/day) group, the highest caffeine intake (≥300 mg/day) group had a 16% increase in risk [132].

In a study of the effects of tea and coffee consumption in cardiovascular diseases and risk factors such as hypertension, hyperglycemia, and hyperlipidemia, Di Lorenzo et al. concluded that data from the clinical literature showed that tea consumption reduced some risk factors, especially in overweight people and obese subjects. For coffee, the results were controversial and did not allow conclusions to be drawn [205].

4 Effects on Liver Disease

4.1 Effects of Green Tea on Liver Disease

4.1.1 Epidemiological Studies

Several human studies have indicated that GTC has beneficial effects in liver diseases such as hepatitis, hepatoma, and liver fibrosis [92]. In a meta-analysis of studies published from 1995 to 2014, Yin et al. found that there was a significant reduction in the risk of liver disease with GTC intake. RRs were 0.74, 0.65, 0.57, 0.56, and 0.49 for hepatocellular carcinoma, liver steatosis, hepatitis, liver cirrhosis, and chronic liver disease, respectively [206].

A retrospective cross-sectional study on 1,018 patients with NAFLD, HCV, and HBV infection found that patients who drank ≥2 cups of coffee per day had a lower liver stiffness, which is indicative of less fibrosis and inflammation, but that tea consumption had no effect [207].

4.1.2 Clinical Studies

Only a few studies have examined the effects of green tea in liver disease. In a clinical experiment on nine cases of intractable chronic hepatitis C with a high viral load of >850 kIU/mL, Sameshima et al. found that combination therapy of 6 g of green tea powder/day and interferon/ribavirin showed 3.5-fold efficacy compared

with interferon/ribavirin therapy alone [208]. This result and that of another clinical trial in which a single 400 mg oral dose of EGCG was safe and well tolerated by all 11 patients with hepatitis C and detectable viremia strongly encourage further studies [209].

4.1.3 Laboratory Studies and Mechanism of Action

Several cell-based and animal experiments demonstrated the hepatoprotective effects of EGCG [5]. For example, our research group showed that consumption of an EGCG-rich green tea beverage reduced liver damage in rats with galactosamine-induced hepatitis [210]. Administration of the beverage restored the plasma levels of inflammatory factors TNF-α and IL-1β and their hepatic expression upregulated by galactosamine. Similarly, in concanavalin A-induced hepatitis mice, oral administration of EGCG (10 or 30 mg/kg) twice daily for 10 days prior to concanavalin A injection was associated with decreased immunoreaction and pathological damage by reducing inflammatory factors, such as TNF-α, IL-6, IFN-γ, and IL-1β (see Fig. 3). EGCG also exhibited antiapoptotic and anti-autophagic effects by inhibiting the expression of proapoptotic protein BNIP3 through the IL-6/JAKs/STAT3 pathway [211].

Steinmann et al. reviewed the antiviral and antibacterial activities of EGCG against human pathogens including HBV, HCV, human immunodeficiency virus, and influenza A virus. In most of the studies, EGCG exhibited antiviral properties within physiological concentrations in vitro [212]. The actions of EGCG were suggested as being mediated through (1) the inhibition of viral entry by interference with binding to target cells, (2) the inhibition of integrase and reverse transcriptase, (3) the destruction of virions by binding to CD4 and interference with gp120 binding, (4) the inactivation of virus particles, (5) the inhibition of intracellular virus growth and viral protease, (6) the alteration of physical integrity of virus particles, and (7) the suppression of viral replication via modulation of cellular redox milieu [212]. One of the studies reviewed by the authors showed that HCV infection was suppressed by EGCG in Huh7.5.1 cells infected with the JFH1-GFP chimeric virus when monitored for HCV RNA and protein expression levels. The inhibitory mechanism was proposed to involve the suppressive effects of EGCG on both the HCV entry and RNA replication steps. In addition, 50 and 25 μM EGCG was shown to eliminate HCV from cell cultures after two and five passages, respectively [213].

Xu et al. demonstrated that EGCG inhibited transcription of the HBV promoter in HEK293 cells co-transfected with expression plasmids of farnesoid X-activated receptor-α and RXR-α, suggesting that EGCG, as an antagonist of farnesoid X-activated receptor-α in liver cells, has the potential to be employed as an effective anti-HBV agent [214].

4.1.4 Hepatotoxicity

Green tea is generally considered to be safe for human health. However, a considerable number of reports have described hepatotoxicity related to GTE. For example, Navarro et al. found that since 2006, there have been more than 50 reports of

clinically apparent acute liver injury with jaundice attributed to GTE [215]. The illness was generally self-limiting, but fatal instances have been reported in up to 10% of cases, typically among those who presented with acute hepatocellular injury and jaundice. These authors pointed out that in most reports of GTE hepatotoxicity, the human dose of EGCG (generally less than 12 mg/kg daily) did not appear to be excessive or in the range that might have direct toxicity (estimated for humans to be 30–90 mg/kg), suggesting that liver injury associated with GTE is an idiosyncratic reaction, typical of conventional drug-induced liver injury. Naturally, the excessive consumption of catechin supplements should be avoided.

A recent investigation by Wang et al. suggested that melatonin may be useful for preventing the potential adverse effects of EGCG in its application for MetS alleviation and body weight reduction [216]. Future studies may explore effective ways to prevent or reduce the possible adverse effects of tea constituents.

4.2 Effects of Coffee on Liver Disease

4.2.1 Epidemiological Studies

Coffee intake may exert beneficial effects in the liver. In population studies among persons with an unknown diagnosis of liver disease, greater coffee intake was associated with lower risk of cirrhosis, chronic liver disease, and HCC [217]. In a large prospective study of patients with chronic hepatitis C and advanced liver disease who had failed to achieve a sustained virological response with peginterferon plus ribavirin treatment, Freedman et al. found an inverse association between coffee intake and liver disease progression. Drinkers of $\geq$3 cups of coffee/day had a 53% lower risk of liver disease progression than non-coffee drinkers. In contrast, no association was observed for the consumption of black or green tea [217].

Yesil et al. identified two cross-sectional studies and three case-control studies investigating the association between coffee consumption and the risk of NAFLD. All of these studies suggest a reduced risk of NAFLD associated with coffee drinking [156].

4.2.2 Clinical Studies

Wadhawan and Anand reviewed the literature for an association between coffee and liver disease and provided clinical evidence of the benefit of coffee consumption in hepatitis B and C, as well as in NAFLD [218]. Coffee consumption was associated with an improvement in liver enzymes such as ALT and AST, especially in individuals at risk for liver disease. Coffee intake of $\geq$2 cups/day in patients with pre-existing liver disease was shown to be associated with lower incidence of fibrosis and cirrhosis, lower HCC rates, and decreased mortality [218]. Similarly, Wijarnpreecha et al. found a significantly decreased risk of NAFLD among coffee drinkers and significantly decreased risk of liver fibrosis among patients with NAFLD who drank coffee on a regular basis [219].

A study to examine whether or not coffee consumption was associated with lower serum aminotransferases in the general Korean population and in those at high risk

for hepatic disease showed that the proportions of individuals with elevated AST were 32.5%, 33.1%, and 26.7% in subjects who drank coffee <1, 1, and ≥2 times/day, respectively. The ORs for elevated ALT and AST were significantly lower in subjects who drank ≥2 cups of coffee/day than in those who drank <1 cup/day. Thus, higher coffee consumption may be associated with lower risk of liver disease [220].

4.2.3 Laboratory Studies and Mechanism of Action

Twenty-five male 129/Sv mice were administered CGA, and an α-naphthylisothiocyanate (ANI) challenge was performed at 75 mg/kg on day 4 after treatment. CGA almost totally attenuated the resulting drug-induced liver damage and cholestasis compared with the untreated group. A dose of 50 mg/kg of CGA significantly prevented drug-induced changes in serum levels of ALT, alkaline phosphatases, total bile acid, direct bilirubin, indirect bilirubin (5.3-, 6.3-, 18.8-, 158-, 41.4-fold, respectively), and AST (4.6-fold). Expression of the altered bile acid metabolism and transport-related genes was normalized by treatment with CGA. The expression of *IL-6*, *TNF-α*, and suppressor of cytokine signaling 3 was found to be significantly decreased (1.2-fold, 11.0-fold, and 4.4-fold, respectively) in the CGA/ANI group. Western blotting revealed that CGA inhibited the activation and expression of signal transducer and activator of transcription 3 and NF-κB. These data suggest that CGA inhibits both ANI-induced intrahepatic cholestasis and liver injury by down-regulating STAT3 and NF-κB signaling [221] (see Fig. 3).

Feng et al. examined the impact of the Chinese herbal medicine Qushi Huayu Decoction (QHD) and its active components (geniposide and CGA) on the NAFLD liver transcriptome and gut microbiota using NAFLD rats. Increased expression of genes required for glutathione production and decreased expression of genes required for lipid synthesis were observed in NAFLD livers treated with QHD and CGA. CGA treatment decreased serum lipopolysaccharide, which could be explained by reduced mucosal damage in the colon of CGA-treated rats. In addition, the results suggested an increased abundance of Treg cell-inducing bacteria, which stimulated Treg cell activity in the CGA-treated colon, which in turn downregulated inflammatory signals, improved gut barrier function, and consequently reduced hepatic exposure to microbial products. These findings suggested that QHD simultaneously enhanced the hepatic antioxidative mechanism, decreased hepatic lipid synthesis, and promoted Treg cell-inducing microbiota in the gut [222].

Watanebe et al. found that coffee intake did not affect obesity or hyperlipidemia in TSOD mice, but that it did cause various degrees of improvement in pancreatic β-cell damage and steatohepatitis with liver carcinogenesis. Most of the effects appeared to be caused by a synergistic effect between caffeine and other components such as polyphenols. The anti-fibrotic effects of coffee were likely attributable to the polyphenols rather than to caffeine [164].

Arauz et al. examined the hepatoprotective properties of coffee and caffeine in a model of chronic bile duct ligation in Wistar rats. Western blot assays showed decreased protein expression levels of transforming growth factor-β1, connective tissue growth factor, α-smooth muscle actin, and collagen 1 in the coffee- and

caffeine-treated ligation groups compared with untreated rats. Similarly, coffee decreased the mRNA levels of these proteins. The results indicated that coffee prevented bile duct ligation-induced liver cirrhosis by attenuating the oxidant processes, blocking hepatic stellate cell activation, and downregulating the main profibrotic molecules involved in extracellular matrix deposition [223].

The beneficial effects of coffee in liver disease have also been supported by a recent review by Salomone et al. [98]. The authors described that in experimental models of fibrosis, caffeine inhibited hepatic stellate cell activation by blocking adenosine receptors and may favorably impact angiogenesis and hepatic hemodynamics, while CGA suppressed liver fibrogenesis and carcinogenesis by reducing oxidative stress and counteracting steatogenesis through the modulation of glucose and lipid homeostasis in the liver.

In contrast, Aoyagi et al. reported that CGA did not contribute to the coffee's suppressive effect on *PPAR-γ* gene expression. They observed that coffee reduced the accumulation of lipids during the adipocytic differentiation of 3T3-L1 cells and that 5% coffee decreased the accumulation of lipids by about 50% compared with control. Coffee inhibited the expression of PPAR-γ, a transcription factor that controls the differentiation of adipocytes, and reduced the expression of other differentiation marker genes *aP2*, adiponectin, *C/EBP-α*, *GLUT-4*, and lipoprotein lipase (*LPL*) during adipocyte differentiation (see Fig. 2). Major bioactive constituents of coffee extracts such as CGA, caffeine, trigonelline, and caffeic acid showed no effect on *PPAR-γ* gene expression. The inhibitory activity of coffee was found to be produced by the roasting of coffee beans [203].

5 Conclusions

Multiple human studies have shown that both green tea and coffee exert beneficial effects in human diseases, and most animal and cell-based experiments support these outcomes. Clearly, GTCs and coffee CGAs are implicated in these activities. Several mechanisms of action have been proposed, among which the involvement of ROS appears to be the most prominent. Two conflicting actions have been proposed: GTCs and CGAs can either scavenge ROS or promote its generation. The scavenging of ROS results in the inhibition of NF-κB activity, leading to the modulation of cytokines and apoptosis-related factors to yield various favorable outcomes such as anti-inflammatory effects and the induction of apoptosis in cancer cells (Fig. 3) [4, 5]. It is also possible that the generation of ROS results in the activation of AMPK, leading to the modulation of various enzymes and factors with roles in health promotion (Fig. 2) [4, 107]. In addition, AMPK activation has been suggested to downregulate NF-κB (Fig. 2), leading to modulation shown in Fig. 3 [224, 225].

At present, no explanation is available for these dual actions of GTCs and CGAs, although some conditions may direct them to act as either prooxidant or antioxidant agents. These conditions include their concentrations, the presence of cations such as Cu^{+} and Fe^{++}, cellular concentrations of antioxidants and oxidoreductive enzymes, and the cellular redox state [4, 107]. These issues remain to be resolved in future studies.

Furthermore, conflicting results in human studies have also been reported. The reasons for these inconsistencies include incomplete adjustment for confounding factors and lack of necessary questions in questionnaires, such as the temperature of tea or coffee, intestinal microbiota, and genetic factors. Nevertheless, we may expect the overall favorable effects of green tea and coffee consumption to promote healthy longevity in view of the growing of evidence presented in this chapter.

References

1. Yang CS, Wang X, Lu G, Picinich SC (2009) Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. Nat Rev Cancer 9:429–439. https://doi.org/10.1038/nrc2641
2. Suzuki Y, Miyoshi N, Isemura M (2012) Health-promoting effects of green tea. Proc Jpn Acad Ser B Phys Biol Sci 88:88–101
3. Khan N, Mukhtar H (2013) Tea and health: studies in humans. Curr Pharm Des 19:6141–6147
4. Hayakawa S, Saito K, Miyoshi N, Ohishi T, Oishi Y, Miyoshi M, Nakamura Y (2016) Anti-cancer effects of green tea by either anti- or pro- oxidative mechanisms. Asian Pac J Cancer Prev 17:1649–1654
5. Ohishi T, Goto S, Monira P, Isemura M, Nakamura Y (2016) Anti-inflammatory action of green tea. Antiinflamm Antiallergy Agents Med Chem 15:74–90. https://doi.org/10.2174/1871523015666160915154443
6. Yang CS, Wang H (2016) Cancer preventive activities of tea catechins. Molecules 21. https://doi.org/10.3390/molecules21121679
7. Yang CS, Zhang J, Zhang L, Huang J, Wang Y (2016) Mechanisms of body weight reduction and metabolic syndrome alleviation by tea. Mol Nutr Food Res 60:160–174. https://doi.org/10.1002/mnfr.201500428
8. Cavalli L, Tavani A (2016) Coffee consumption and its impact on health. In: Wilson T, Temple NJ (eds) Beverage impacts on health and nutrition, 2nd edn. Springer, Cham. https://doi.org/10.1007/978-3-319-23672-8
9. Takeshi T, Yosuke M, Isao K (2013) Biochemical and physicochemical characteristics of green tea polyphenols. In: Juneja LR, Kapoor MP, Okubo T, Rao T (eds) Green tea polyphenols: nutraceuticals of modern life. CRC Press, Boca Raton
10. Cano-Marquina A, Tarin JJ, Cano A (2013) The impact of coffee on health. Maturitas 75:7–21. https://doi.org/10.1016/j.maturitas.2013.02.002
11. Meinhart AD, Damin FM, Caldeirao L, da Silveira TFF, Filho JT, Godoy HT (2017) Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. Food Res Int 99:522–530. https://doi.org/10.1016/j.foodres.2017.06.017
12. Temple JL, Bernard C, Lipshultz SE, Czachor JD, Westphal JA, Mestre MA (2017) The safety of ingested caffeine: a comprehensive review. Front Psych 8:80. https://doi.org/10.3389/fpsyt.2017.00080
13. Yuan JM, Sun C, Butler LM (2011) Tea and cancer prevention: epidemiological studies. Pharmacol Res 64:123–135. https://doi.org/10.1016/j.phrs.2011.03.002
14. Yuan JM (2013) Cancer prevention by green tea: evidence from epidemiologic studies. Am J Clin Nutr 98:1676S–1681S. https://doi.org/10.3945/ajcn.113.058271
15. Bamia C, Lagiou P, Jenab M et al (2015) Coffee, tea and decaffeinated coffee in relation to hepatocellular carcinoma in a European population: multicentre, prospective cohort study. Int J Cancer 136:1899–1908. https://doi.org/10.1002/ijc.29214
16. Zhang YF, Xu Q, Lu J et al (2015) Tea consumption and the incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Eur J Cancer Prev 24:353–362. https://doi.org/10.1097/CEJ.0000000000000094

17. Wang Y, Duan H, Yang H (2015) A case-control study of stomach cancer in relation to Camellia sinensis in China. Surg Oncol 24:67–70. https://doi.org/10.1016/j.suronc.2015.02.002
18. Lee PMY, Ng CF, Liu ZM et al (2017) Reduced prostate cancer risk with green tea and epigallocatechin 3-gallate intake among Hong Kong Chinese men. Prostate Cancer Prostatic Dis 20:318–322. https://doi.org/10.1038/pcan.2017.18
19. Sawada N (2017) Risk and preventive factors for prostate cancer in Japan: The Japan Public Health Center-based prospective (JPHC) study. J Epidemiol 27:2–7. https://doi.org/10.1016/j.je.2016.09.001
20. Hoang VD, Lee AH, Pham NM, Xu D, Binns CW (2016) Habitual tea consumption reduces prostate cancer risk in Vietnamese men: a Case-Control Study. Asian Pac J Cancer Prev 17:4939–4944. https://doi.org/10.22034/APJCP.2016.17.11.4939
21. Guo Y, Zhi F, Chen P et al (2017) Green tea and the risk of prostate cancer: a systematic review and meta-analysis. Medicine (Baltimore) 96:e6426. https://doi.org/10.1097/MD.0000000000006426
22. Xiong J, Lin J, Wang A et al (2017) Tea consumption and the risk of biliary tract cancer: a systematic review and dose-response meta-analysis of observational studies. Oncotarget 8:39649–39657. https://doi.org/10.18632/oncotarget.16963
23. Chen Y, Wu Y, Du M et al (2017) An inverse association between tea consumption and colorectal cancer risk. Oncotarget 8:37367–37376. https://doi.org/10.18632/oncotarget.16959
24. Zhan X, Wang J, Pan S, Lu C (2017) Tea consumption and the risk of ovarian cancer: a meta-analysis of epidemiological studies. Oncotarget 8:37796–37806. https://doi.org/10.18632/oncotarget.16890
25. Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A (2006) Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. Cancer Res 66:1234–1240. https://doi.org/10.1158/0008-5472.CAN-05-1145
26. Jacob SA, Khan TM, Lee LH (2017) The effect of green tea consumption on prostate cancer risk and progression: a systematic review. Nutr Cancer 69:353–364. https://doi.org/10.1080/01635581.2017.1285037
27. Lassed S, Deus CM, Djebbari R et al (2017) Protective effect of green tea (Camellia sinensis (L.) Kuntze) against prostate cancer: from in vitro data to Algerian patients. Evid Based Complement Alternat Med 2017:1691568. https://doi.org/10.1155/2017/1691568
28. D'Arena G, Simeon V, De Martino L et al (2013) Regulatory T-cell modulation by green tea in chronic lymphocytic leukemia. Int J Immunopathol Pharmacol 26:117–125. https://doi.org/10.1177/039463201302600111
29. Xue KS, Tang L, Cai Q, Shen Y, Su J, Wang JS (2015) Mitigation of fumonisin biomarkers by green tea polyphenols in a high-risk population of hepatocellular carcinoma. Sci Rep 5:17545. https://doi.org/10.1038/srep17545
30. Garcia FA, Cornelison T, Nuno T et al (2014) Results of a phase II randomized, double-blind, placebo-controlled trial of Polyphenon E in women with persistent high-risk HPV infection and low-grade cervical intraepithelial neoplasia. Gynecol Oncol 132:377–382. https://doi.org/10.1016/j.ygyno.2013.12.034
31. Gee JR, Saltzstein DR, Kim K et al (2017) A phase II randomized, double-blind, presurgical trial of polyphenon E in bladder cancer patients to evaluate pharmacodynamics and bladder tissue biomarkers. Cancer Prev Res 10:298–307. https://doi.org/10.1158/1940-6207.CAPR-16-0167
32. Shin CM, Lee DH, Seo AY et al (2017) Green tea extracts for the prevention of metachronous colorectal polyps among patients who underwent endoscopic removal of colorectal adenomas: a randomized clinical trial. Clin Nutr. https://doi.org/10.1016/j.clnu.2017.01.014
33. Je Y, Park T (2015) Tea consumption and endometrial cancer risk: meta-analysis of prospective cohort studies. Nutr Cancer 67:825–830. https://doi.org/10.1080/01635581.2015.1040521

34. Weng H, Zeng XT, Li S, Kwong JS, Liu TZ, Wang XH (2016) Tea consumption and risk of bladder cancer: a dose-response meta-analysis. Front Physiol 7:693. https://doi.org/10.3389/fphys.2016.00693
35. Gontero P, Marra G, Soria F et al (2015) A randomized double-blind placebo controlled phase I-II study on clinical and molecular effects of dietary supplements in men with precancerous prostatic lesions. Chemoprevention or "chemopromotion"? Prostate 75:1177–1186. https://doi.org/10.1002/pros.22999
36. Azimi S, Mansouri Z, Bakhtiari S, Tennant M, Kruger E, Rajabibazl M, Daraei A (2017) Does green tea consumption improve the salivary antioxidant status of smokers? Arch Oral Biol 78:1–5. https://doi.org/10.1016/j.archoralbio.2017.02.002
37. Rob F, Juzlova K, Secnikova Z, Jirakova A, Hercogova J (2017) Successful treatment with 10% sinecatechins ointment for recurrent anogenital warts in an eleven-year-old child. Pediatr Infect Dis J 36:235–236. https://doi.org/10.1097/INF.0000000000001397
38. Shimizu M, Adachi S, Masuda M, Kozawa O, Moriwaki H (2011) Cancer chemoprevention with green tea catechins by targeting receptor tyrosine kinases. Mol Nutr Food Res 55:832–843. https://doi.org/10.1002/mnfr.201000622
39. Shirakami Y, Sakai H, Kochi T, Seishima M, Shimizu M (2016) Catechins and its role in chronic diseases. Adv Exp Med Biol 929:67–90. https://doi.org/10.1007/978-3-319-41342-6_4
40. Hibasami H, Achiwa Y, Fujikawa T, Komiya T (1996) Induction of programmed cell death (apoptosis) in human lymphoid leukemia cells by catechin compounds. Anticancer Res 16:1943–1946
41. Hayakawa S, Saeki K, Sazuka M et al (2001) Apoptosis induction by epigallocatechin gallate involves its binding to Fas. Biochem Biophys Res Commun 285:1102–1106. https://doi.org/10.1006/bbrc.2001.5293
42. Tachibana H (2011) Green tea polyphenol sensing. Proc Jpn Acad Ser B Phys Biol Sci 87:66–80
43. Matsuo T, Miyata Y, Asai A, Sagara Y, Furusato B, Fukuoka J, Sakai H (2017) Green tea polyphenol induces changes in cancer-related factors in an animal model of bladder cancer. PLoS One 12:e0171091. https://doi.org/10.1371/journal.pone.0171091
44. Liu SM, SY O, Huang HH (2017) Green tea polyphenols induce cell death in breast cancer MCF-7 cells through induction of cell cycle arrest and mitochondrial-mediated apoptosis. J Zhejiang Univ Sci B 18:89–98. https://doi.org/10.1631/jzus.B1600022
45. Hao X, Xiao H, Ju J, Lee MJ, Lambert JD, Yang CS (2017) Green tea polyphenols inhibit colorectal tumorigenesis in azoxymethane-treated F344 rats. Nutr Cancer 69:623–631. https://doi.org/10.1080/01635581.2017.1295088
46. Posadino AM, Phu HT, Cossu A et al (2017) Oxidative stress-induced Akt downregulation mediates green tea toxicity towards prostate cancer cells. Toxicol In Vitro 42:255–262. https://doi.org/10.1016/j.tiv.2017.05.005
47. Moradzadeh M, Hosseini A, Erfanian S, Rezaei H (2017) Epigallocatechin-3-gallate promotes apoptosis in human breast cancer T47D cells through down-regulation of PI3K/AKT and Telomerase. Pharmacol Rep 69:924–928. https://doi.org/10.1016/j.pharep.2017.04.008
48. Chen Y, Wang XQ, Zhang Q et al (2017) (−)-Epigallocatechin-3-gallate inhibits colorectal cancer stem cells by suppressing Wnt/beta-catenin pathway. Forum Nutr 9. https://doi.org/10.3390/nu9060572
49. Shin YS, Kang SU, Park JK et al (2016) Anti-cancer effect of (−)-epigallocatechin-3-gallate (EGCG) in head and neck cancer through repression of transactivation and enhanced degradation of beta-catenin. Phytomedicine 23:1344–1355. https://doi.org/10.1016/j.phymed.2016.07.005
50. Harati K, Behr B, Wallner C et al (2017) Antiproliferative activity of epigallocatechin3gallate and silibinin on soft tissue sarcoma cells. Mol Med Rep 15:103–110. https://doi.org/10.3892/mmr.2016.5969

51. Cornwall S, Cull G, Joske D, Ghassemifar R (2016) Green tea polyphenol "epigallocatechin-3-gallate", differentially induces apoptosis in CLL B-and T-Cells but not in healthy B-and T-Cells in a dose dependant manner. Leuk Res 51:56–61. https://doi.org/10.1016/j.leukres.2016.10.011
52. Kwak TW, Park SB, Kim HJ, Jeong YI, Kang DH (2017) Anticancer activities of epigallocatechin-3-gallate against cholangiocarcinoma cells. Onco Targets Ther 10:137–144. https://doi.org/10.2147/OTT.S112364
53. Luo KW, Wei C, Lung WY, Wei XY, Cheng BH, Cai ZM, Huang WR (2017) EGCG inhibited bladder cancer SW780 cell proliferation and migration both in vitro and in vivo via down-regulation of NF-kappaB and MMP-9. J Nutr Biochem 41:56–64. https://doi.org/10.1016/j.jnutbio.2016.12.004
54. Okada N, Tanabe H, Tazoe H, Ishigami Y, Fukutomi R, Yasui K, Isemura M (2009) Differentiation-associated alteration in sensitivity to apoptosis induced by (−)-epigallocatechin-3-O-gallate in HL-60 cells. Biomed Res 30:201–206
55. Ward RE, Benninghoff AD, Healy BJ, Li M, Vagu B, Hintze KJ (2017) Consumption of the total Western diet differentially affects the response to green tea in rodent models of chronic disease compared to the AIN93G diet. Mol Nutr Food Res 61. https://doi.org/10.1002/mnfr.201600720
56. Mbuthia KS, Mireji PO, Ngure RM, Stomeo F, Kyallo M, Muoki C, Wachira FN (2017) Tea (Camellia sinensis) infusions ameliorate cancer in 4TI metastatic breast cancer model. BMC Complement Altern Med 17:202. https://doi.org/10.1186/s12906-017-1683-6
57. Spano D, Heck C, De Antonellis P, Christofori G, Zollo M (2012) Molecular networks that regulate cancer metastasis. Semin Cancer Biol 22:234–249. https://doi.org/10.1016/j.semcancer.2012.03.006
58. Taniguchi S, Fujiki H, Kobayashi H, Go H, Miyado K, Sadano H, Shimokawa R (1992) Effect of (−)-epigallocatechin gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines. Cancer Lett 65:51–54
59. Sazuka M, Murakami S, Isemura M, Satoh K, Nukiwa T (1995) Inhibitory effects of green tea infusion on in vitro invasion and in vivo metastasis of mouse lung carcinoma cells. Cancer Lett 98:27–31
60. Gupta S, Hastak K, Ahmad N, Lewin JS, Mukhtar H (2001) Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. Proc Natl Acad Sci U S A 98:10350–10355. https://doi.org/10.1073/pnas.171326098
61. Kim SJ, Amankwah E, Connors S et al (2014) Safety and chemopreventive effect of Polyphenon E in preventing early and metastatic progression of prostate cancer in TRAMP mice. Cancer Prev Res 7:435–444. https://doi.org/10.1158/1940-6207.CAPR-13-0427-T
62. Sazuka M, Imazawa H, Shoji Y, Mita T, Hara Y, Isemura M (1997) Inhibition of collagenases from mouse lung carcinoma cells by green tea catechins and black tea theaflavins. Biosci Biotechnol Biochem 61:1504–1506
63. Shao J, Meng Q, Li Y (2016) Theaflavins suppress tumor growth and metastasis via the blockage of the STAT3 pathway in hepatocellular carcinoma. Onco Targets Ther 9:4265–4275. https://doi.org/10.2147/OTT.S102858
64. Rashidi B, Malekzadeh M, Goodarzi M, Masoudifar A, Mirzaei H (2017) Green tea and its anti-angiogenesis effects. Biomed Pharmacother 89:949–956. https://doi.org/10.1016/j.biopha.2017.01.161
65. Wierzejska R (2015) Coffee consumption vs. cancer risk – a review of scientific data. Rocz Panstw Zakl Hig 66:293–298
66. MC Y, Mack TM, Hanisch R, Cicioni C, Henderson BE (1986) Cigarette smoking, obesity, diuretic use, and coffee consumption as risk factors for renal cell carcinoma. J Natl Cancer Inst 77:351–356
67. Nilsson LM, Johansson I, Lenner P, Lindahl B, Van Guelpen B (2010) Consumption of filtered and boiled coffee and the risk of incident cancer: a prospective cohort study. Cancer Causes Control 21:1533–1544. https://doi.org/10.1007/s10552-010-9582-x
68. Setiawan VW, Wilkens LR, Lu SC, Hernandez BY, Le Marchand L, Henderson BE (2015) Association of coffee intake with reduced incidence of liver cancer and death from chronic

liver disease in the US multiethnic cohort. Gastroenterology 148:118–125; quiz e115. https://doi.org/10.1053/j.gastro.2014.10.005
69. Budhathoki S, Iwasaki M, Yamaji T, Sasazuki S, Tsugane S (2015) Coffee intake and the risk of colorectal adenoma: the colorectal adenoma study in Tokyo. Int J Cancer 137:463–470. https://doi.org/10.1002/ijc.29390
70. Gosvig CF, Kjaer SK, Blaakaer J, Hogdall E, Hogdall C, Jensen A (2015) Coffee, tea, and caffeine consumption and risk of epithelial ovarian cancer and borderline ovarian tumors: results from a Danish case-control study. Acta Oncol 54:1144–1151. https://doi.org/10.3109/0284186X.2014.1001035
71. Bhoo-Pathy N, Peeters PH, Uiterwaal CS et al (2015) Coffee and tea consumption and risk of pre- and postmenopausal breast cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study. Breast Can Res 17:15. https://doi.org/10.1186/s13058-015-0521-3
72. Oh JK, Sandin S, Strom P, Lof M, Adami HO, Weiderpass E (2015) Prospective study of breast cancer in relation to coffee, tea and caffeine in Sweden. Int J Cancer 137:1979–1989. https://doi.org/10.1002/ijc.29569
73. Guercio BJ, Sato K, Niedzwiecki D et al (2015) Coffee intake, recurrence, and mortality in stage III colon cancer: results from CALGB 89803 (Alliance). J Clin Oncol 33:3598–3607. https://doi.org/10.1200/JCO.2015.61.5062
74. Nakamura T, Ishikawa H, Mutoh M, Wakabayashi K, Kawano A, Sakai T, Matsuura N (2016) Coffee prevents proximal colorectal adenomas in Japanese men: a prospective cohort study. Eur J Cancer Prev 25:388–394. https://doi.org/10.1097/CEJ.0000000000000203
75. Zhou Q, Luo ML, Li H, Li M, Zhou JG (2015) Coffee consumption and risk of endometrial cancer: a dose-response meta-analysis of prospective cohort studies. Sci Rep 5:13410. https://doi.org/10.1038/srep13410
76. Yew YW, Lai YC, Schwartz RA (2016) Coffee consumption and melanoma: a systematic review and meta-analysis of observational studies. Am J Clin Dermatol 17:113–123. https://doi.org/10.1007/s40257-015-0165-1
77. Bravi F, Tavani A, Bosetti C, Boffetta P, La Vecchia C (2017) Coffee and the risk of hepatocellular carcinoma and chronic liver disease: a systematic review and meta-analysis of prospective studies. Eur J Cancer Prev 26:368–377. https://doi.org/10.1097/CEJ.0000000000000252
78. Caini S, Cattaruzza S, Bendinelli B et al (2017) Coffee, tea and caffeine intake and the risk of non-melanoma skin cancer: a review of the literature and meta-analysis. Eur J Nutr 56:1–12. https://doi.org/10.1007/s00394-016-1253-6
79. Xie Y, Huang S, He T, Su Y (2016) Coffee consumption and risk of gastric cancer: an updated meta-analysis. Asia Pac J Clin Nutr 25:578–588. https://doi.org/10.6133/apjcn.092015.07
80. Vaseghi G, Haghjoo-Javanmard S, Naderi J, Eshraghi A, Mahdavi M, Mansourian M (2016) Coffee consumption and risk of nonmelanoma skin cancer: a dose-response meta-analysis. Eur J Cancer Prev. https://doi.org/10.1097/CEJ.0000000000000322
81. Kennedy OJ, Roderick P, Buchanan R, Fallowfield JA, Hayes PC, Parkes J (2017) Coffee, including caffeinated and decaffeinated coffee, and the risk of hepatocellular carcinoma: a systematic review and dose-response meta-analysis. BMJ Open 7:e013739. https://doi.org/10.1136/bmjopen-2016-013739
82. Yang TO, Crowe F, Cairns BJ, Reeves GK, Beral V (2015) Tea and coffee and risk of endometrial cancer: cohort study and meta-analysis. Am J Clin Nutr 101:570–578. https://doi.org/10.3945/ajcn.113.081836
83. Chen J, Long S (2014) Tea and coffee consumption and risk of laryngeal cancer: a systematic review meta-analysis. PLoS One 9:e112006. https://doi.org/10.1371/journal.pone.0112006
84. Liu H, Hua Y, Zheng X, Shen Z, Luo H, Tao X, Wang Z (2015) Effect of coffee consumption on the risk of gastric cancer: a systematic review and meta-analysis of prospective cohort studies. PLoS One 10:e0128501. https://doi.org/10.1371/journal.pone.0128501
85. Xie Y, Qin J, Nan G, Huang S, Wang Z, Su Y (2016) Coffee consumption and the risk of lung cancer: an updated meta-analysis of epidemiological studies. Eur J Clin Nutr 70:199–206. https://doi.org/10.1038/ejcn.2015.96

86. Parodi S, Merlo DF, Stagnaro E, Working Group for the Epidemiology of Hematolymphopoietic Malignancies in I (2017) Coffee and tea consumption and risk of leukaemia in an adult population: a reanalysis of the Italian multicentre case-control study. Cancer Epidemiol 47:81–87. https://doi.org/10.1016/j.canep.2017.01.005
87. Thomopoulos TP, Ntouvelis E, Diamantaras AA et al (2015) Maternal and childhood consumption of coffee, tea and cola beverages in association with childhood leukemia: a meta-analysis. Cancer Epidemiol 39:1047–1059. https://doi.org/10.1016/j.canep.2015.08.009
88. Turati F, Bosetti C, Polesel J et al (2015) Coffee, tea, cola, and bladder cancer risk: dose and time relationships. Urology 86:1179–1184. https://doi.org/10.1016/j.urology.2015.09.017
89. Li L, Gan Y, Wu C, Qu X, Sun G, Lu Z (2015) Coffee consumption and the risk of gastric cancer: a meta-analysis of prospective cohort studies. BMC Cancer 15:733. https://doi.org/10.1186/s12885-015-1758-z
90. Makiuchi T, Sobue T, Kitamura T et al (2016) Association between green tea/coffee consumption and biliary tract cancer: a population-based cohort study in Japan. Cancer Sci 107:76–83. https://doi.org/10.1111/cas.12843
91. Akter S, Kashino I, Mizoue T et al (2016) Coffee drinking and colorectal cancer risk: an evaluation based on a systematic review and meta-analysis among the Japanese population. Jpn J Clin Oncol 46:781–787. https://doi.org/10.1093/jjco/hyw059
92. Miyoshi N, Pervin M, Suzuki T, Unno K, Isemura M, Nakamura Y (2015) Green tea catechins for well-being and therapy: prospects and opportunities. Botanics 5:85–96. https://doi.org/10.2147/btat.s91784
93. Grubben MJ, Van Den Braak CC, Broekhuizen R et al (2000) The effect of unfiltered coffee on potential biomarkers for colonic cancer risk in healthy volunteers: a randomized trial. Aliment Pharmacol Ther 14:1181–1190
94. Misik M, Hoelzl C, Wagner KH et al (2010) Impact of paper filtered coffee on oxidative DNA-damage: results of a clinical trial. Mutat Res 692:42–48. https://doi.org/10.1016/j.mrfmmm.2010.08.003
95. Steinkellner H, Hoelzl C, Uhl M et al (2005) Coffee consumption induces GSTP in plasma and protects lymphocytes against (+/−)-anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide induced DNA-damage: results of controlled human intervention trials. Mutat Res 591:264–275. https://doi.org/10.1016/j.mrfmmm.2005.04.016
96. Shaposhnikov S, Hatzold T, Yamani NE et al (2016) Coffee and oxidative stress: a human intervention study. Eur J Nutr. https://doi.org/10.1007/s00394-016-1336-4
97. Deka SJ, Gorai S, Manna D, Trivedi V (2017) Evidence of PKC Binding and Translocation to explain the anticancer mechanism of chlorogenic acid in breast cancer cells. Curr Mol Med. https://doi.org/10.2174/1566524017666170209160619
98. Salomone F, Galvano F, Li Volti G (2017) Molecular basesunderlying the hepatoprotective effects of coffee. Forum Nutr 9. https://doi.org/10.3390/nu9010085
99. Xue N, Zhou Q, Ji M et al (2017) Chlorogenic acid inhibits glioblastoma growth through repolarizating macrophage from M2 to M1 phenotype. Sci Rep 7:39011. https://doi.org/10.1038/srep39011
100. Xu R, Kang Q, Ren J, Li Z, Xu X (2013) Antitumor molecular mechanism of chlorogenic acid on inducting genes GSK-3 beta and APC and inhibiting gene beta -catenin. J Anal Methods Chem 2013:951319. https://doi.org/10.1155/2013/951319
101. Ojha D, Mukherjee H, Mondal S et al (2014) Anti-inflammatory activity of Odina wodier Roxb, an Indian folk remedy, through inhibition of toll-like receptor 4 signaling pathway. PLoS One 9:e104939. https://doi.org/10.1371/journal.pone.0104939
102. Choi DW, Lim MS, Lee JW et al (2015) The cytotoxicity of kahweol in HT-29 human colorectal cancer cells is mediated by apoptosis and suppression of heat shock protein 70 expression. Biomol Ther (Seoul) 23:128–133. https://doi.org/10.4062/biomolther.2014.133
103. Weng CJ, Yen GC (2012) Chemopreventive effects of dietary phytochemicals against cancer invasion and metastasis: phenolic acids, monophenol, polyphenol, and their derivatives. Cancer Treat Rev 38:76–87. https://doi.org/10.1016/j.ctrv.2011.03.001

104. Hashibe M, Galeone C, Buys SS, Gren L, Boffetta P, Zhang ZF, La Vecchia C (2015) Coffee, tea, caffeine intake, and the risk of cancer in the PLCO cohort. Br J Cancer 113:809–816. https://doi.org/10.1038/bjc.2015.276
105. Ogawa T, Sawada N, Iwasaki M et al (2016) Coffee and green tea consumption in relation to brain tumor risk in a Japanese population. Int J Cancer 139:2714–2721. https://doi.org/10.1002/ijc.30405
106. Inoue M, Kurahashi N, Iwasaki M et al (2009) Effect of coffee and green tea consumption on the risk of liver cancer: cohort analysis by hepatitis virus infection status. Cancer Epidemiol Biomark Prev 18:1746–1753. https://doi.org/10.1158/1055-9965.EPI-08-0923
107. Suzuki T, Pervin M, Goto S, Isemura M, Nakamura Y (2016) Beneficial effects of tea and the green tea catechin epigallocatechin-3-gallate on obesity. Molecules 21. https://doi.org/10.3390/molecules21101305
108. Grosso G, Marventano S, Galvano F, Pajak A, Mistretta A (2014) Factors associated with metabolic syndrome in a mediterranean population: role of caffeinated beverages. J Epidemiol 24:327–333
109. Grosso G, Stepaniak U, Micek A, Topor-Madry R, Pikhart H, Szafraniec K, Pajak A (2015) Association of daily coffee and tea consumption and metabolic syndrome: results from the Polish arm of the HAPIEE study. Eur J Nutr 54:1129–1137. https://doi.org/10.1007/s00394-014-0789-6
110. Tsubono Y, Tsugane S (1997) Green tea intake in relation to serum lipid levels in Middle-aged Japanese men and women. Ann Epidemiol 7:280–284
111. Hino A, Adachi H, Enomoto M et al (2007) Habitual coffee but not green tea consumption is inversely associated with metabolic syndrome: an epidemiological study in a general Japanese population. Diabetes Res Clin Pract 76:383–389. https://doi.org/10.1016/j.diabres.2006.09.033
112. Takami H, Nakamoto M, Uemura H et al (2013) Inverse correlation between coffee consumption and prevalence of metabolic syndrome: baseline survey of the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study in Tokushima, Japan. J Epidemiol 23:12–20
113. Legeay S, Rodier M, Fillon L, Faure S, Clere N (2015) Epigallocatechin gallate: a review of its beneficial properties to prevent metabolic syndrome. Forum Nutr 7:5443–5468. https://doi.org/10.3390/nu7075230
114. Chen IJ, Liu CY, Chiu JP, Hsu CH (2016) Therapeutic effect of high-dose green tea extract on weight reduction: a randomized, double-blind, placebo-controlled clinical trial. Clin Nutr 35:592–599. https://doi.org/10.1016/j.clnu.2015.05.003
115. Amiot MJ, Riva C, Vinet A (2016) Effects of dietary polyphenols on metabolic syndrome features in humans: a systematic review. Obes Rev 17:573–586. https://doi.org/10.1111/obr.12409
116. Vieira Senger AE, Schwanke CH, Gomes I, Valle Gottlieb MG (2012) Effect of green tea (Camellia sinensis) consumption on the components of metabolic syndrome in elderly. J Nutr Health Aging 16:738–742. https://doi.org/10.1007/s12603-012-0081-5
117. Razavi BM, Lookian F, Hosseinzadeh H (2017) Protective effects of green tea on olanzapine-induced-metabolic syndrome in rats. Biomed Pharmacother 92:726–731. https://doi.org/10.1016/j.biopha.2017.05.113
118. Chen J, Song H (2016) Protective potential of epigallocatechin-3-gallate against benign prostatic hyperplasia in metabolic syndrome rats. Environ Toxicol Pharmacol 45:315–320. https://doi.org/10.1016/j.etap.2016.06.015
119. Tian C, Ye X, Zhang R et al (2013) Green tea polyphenols reduced fat deposits in high fat-fed rats via erk1/2-PPARgamma-adiponectin pathway. PLoS One 8:e53796. https://doi.org/10.1371/journal.pone.0053796
120. Collins QF, Liu HY, Pi J, Liu Z, Quon MJ, Cao W (2007) Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5′-AMP-activated protein kinase. J Biol Chem 282:30143–30149. https://doi.org/10.1074/jbc.M702390200
121. Vernarelli JA, Lambert JD (2013) Tea consumption is inversely associated with weight status and other markers for metabolic syndrome in US adults. Eur J Nutr 52:1039–1048. https://doi.org/10.1007/s00394-012-0410-9

122. Hursel R, Viechtbauer W, Westerterp-Plantenga MS (2009) The effects of green tea on weight loss and weight maintenance: a meta-analysis. Int J Obes 33:956–961. https://doi.org/10.1038/ijo.2009.135
123. Nagao T, Hase T, Tokimitsu I (2007) A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. Obesity 15:1473–1483. https://doi.org/10.1038/oby.2007.176
124. Ferreira MA, Silva DM, de Morais AC Jr, Mota JF, Botelho PB (2016) Therapeutic potential of green tea on risk factors for type 2 diabetes in obese adults – a review. Obes Rev 17:1316–1328. https://doi.org/10.1111/obr.12452
125. Suliburska J, Bogdanski P, Szulinska M, Stepien M, Pupek-Musialik D, Jablecka A (2012) Effects of green tea supplementation on elements, total antioxidants, lipids, and glucose values in the serum of obese patients. Biol Trace Elem Res 149:315–322. https://doi.org/10.1007/s12011-012-9448-z
126. Igarashi Y, Obara T, Ishikuro M et al (2017) Randomized controlled trial of the effects of consumption of 'Yabukita' or 'Benifuuki' encapsulated tea-powder on low-density lipoprotein cholesterol level and body weight. Food Nutr Res 61:1334484. https://doi.org/10.1080/16546628.2017.1334484
127. Huang J, Wang Y, Xie Z, Zhou Y, Zhang Y, Wan X (2014) The anti-obesity effects of green tea in human intervention and basic molecular studies. Eur J Clin Nutr 68:1075–1087. https://doi.org/10.1038/ejcn.2014.143
128. Kim SN, Kwon HJ, Akindehin S, Jeong HW, Lee YH (2017) Effects of Epigallocatechin-3-Gallate on Autophagic Lipolysis in Adipocytes. Forum Nutr 9. https://doi.org/10.3390/nu9070680
129. Lee MS, Shin Y, Jung S, Kim Y (2017) Effects of epigallocatechin-3-gallate on thermogenesis and mitochondrial biogenesis in brown adipose tissues of diet-induced obese mice. Food Nutr Res 61:1325307. https://doi.org/10.1080/16546628.2017.1325307
130. Pan H, Gao Y, Tu Y (2016) Mechanisms of body weight reduction by black tea polyphenols. Molecules 21. https://doi.org/10.3390/molecules21121659
131. Li W, Yang J, Zhu XS, Li SC, Ho PC (2016) Correlation between tea consumption and prevalence of hypertension among Singaporean Chinese residents aged 40 years. J Hum Hypertens 30:11–17. https://doi.org/10.1038/jhh.2015.45
132. Chei CL, Loh JK, Soh A, Yuan JM, Koh WP (2017) Coffee, tea, caffeine, and risk of hypertension: The Singapore Chinese Health Study. Eur J Nutr. https://doi.org/10.1007/s00394-017-1412-4
133. Yarmolinsky J, Gon G, Edwards P (2015) Effect of tea on blood pressure for secondary prevention of cardiovascular disease: a systematic review and meta-analysis of randomized controlled trials. Nutr Rev 73:236–246. https://doi.org/10.1093/nutrit/nuv001
134. Li G, Zhang Y, Thabane L, Mbuagbaw L, Liu A, Levine MA, Holbrook A (2015) Effect of green tea supplementation on blood pressure among overweight and obese adults: a systematic review and meta-analysis. J Hypertens 33:243–254. https://doi.org/10.1097/HJH.0000000000000426
135. Nogueira LP, Nogueira Neto JF, Klein MR, Sanjuliani AF (2017) Short-term effects of green tea on blood pressure, endothelial function, and metabolic profile in obese prehypertensive women: a crossover randomized clinical trial. J Am Coll Nutr 36:108–115. https://doi.org/10.1080/07315724.2016.1194236
136. Yi QY, Li HB, Qi J et al (2016) Chronic infusion of epigallocatechin-3-O-gallate into the hypothalamic paraventricular nucleus attenuates hypertension and sympathoexcitation by restoring neurotransmitters and cytokines. Toxicol Lett 262:105–113. https://doi.org/10.1016/j.toxlet.2016.09.010
137. Szulinska M, Stepien M, Kregielska-Narozna M et al (2017) Effects of green tea supplementation on inflammation markers, antioxidant status and blood pressure in NaCl-induced hypertensive rat model. Food Nutr Res 61:1295525. https://doi.org/10.1080/16546628.2017.1295525

138. Kluknavsky M, Balis P, Puzserova A et al (2016) (−)-Epicatechin prevents blood pressure increase and reduces locomotor hyperactivity in young spontaneously hypertensive rats. Oxidative Med Cell Longev 2016:6949020. https://doi.org/10.1155/2016/6949020
139. Takagaki A, Nanjo F (2015) Effects of metabolites produced from (−)-Epigallocatechin gallate by rat intestinal bacteria on angiotensin I-converting enzyme activity and blood pressure in spontaneously hypertensive rats. J Agric Food Chem 63:8262–8266. https://doi.org/10.1021/acs.jafc.5b03676
140. Ke Z, Su Z, Zhang X et al (2017) Discovery of a potent angiotensin converting enzyme inhibitor via virtual screening. Bioorg Med Chem Lett 27:3688–3692. https://doi.org/10.1016/j.bmcl.2017.07.016
141. Panagiotakos DB, Lionis C, Zeimbekis A, Gelastopoulou K, Papairakleous N, Das UN, Polychronopoulos E (2009) Long-term tea intake is associated with reduced prevalence of (type 2) diabetes mellitus among elderly people from Mediterranean islands: MEDIS epidemiological study. Yonsei Med J 50:31–38. https://doi.org/10.3349/ymj.2009.50.1.31
142. Fu QY, Li QS, Lin XM et al (2017) Antidiabetic effects of tea. Molecules 22. https://doi.org/10.3390/molecules22050849
143. Pham NM, Nanri A, Kochi T et al (2014) Coffee and green tea consumption is associated with insulin resistance in Japanese adults. Metabolism 63:400–408. https://doi.org/10.1016/j.metabol.2013.11.008
144. Fukino Y, Shimbo M, Aoki N, Okubo T, Iso H (2005) Randomized controlled trial for an effect of green tea consumption on insulin resistance and inflammation markers. J Nutr Sci Vitaminol 51:335–342
145. Fukino Y, Ikeda A, Maruyama K, Aoki N, Okubo T, Iso H (2008) Randomized controlled trial for an effect of green tea-extract powder supplementation on glucose abnormalities. Eur J Clin Nutr 62:953–960. https://doi.org/10.1038/sj.ejcn.1602806
146. Liu CY, Huang CJ, Huang LH, Chen IJ, Chiu JP, Hsu CH (2014) Effects of green tea extract on insulin resistance and glucagon-like peptide 1 in patients with type 2 diabetes and lipid abnormalities: a randomized, double-blinded, and placebo-controlled trial. PLoS One 9:e91163. https://doi.org/10.1371/journal.pone.0091163
147. Brown AL, Lane J, Coverly J et al (2009) Effects of dietary supplementation with the green tea polyphenol epigallocatechin-3-gallate on insulin resistance and associated metabolic risk factors: randomized controlled trial. Br J Nutr 101:886–894. https://doi.org/10.1017/S0007114508047727
148. Borges CM, Papadimitriou A, Duarte DA, Lopes de Faria JM, Lopes de Faria JB (2016) The use of green tea polyphenols for treating residual albuminuria in diabetic nephropathy: a double-blind randomised clinical trial. Sci Rep 6:28282. https://doi.org/10.1038/srep28282
149. Mackenzie T, Leary L, Brooks WB (2007) The effect of an extract of green and black tea on glucose control in adults with type 2 diabetes mellitus: double-blind randomized study. Metabolism 56:1340–1344. https://doi.org/10.1016/j.metabol.2007.05.018
150. Josic J, Olsson AT, Wickeberg J, Lindstedt S, Hlebowicz J (2010) Does green tea affect postprandial glucose, insulin and satiety in healthy subjects: a randomized controlled trial. Nutr J 9:63. https://doi.org/10.1186/1475-2891-9-63
151. Wang X, Tian J, Jiang J, Li L, Ying X, Tian H, Nie M (2014) Effects of green tea or green tea extract on insulin sensitivity and glycaemic control in populations at risk of type 2 diabetes mellitus: a systematic review and meta-analysis of randomised controlled trials. J Hum Nutr Diet 27:501–512. https://doi.org/10.1111/jhn.12181
152. Williamson G (2013) Possible effects of dietary polyphenols on sugar absorption and digestion. Mol Nutr Food Res 57:48–57. https://doi.org/10.1002/mnfr.201200511
153. Anderson RA, Polansky MM (2002) Tea enhances insulin activity. J Agric Food Chem 50:7182–7186
154. Han MK (2003) Epigallocatechin gallate, a constituent of green tea, suppresses cytokine-induced pancreatic beta-cell damage. Exp Mol Med 35:136–139. https://doi.org/10.1038/emm.2003.19

155. Waltner-Law ME, Wang XL, Law BK, Hall RK, Nawano M, Granner DK (2002) Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J Biol Chem 277:34933–34940. https://doi.org/10.1074/jbc.M204672200
156. Yesil A, Yilmaz Y (2013) Review article: coffee consumption, the metabolic syndrome and non-alcoholic fatty liver disease. Aliment Pharmacol Ther 38:1038–1044. https://doi.org/10.1111/apt.12489
157. Nordestgaard AT, Thomsen M, Nordestgaard BG (2015) Coffee intake and risk of obesity, metabolic syndrome and type 2 diabetes: a Mendelian randomization study. Int J Epidemiol 44:551–565. https://doi.org/10.1093/ije/dyv083
158. Shang F, Li X, Jiang X (2016) Coffee consumption and risk of the metabolic syndrome: a meta-analysis. Diabetes Metab 42:80–87. https://doi.org/10.1016/j.diabet.2015.09.001
159. Micek A, Grosso G, Polak M et al (2017) Association between tea and coffee consumption and prevalence of metabolic syndrome in Poland - results from the WOBASZ II study (2013–2014). Int J Food Sci Nutr 1–11. https://doi.org/10.1080/09637486.2017.1362690
160. Kim HJ, Cho S, Jacobs DR Jr, Park K (2014) Instant coffee consumption may be associated with higher risk of metabolic syndrome in Korean adults. Diabetes Res Clin Pract 106:145–153. https://doi.org/10.1016/j.diabres.2014.07.007
161. Patti AM, Al-Rasadi K, Katsiki N et al (2015) Effect of a Natural Supplement Containing Curcuma Longa, Guggul, and Chlorogenic Acid in Patients With Metabolic Syndrome. Angiology 66:856–861. https://doi.org/10.1177/0003319714568792
162. Santana-Galvez J, Cisneros-Zevallos L, Jacobo-Velazquez DA (2017) Chlorogenic acid: recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. Molecules 22. https://doi.org/10.3390/molecules22030358
163. Panchal SK, Poudyal H, Waanders J, Brown L (2012) Coffee extract attenuates changes in cardiovascular and hepatic structure and function without decreasing obesity in high-carbohydrate, high-fat diet-fed male rats. J Nutr 142:690–697. https://doi.org/10.3945/jn.111.153577
164. Watanabe S, Takahashi T, Ogawa H et al (2017) Daily Coffee Intake Inhibits Pancreatic Beta Cell Damage and Nonalcoholic Steatohepatitis in a Mouse Model of Spontaneous Metabolic Syndrome, Tsumura-Suzuki Obese Diabetic Mice. Metab Syndr Relat Disord 15:170–177. https://doi.org/10.1089/met.2016.0114
165. Ma Y, Gao M, Liu D (2015) Chlorogenic acid improves high fat diet-induced hepatic steatosis and insulin resistance in mice. Pharm Res 32:1200–1209. https://doi.org/10.1007/s11095-014-1526-9
166. Mubarak A, Hodgson JM, Considine MJ, Croft KD, Matthews VB (2013) Supplementation of a high-fat diet with chlorogenic acid is associated with insulin resistance and hepatic lipid accumulation in mice. J Agric Food Chem 61:4371–4378. https://doi.org/10.1021/jf400920x
167. Catalano D, Martines GF, Tonzuso A, Pirri C, Trovato FM, Trovato GM (2010) Protective role of coffee in non-alcoholic fatty liver disease (NAFLD). Dig Dis Sci 55:3200–3206. https://doi.org/10.1007/s10620-010-1143-3
168. Onakpoya I, Terry R, Ernst E (2011) The use of green coffee extract as a weight loss supplement: a systematic review and meta-analysis of randomised clinical trials. Gastroenterol Res Pract 2011. https://doi.org/10.1155/2011/382852
169. Ohnaka K, Ikeda M, Maki T et al (2012) Effects of 16-week consumption of caffeinated and decaffeinated instant coffee on glucose metabolism in a randomized controlled trial. J Nutr Metab 2012:207426. https://doi.org/10.1155/2012/207426
170. Thom E (2007) The effect of chlorogenic acid enriched coffee on glucose absorption in healthy volunteers and its effect on body mass when used long-term in overweight and obese people. J Int Med Res 35:900–908. https://doi.org/10.1177/147323000703500620
171. Soga S, Ota N, Shimotoyodome A (2013) Stimulation of postprandial fat utilization in healthy humans by daily consumption of chlorogenic acids. Biosci Biotechnol Biochem 77:1633–1636. https://doi.org/10.1271/bbb.130147
172. Hsu CL, Huang SL, Yen GC (2006) Inhibitory effect of phenolic acids on the proliferation of 3T3-L1 preadipocytes in relation to their antioxidant activity. J Agric Food Chem 54:4191–4197. https://doi.org/10.1021/jf0609882

173. Murase T, Misawa K, Minegishi Y et al (2011) Coffee polyphenols suppress diet-induced body fat accumulation by downregulating SREBP-1c and related molecules in C57BL/6J mice. Am J Phys Endocrinol Metab 300:E122–E133. https://doi.org/10.1152/ajpendo.00441.2010
174. Huang CC, Tung YT, Huang WC, Chen YM, Hsu YJ, Hsu MC (2016) Beneficial effects of cocoa, coffee, green tea, and garcinia complex supplement on diet induced obesity in rats. BMC Complement Altern Med 16:100. https://doi.org/10.1186/s12906-016-1077-1
175. H VS, K V, Patel D, K S (2016) Biomechanism of chlorogenic acid complex mediated plasma free fatty acid metabolism in rat liver. BMC Complement Altern Med 16:274. https://doi.org/10.1186/s12906-016-1258-y
176. Maki C, Funakoshi-Tago M, Aoyagi R et al (2017) Coffee extract inhibits adipogenesis in 3T3-L1 preadipocyes by interrupting insulin signaling through the downregulation of IRS1. PLoS One 12:e0173264. https://doi.org/10.1371/journal.pone.0173264
177. Li Kwok Cheong JD, Croft KD, Henry PD, Matthews V, Hodgson JM, Ward NC (2014) Green coffee polyphenols do not attenuate features of the metabolic syndrome and improve endothelial function in mice fed a high fat diet. Arch Biochem Biophys 559:46–52. https://doi.org/10.1016/j.abb.2014.02.005
178. Grosso G, Micek A, Godos J et al (2017) Long-term coffee consumption is associated with decreased incidence of new-onset hypertension: a dose-response meta-analysis. Forum Nutr 9. https://doi.org/10.3390/nu9080890
179. Rhee JJ, Qin F, Hedlin HK et al (2016) Coffee and caffeine consumption and the risk of hypertension in postmenopausal women. Am J Clin Nutr 103:210–217. https://doi.org/10.3945/ajcn.115.120147
180. Lopez-Garcia E, Orozco-Arbelaez E, Leon-Munoz LM, Guallar-Castillon P, Graciani A, Banegas JR, Rodriguez-Artalejo F (2016) Habitual coffee consumption and 24-h blood pressure control in older adults with hypertension. Clin Nutr 35:1457–1463. https://doi.org/10.1016/j.clnu.2016.03.021
181. Tajik N, Tajik M, Mack I, Enck P (2017) The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. Eur J Nutr. https://doi.org/10.1007/s00394-017-1379-1
182. Revuelta-Iniesta R, Al-Dujaili EA (2014) Consumption of green coffee reduces blood pressure and body composition by influencing 11beta-HSD1 enzyme activity in healthy individuals: a pilot crossover study using green and black coffee. Biomed Res Int 2014:482704. https://doi.org/10.1155/2014/482704
183. Suzuki A, Yamamoto N, Jokura H, Yamamoto M, Fujii A, Tokimitsu I, Saito I (2006) Chlorogenic acid attenuates hypertension and improves endothelial function in spontaneously hypertensive rats. J Hypertens 24:1065–1073. https://doi.org/10.1097/01.hjh.0000226196.67052.c0
184. Zhao Y, Wang J, Ballevre O, Luo H, Zhang W (2012) Antihypertensive effects and mechanisms of chlorogenic acids. Hypertens Res 35:370–374. https://doi.org/10.1038/hr.2011.195
185. Ding M, Bhupathiraju SN, Chen M, van Dam RM, FB H (2014) Caffeinated and decaffeinated coffee consumption and risk of type 2 diabetes: a systematic review and a dose-response meta-analysis. Diabetes Care 37:569–586. https://doi.org/10.2337/dc13-1203
186. Bhupathiraju SN, Pan A, Manson JE, Willett WC, van Dam RM, FB H (2014) Changes in coffee intake and subsequent risk of type 2 diabetes: three large cohorts of US men and women. Diabetologia 57:1346–1354. https://doi.org/10.1007/s00125-014-3235-7
187. Loftfield E, Freedman ND, Graubard BI et al (2015) Association of coffee consumption with overall and cause-specific mortality in a large US Prospective Cohort Study. Am J Epidemiol 182:1010–1022. https://doi.org/10.1093/aje/kwv146
188. Koloverou E, Panagiotakos DB, Pitsavos C et al (2015) The evaluation of inflammatory and oxidative stress biomarkers on coffee-diabetes association: results from the 10-year follow-up of the ATTICA Study (2002-2012). Eur J Clin Nutr 69:1220–1225. https://doi.org/10.1038/ejcn.2015.98

189. Yarmolinsky J, Mueller NT, Duncan BB, Bisi Molina Mdel C, Goulart AC, Schmidt MI (2015) Coffee consumption, newly diagnosed diabetes, and other alterations in glucose homeostasis: a cross-sectional analysis of the longitudinal study of adult health (ELSA-Brasil). PLoS One 10:e0126469. https://doi.org/10.1371/journal.pone.0126469
190. Lee JK, Kim K, Ahn Y, Yang M, Lee JE (2015) Habitual coffee intake, genetic polymorphisms, and type 2 diabetes. Eur J Endocrinol 172:595–601. https://doi.org/10.1530/EJE-14-0805
191. Jacobs S, Kroger J, Floegel A et al (2014) Evaluation of various biomarkers as potential mediators of the association between coffee consumption and incident type 2 diabetes in the EPIC-Potsdam Study. Am J Clin Nutr 100:891–900. https://doi.org/10.3945/ajcn.113.080317
192. Hinkle SN, Laughon SK, Catov JM, Olsen J, Bech BH (2015) First trimester coffee and tea intake and risk of gestational diabetes mellitus: a study within a national birth cohort. BJOG 122:420–428. https://doi.org/10.1111/1471-0528.12930
193. Santos RM, Lima DR (2016) Coffee consumption, obesity and type 2 diabetes: a mini-review. Eur J Nutr 55:1345–1358. https://doi.org/10.1007/s00394-016-1206-0
194. Dickson JC, Liese AD, Lorenzo C et al (2015) Associations of coffee consumption with markers of liver injury in the insulin resistance atherosclerosis study. BMC Gastroenterol 15:88. https://doi.org/10.1186/s12876-015-0321-3
195. Chrysant SG (2017) The impact of coffee consumption on blood pressure, cardiovascular disease and diabetes mellitus. Expert Rev Cardiovasc Ther 15:151–156. https://doi.org/10.1080/14779072.2017.1287563
196. van Dijk AE, Olthof MR, Meeuse JC, Seebus E, Heine RJ, van Dam RM (2009) Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance. Diabetes Care 32:1023–1025. https://doi.org/10.2337/dc09-0207
197. Meng S, Cao J, Feng Q, Peng J, Hu Y (2013) Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. Evid Based Complement Alternat Med 2013:801457. https://doi.org/10.1155/2013/801457
198. Jin S, Chang C, Zhang L, Liu Y, Huang X, Chen Z (2015) Chlorogenic acid improves late diabetes through adiponectin receptor signaling pathways in db/db mice. PLoS One 10: e0120842. https://doi.org/10.1371/journal.pone.0120842
199. Taguchi K, Hida M, Matsumoto T, Ikeuchi-Takahashi Y, Onishi H, Kobayashi T (2014) Effect of short-term polyphenol treatment on endothelial dysfunction and thromboxane A2 levels in streptozotocin-induced diabetic mice. Biol Pharm Bull 37:1056–1061
200. Hong BN, Nam YH, Woo SH, Kang TH (2017) Chlorogenic acid rescues sensorineural auditory function in a diabetic animal model. Neurosci Lett 640:64–69. https://doi.org/10.1016/j.neulet.2017.01.030
201. Ye HY, Li ZY, Zheng Y, Chen Y, Zhou ZH, Jin J (2016) The attenuation of chlorogenic acid on oxidative stress for renal injury in streptozotocin-induced diabetic nephropathy rats. Arch Pharm Res 39:989–997. https://doi.org/10.1007/s12272-016-0771-3
202. Boudjelal A, Siracusa L, Henchiri C, Sarri M, Abderrahim B, Baali F, Ruberto G (2015) Antidiabetic effects of aqueous infusions of *Artemisia herba-alba* and *Ajuga iva* in alloxan-induced diabetic rats. Planta Med 81:696–704. https://doi.org/10.1055/s-0035-1546006
203. Aoyagi R, Funakoshi-Tago M, Fujiwara Y, Tamura H (2014) Coffee inhibits adipocyte differentiation via inactivation of PPARgamma. Biol Pharm Bull 37:1820–1825
204. Mellbye FB, Jeppesen PB, Hermansen K, Gregersen S (2015) Cafestol, a bioactive substance in coffee, stimulates insulin secretion and increases glucose uptake in muscle cells: studies in vitro. J Nat Prod 78:2447–2451. https://doi.org/10.1021/acs.jnatprod.5b00481
205. Di Lorenzo A, Curti V, Tenore GC, Nabavi SM, Daglia M (2017) Effects of tea and coffee consumption on cardiovascular diseases and relative risk factors: an update. Curr Pharm Des 23:2474–2487. https://doi.org/10.2174/1381612823666170215145855
206. Yin X, Yang J, Li T et al (2015) The effect of green tea intake on risk of liver disease: a meta analysis. Int J Clin Exp Med 8:8339–8346
207. Hodge A, Lim S, Goh E et al (2017) Coffee intake is associated with a lower liver stiffness in patients with non-alcoholic fatty liver disease, hepatitis C, and hepatitis B. Forum Nutr 9. https://doi.org/10.3390/nu9010056

208. Sameshima Y, Ishidu Y, Ono Y, Hujita M, Kuriki Y (2008) Green tea powder enhances the safety and efficacy of interferon α-2b plus ribavirin combination therapy in chronic hepatitis C patients with a very high genotype 1 HCV load. In: Mamoru I (ed) Beneficial health effect of green tea. Research Signpost, Trivandrum
209. Halegoua-De Marzio D, Kraft WK, Daskalakis C, Ying X, Hawke RL, Navarro VJ (2012) Limited sampling estimates of epigallocatechin gallate exposures in cirrhotic and noncirrhotic patients with hepatitis C after single oral doses of green tea extract. Clin Ther 34(2279–2285): e2271. https://doi.org/10.1016/j.clinthera.2012.10.009
210. Abe K, Ijiri M, Suzuki T, Taguchi K, Koyama Y, Isemura M (2005) Green tea with a high catechin content suppresses inflammatory cytokine expression in the galactosamine-injured rat liver. Biomed Res 26:187–192
211. Li S, Xia Y, Chen K et al (2016) Epigallocatechin-3-gallate attenuates apoptosis and autophagy in concanavalin A-induced hepatitis by inhibiting BNIP3. Drug Des Devel Ther 10:631–647. https://doi.org/10.2147/DDDT.S99420
212. Steinmann J, Buer J, Pietschmann T, Steinmann E (2013) Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. Br J Pharmacol 168:1059–1073. https://doi.org/10.1111/bph.12009
213. Chen C, Qiu H, Gong J et al (2012) Epigallocatechin-3-gallate inhibits the replication cycle of hepatitis C virus. Arch Virol 157:1301–1312. https://doi.org/10.1007/s00705-012-1304-0
214. Xu J, Gu W, Li C et al (2016) Epigallocatechin gallate inhibits hepatitis B virus via farnesoid X receptor alpha. J Nat Med 70:584–591. https://doi.org/10.1007/s11418-016-0980-6
215. Navarro VJ, Khan I, Bjornsson E, Seeff LB, Serrano J, Hoofnagle JH (2017) Liver injury from herbal and dietary supplements. Hepatology 65:363–373. https://doi.org/10.1002/hep.28813
216. Wang D, Wei Y, Wang T, Wan X, Yang CS, Reiter RJ, Zhang J (2015) Melatonin attenuates (−)-epigallocatehin-3-gallate-triggered hepatotoxicity without compromising its down-regulation of hepatic gluconeogenic and lipogenic genes in mice. J Pineal Res 59:497–507. https://doi.org/10.1111/jpi.12281
217. Freedman ND, Everhart JE, Lindsay KL et al (2009) Coffee intake is associated with lower rates of liver disease progression in chronic hepatitis C. Hepatology 50:1360–1369. https://doi.org/10.1002/hep.23162
218. Wadhawan M, Anand AC (2016) Coffee and liver disease. J Clin Exp Hepatol 6:40–46. https://doi.org/10.1016/j.jceh.2016.02.003
219. Wijarnpreecha K, Thongprayoon C, Ungprasert P (2017) Coffee consumption and risk of nonalcoholic fatty liver disease: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol 29:e8–e12. https://doi.org/10.1097/MEG.0000000000000776
220. MG O, Han MA, Kim MW, Park CG, Kim YD, Lee J (2016) Coffee consumption is associated with lower serum aminotransferases in the general Korean population and in those at high risk for hepatic disease. Asia Pac J Clin Nutr 25:767–775. https://doi.org/10.6133/apjcn.092015.36
221. Tan Z, Luo M, Yang J et al (2016) Chlorogenic acid inhibits cholestatic liver injury induced by alpha-naphthylisothiocyanate: involvement of STAT3 and NFkappaB signalling regulation. J Pharm Pharmacol 68:1203–1213. https://doi.org/10.1111/jphp.12592
222. Feng Q, Liu W, Baker SS et al (2017) Multi-targeting therapeutic mechanisms of the Chinese herbal medicine QHD in the treatment of non-alcoholic fatty liver disease. Oncotarget 8:27820–27838. https://doi.org/10.18632/oncotarget.15482
223. Arauz J, Zarco N, Hernandez-Aquino E, Galicia-Moreno M, Favari L, Segovia J, Muriel P (2017) Coffee consumption prevents fibrosis in a rat model that mimics secondary biliary cirrhosis in humans. Nutr Res 40:65–74. https://doi.org/10.1016/j.nutres.2017.03.008
224. Sag D, Carling D, Stout RD, Suttles J (2008) Adenosine 5′-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. J Immunol 181:8633–8641
225. Salminen A, Hyttinen JM, Kaarniranta K (2011) AMP-activated protein kinase inhibits NF-kappaB signaling and inflammation: impact on healthspan and lifespan. J Mol Med (Berl) 89:667–676. https://doi.org/10.1007/s00109-011-0748-0

Theobroma cacao and *Theobroma grandiflorum*: Bioactive Compounds and Associated Health Benefits

36

Maria Inés Genovese and Helena Rudge de Moraes Barros

Contents

Abstract

The genus Theobroma comprises about 20 species, among them cocoa (*Theobroma cacao* L.), with the highest economic importance, and cupuassu (*T. grandiflorum*), of growing interest. Chemical compositions of cocoa and cupuassu unprocessed fresh seeds, pulps, and products (chocolate and cupulate) are presented, and the effects of processing in profile and quantity of the bioactive compounds, namely polyphenols and methylxanthines, are discussed. Dietary consumption of cocoa and dark chocolate has been associated to beneficial effects on health, mainly related to polyphenols and their antioxidant and anti-inflammatory activities affecting important signaling pathways and also modulating intestinal microbiota. Vasodilation and cardioprotective effects of cocoa polyphenols are related to release of nitric oxide (NO) through activation of

M. I. Genovese (✉) · H. R. d. M. Barros
Department of Food Science and Experimental Nutrition, University of São Paulo, São Paulo, Brazil
e-mail: genovese@usp.br; lebarros79@hotmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_15

endothelial NO synthase. Significant improvement of insulin resistance and flow-mediated dilatation (FMD) and reductions in diastolic blood pressure and mean arterial pressure were reported. In particular, the effects of cocoa and cupuassu polyphenols on obesity and glucose metabolism are reviewed.

Keywords
Cocoa · Cupuassu · Polyphenols · Obesity · Glucose metabolism

Abbreviations

AA	Arachidonic acid
ALT	Alanine aminotransferase
AMPK	AMP-activated protein kinase
AST	Aspartate aminotransferase
BMI	Body mass index
DW	Dry weight
FA	Fatty acid
FFA	Free fatty acids
FMD	Flow-mediated dilatation
FW	Fresh weight
GK	Glucokinase
GLP	Glucagon-like peptide
GLUT	Glucose transporter
GPx	Glutathione peroxidase
GR	Glutathione reductase
GS	Glycogen synthase
GSK3	Glycogen synthase kinase 3
HDLc	High-density lipoprotein
HF	High fat
HO-1	Heme oxygenase-1
HOMA-B	Homeostatic model assessment of cell function
IRS	Insulin receptor substrate
JNK	Jun N-terminal kinase
LDLc	Low-density lipoprotein
MDA	Malondialdehyde
MES-WAT	Mesenteric white adipose tissue
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
Nrf2	Nuclear factor erythroid 2-related factor
PEPCK	Phosphoenolpyruvate carboxykinase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
T2D	Type 2 diabetes
Tb/Cf ratio	Theobromine/caffeine ratio
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol

TG	Triacylglycerol
TLR4	Toll-like receptor 4
UCP	Uncoupling protein
VLDLc	Very low-density lipoprotein

1 Introduction

The genus *Theobroma* comprises about 20 species, and several of them produce edible seeds, notably cacao and cupuaçu. The highest economic importance is of the cultivars (varieties) "Criollo," "Forastero" and "Trinitario," a hybrid of Criollo and Forastero, of the species *Theobroma cacao* L., which are used for chocolate production [1]. On the other side, the species *T. grandiflorum* (cupuassu or cupuaçu) is getting more relevance for the South American market where the fruit pulp is highly appreciated and seeds are used for the manufacture of chocolate-like products. Cupuassu pulp can be consumed as juices, mousses, drinks, ice-creams, jellies and candies, although not usually consumed directly due to its strong acidity. The seeds contain high amounts of fat and may be used in food products and in a variety of cosmetics. The seeds can also be processed in a similar way as cocoa to yield a chocolate-like product called "cupulate®," firstly described and patented by the Brazilian Research Institute Embrapa (Empresa Brasileira de Pesquisa Agropecuária) [2].

Processing of raw cocoa involves several stages and starts with a fermentation step, crucial for the development of the desirable flavor of cocoa products. After drying/roasting and separation of shells, the grinding of beans results in cocoa nibs, which are then milled to a liquid paste known as cocoa liquor. Pressing of cocoa liquor removes cocoa butter and leaves a solid cake which is pulverized into cocoa powder. Chocolate is produced adding more cocoa butter and other ingredients such as sugar, milk, and emulsifying agents to cocoa liquor, in different proportions depending on the type of chocolate desired. All these processing steps result in changes in composition and quantity of the bioactive compounds present in cocoa, mainly a sharp decrease in concentration after fermentation and drying [3].

2 Chemical Composition of Cocoa and Cupuassu

Cocoa butter is the major nutrient in cocoa beans. Differences in chemical composition of cocoa beans are due to different geographic origin, genetic variability and post-harvesting processing. Cocoa beans from five regions of Cameroon, the fourth largest producer of cocoa in the world, variety Trinitario, showed fat contents ranging from 24.6% to 43.1%, moisture from 3.1% to 4.2%, carbohydrates from 24.4% to 43.4%, and proteins from 11.7% to 13.4% [4]. Ecuadorian unroasted cocoa beans showed higher moisture (5.95%), fibre (19.5%) and total fat (43.4%) content than Ghanaian beans, lower carbohydrates (33.8%), and similar contents of protein (12.8%) and ash (~4%) [5].

Table 1 Chemical compositions of cocoa and cupuassu fresh seeds and products (%)

Sample	Moisture	Ashes	Lipids	Proteins	Carbohydrates
Cupuassu					
Fresh seeds	53 ± 1^{a}	1.46 ± 0.06^{a}	22 ± 1^{a}	4.3 ± 0.1^{a}	16 ± 1^{a}
Fermented and dried seeds	8.6 ± 0.1^{b}	2.76 ± 0.01^{b}	45 ± 3^{b}	10.0 ± 0.4^{b}	31 ± 1^{b}
Fermented and roasted seeds	3.2 ± 0.1^{c}	3.02 ± 0.02^{c}	48 ± 2^{b}	10.0 ± 0.2^{b}	31 ± 2^{b}
Liquor	2.64 ± 0.03^{d}	3.0 ± 0.2^{c}	54 ± 2^{c}	10.5 ± 0.3^{b}	27 ± 1^{c}
Cupulate	1.12 ± 0.01^{e}	1.78 ± 0.05^{d}	37 ± 2^{d}	7.0 ± 0.2^{c}	52 ± 3^{d}
Cocoa					
Fresh seeds	46 ± 2^{f}	2.09 ± 0.05^{e}	20 ± 1^{a}	9.1 ± 0.2^{b}	19 ± 1^{a}
Chocolate	1.5 ± 0.1^{g}	1.55 ± 0.04^{a}	35 ± 2^{d}	7.8 ± 0.6^{c}	53 ± 4^{d}

Values in the same column with different letters are statistically different ($p < 0.05$). Data from Pugliese [7]

Fresh seeds of cupuassu, from three different harvest seasons, presented ca 53% moisture, 1.5% ashes, 22% lipids, 3.8–4.2% proteins, and 14–18% carbohydrates. Fresh pulps presented higher moisture (90%), as expected, and lower ashes (0.5%), lipids (1%), proteins (0.7%) and carbohydrates contents (5–6%) [6]. For comparison, cocoa fresh pulp from the same origin (Bahia, Brazil) presented 85% moisture, 0.43% ashes, 0.22% lipids, 0.77% proteins, and 12% carbohydrates [7].

Chemical compositions of cocoa and cupuassu unprocessed fresh seeds and products prepared by CEPLAC (Comissão Executiva do Plano da Lavoura Cacaueira, Cocoa Processing Unit, Fazenda Riachuelo, Bahia, Brazil) are presented in Table 1. Although with similar lipid content, protein content of cocoa fresh seeds was more than double that of cupuassu fresh seeds. Cupulate and chocolate, on the other side, presented a similar composition.

Similar to what happens to cocoa, processing results in alteration of the chemical composition of cupuassu seeds. Fermentation and sun-drying reduce drastically moisture content of seeds, leading to a proportional increase of the other constituents. After the elimination of shells from roasted seeds, there is a reduction in carbohydrate content (fiber) and an increase in lipid content of the resulting nibs, which are then ground to produce the liquor (Table 1). Finally, cupuassu liquor (52%) is mixed with cupuassu butter (8%), milk powder (6%), lecithin (0.5%) and sugar (33.5%), resulting in an increase in carbohydrates content in the final product, cupulate [7].

Quantitatively C16:0 (hexadecanoic acid or palmitic acid) (>25%), C18:0 (octadecanoic acid or stearic acid) (>33%) and C18:1 (octadecenoic acid or oleic acid) (>34%) are the most important fatty acids in unroasted cocoa beans of both origins Ecuadorian and Ghanaian [5]. Among the fatty acids detected in cocoa beans from five different regions of Cameroon, stearic acid was the most abundant (37.6–39.5%) followed by oleic acid (30.4–33.2%) and palmitic acid (24.8–28.2%), and saturated fatty acids were most abundant (63.9–66.8%), followed by monounsaturated (30.4–33.2%) and finally by polyunsaturated fatty acids (2.7–3.2%) [4].

Table 2 Fatty acid composition of cupuassu fresh seeds and cupuassu liquor (% total lipids)

Formula	Fatty acid	Fresh seeds %	Liquor %
16:0	Palmitic	7.54 ± 0.08[a]	6.86 ± 0.05[b]
17:0	Margaric	0.19 ± 0.01[a]	0.18 ± 0.01[a]
18:0	Stearic	32.6 ± 0.1[a]	31.4 ± 0.2[b]
18:1 (n-9)	Oleic	41.31 ± 0.06[a]	43.5 ± 0.1[b]
18:1 (n-7)	Vaccenic	0.47 ± 0.02[a]	0.46 ± 0.01[a]
18:2 (n-6)	Linoleic	4.9 ± 0.1[a]	5.04 ± 0.01[a]
18:3 (n-3)	α-linolenic	0.20 ± 0.01[a]	0.13 ± 0.00[b]
20:0	Eicosanoic	10.51 ± 0.04[a]	10.23 ± 0.09[b]
20:1 (n-9)	Eicosenoic	0.34 ± 0.01[a]	0.37 ± 0.01[b]
22:0	Docosanoic	1.72 ± 0.01[a]	1.70 ± 0.05[a]
24:0	Tetracosanoic	0.19 ± 0.00[a]	0.17 ± 0.01[a]
Total	Saturated	52.81 ± 0.07[a]	50.5 ± 0.4[b]
	Monounsaturated	42.12 ± 0.04[a]	44.4 ± 0.1[b]
	Polyunsaturated	5.07 ± 0.11[a]	5.17 ± 0.01[a]

Values in the same row with different letters are statistically different ($p < 0.05$). Data from Pugliese [7]

Fatty acid composition of cupuassu fresh seeds and liquor is shown in Table 2. Although the fatty acid composition is very similar to cocoa, as it can be seen, cupuassu fat stands out for the prevalence of oleic acid (18:1, 41%), followed by stearic acid (18:0, 33%), and much lower amounts of palmitic acid (16:0, 7.5%) than cocoa fat (~26%). Cupuassu fat also presents ten times more arachidic acid (20:0) than cocoa fat [8]. The fatty acid composition of cupuassu fat provides softness and enables its use in chocolates, replacing cocoa butter [9]. According to Lucas [10], in spite of the higher melting point of cupuassu butter (33.9 °C) compared to cocoa butter (31 °C), the high content of monounsaturated fatty acids, mainly oleic acid, of cupuassu fat turns it softer than cocoa fat.

Bezerra et al. [11] also found that the primary fatty acids in cupuassu fat are oleic (41.6%) and stearic (31.6%) acids, and that the fat has low atherogenicity and thrombogenicity indexes, melting point of 38.1 °C, and can be combined in blends with suitable physicochemical properties for many applications.

Among by-products, cupuassu peel flour stands out for presenting an elevated dietary fiber content (~80%), mainly insoluble fiber (~78%), higher than that of cocoa peel (~64%, 52% insoluble fiber), and for being able to be incorporated in whole-bread at 6% level without affecting color, aroma, texture and flavor attributes [12].

3 Bioactive Compounds of Cocoa and Cupuassu

Cocoa is rich in polyphenols and methylxanthines, and the higher the amount of cocoa solids, the higher the content of methylxanthines and polyphenols in cocoa products such as cocoa liquor, cocoa powder, dark, milk, and powdered chocolate [13].

Besides, processed cocoa beans and cocoa-based products may present other substances and chemical compounds of microbial and fungal origin which can be also related to human health benefits. Bacteria and fungi which drive spontaneous postharvest fermentation of cocoa beans immediately after bean harvesting produce various primary and secondary metabolites with antifungal, antineoplastic, anti-infective and cholesterol-lowering activities [14].

The physiological effects of methylxanthines are mainly mediated by adenosine receptors blockage and include central nervous system stimulation, diuresis, cardiovascular and metabolic effects, bronchial relaxation and increased secretion of gastric acids [15]. The main methylxanthines of cocoa are theobromine (0.8–2% on a dry weight basis, DW) and caffeine (about 0.2%) and concentrations vary depending on bean type, plant species, and degree of fermentation [16]. As expected, concentrations of theobromine and caffeine decrease in cocoa products, following the order cocoa > baking chocolate > dark chocolate > milk chocolate > cocoa butter, and theophylline is normally below limit of detection. The benefits of chocolate on mood seem to be mainly exerted by caffeine whereas theobromine may be related to other effects [15].

Methylxanthine contents of fresh cocoa beans from different cocoa varieties grown in Peru were in the range of 1.09–1.45%, for theobromine, and 0.20–0.41% DW, for caffeine, decreasing during fermentation and drying. Liquor from these samples presented theobromine contents from 0.84 to 1.14, and caffeine contents from 0.08 to .165% DW [17]. The average contents of total methylxanthines decreased from 15.05 mg/g dry matter in non-fermented to 12.94 mg/g dry matter in fermented cocoa samples from Nicaragua [18]. Although methylxanthines do not undergo chemical degradation during fermentation, about 30% of alkaloids are lost due to diffusion out of the bean cotyledons [19].

The theobromine/caffeine (Tb/Cf) ratio is considered an important parameter for cocoa, and lower ratios (<3) are typical of Criollo cocoa (less bitter), as it presents lower concentration of theobromine and higher levels of caffeine relative to other genetic groups, Trinitario (< 9 Tb/Cf >3) and Forastero (Tb/Cf >9) cocoas [16–18].

Caffeic acid was reported to be present in a concentration range of 1.1–2.1 mg/g dried extract, theobromine in a concentration range of 4.7–11.6 mg/g dried extract and (−)-epicatechin in a concentration range of 1.1–142.9 mg/g dried extract in cocoa beans from five different regions of Cameroon [4].

Cupuassu presents a high amount of phenolics, especially on seeds, and of ascorbic acid on pulp. However, a much lower content of theobromine (1 mg/g) and caffeine (0.5 mg/g) was reported for ground seeds (both raw and roasted) of cupuassu in comparison to cacao seeds (33–36 mg/g theobromine, 3.3–5.6 mg/g caffeine) [20]. Fresh pulp of cupuassu showed a high content of ascorbic acid (ca 102 mg/100 g DW sample, or 17 mg/100 g FW) but commercial frozen pulps seem to suffer a drastic loss during processing, presenting a ten times lower ascorbic acid content (9–13 mg/100 g DW), probably as a result of oxidative reactions during pulp separation and thermal processing [6].

Cocoa by-products are also important sources of bioactive compounds. Cocoa husks are part of the cocoa bean and are separated from the cotyledons after the

roasting process. They have a high content of total dietary fibre, mainly insoluble fibre, and represent a secondary source of theobromine, caffeine, and phenolics (these last in the range 2.6–4.1 mg/g DW) [21].

4 Cocoa Polyphenolic Composition

Polyphenols in cocoa beans can be classified into three main groups: flavan-3-ols or catechins (37%), anthocyanins (4%) and proanthocyanidins (58%), and the main catechin is (−)-epicatechin with up to 35% of polyphenol content [3]. Polyphenols represent about 10% of the whole bean's dry weight, with epicatechin concentrations among freshly harvested beans ranging from 21.9 to 43.3 mg/g of dry defatted sample [1].

Besides these major compounds (catechin, epicatechin and their dimers procyanidin B1 and procyanidin B2), other polyphenols have been reported in cocoa beans or cocoa products: procyanidin B3 = catechin-(4α → 8)-catechin, procyanidin B4 = catechin-(4α → 8)-epicatechin, procyanidin B5 = epicatechin-(4 β → 6)-epicatechin, procyanidin C1 = epicatechin-(4β → 8)-epicatechin-(4β → 8)-epicatechin, procyanidin D = epicatechin-(4 β → 8)-epicatechin-(4 β → 8)-epicatechin-(4 β → 8)-epicatechin, higher oligo- and polymers, mostly homologues of epicatechin with 2–18 monomeric units, as well the flavanols quercetin, quercetin-3-O-glucoside (isoquercitin), quercetin-3-O-galactoside (hyperoside), quercetin-3-O-arabinoside, the flavones apigenin, apigenin-8-C-glucoside (vitexin), apigenin-6-C-glucoside (isovitexin), luteolin and luteolin-7-O-glucoside, dihydroquercetin, dihydroxykaempferol, kaempferolrutinoside, naringenin, naringenin-glucoside, myricetin-glucoside, and also caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, phenylacetic acid, phloretic acid, protocatechuic acid, syringic acid, vanillic acid, clovamide and dideoxyclovamide [1].

5 Cupuassu Polyphenolic Composition

Nine known flavonoids were found in cupuassu seeds: (+)-catechin, (−)-epicatechin, isoscutellarein 8-*O*-*β*-D-glucuronide, hypolaetin 8-*O*-*β* glucuronide, quercetin 3-*O*-*β*-D-glucuronide, quercetin 3-*O*-*β*-D-glucuronide 6″-methyl ester, quercetin, kaempferol, and isoscutellarein 8-*O*-*β*-D-glucuronide 6″-methyl ester. Two new sulphated flavonoid glycosides, theograndins I and II, were also described [22].

Pugliese et al. [6] reported that the main flavonoids both in seeds and pulp of cupuassu were the glycosyl flavones, mainly the glucuronide and glucuronide-sulfates: Hypolaetin-8-O-β-D-glucuronide, Hypolaetin-8-O-β-D-glucuronide-3″-O-sulfate (Theograndin II); Isoscutellarein-8-O-β-D-glucuronide; Hypolaetin-3′-methyl ether-8-O-β-D-glucuronide; Isoscutellarein-8-O-β-D-glucuronide-3″-O-sulfate (Theograndin I); Hypolaetin-3′-methyl ether −8-O-β-D-glucuronide- 3″-O-sulfate, with a total flavone concentration above 31 mg/g of unfermented cupuassu

seeds. Similar flavonoid profiles were observed for fresh and commercial frozen pulps, but commercial frozen pulps presented lower contents than did fresh pulps, on average 0.5 and 2.2 mg/g, respectively.

Cupuassu did not show any anthocyanidins, which are common in cocoa seeds. The total proanthocyanidin content of unfermented cupuassu seeds was 23 mg/g. Cupuassu pulp has a phenolic content similar to that found in other tropical fruits, like passion fruit [23].

6 Effect of Processing on Polyphenols

Polyphenols in the cocoa beans are stored in the cotyledons. Processing of cocoa seeds for chocolate manufacture is responsible for a significant decrease in polyphenol content, mainly during fermentation and drying. Polyphenols diffusion from storage cells results in exposition to other bean constituents and oxidation reactions to produce insoluble high molecular weight tannins, both nonenzymatic and catalyzed by polyphenol-oxidase. Fermentation of cocoa beans is crucial for the development of precursors for chocolate flavour. Anthocyanins that represent ca. 4% of the total polyphenol content are hydrolyzed during the fermentation process of cocoa bean. During drying, additional loss of polyphenol occurs, mainly due to nonenzymatic browning reactions. After fermentation and drying, the nibs obtained are roasted and ground, resulting in the cocoa liquor. Removal of part of the cocoa butter from the liquor results in cocoa powder, and these products can be further alkalinized (*Dutching*), a process that also leads to significant losses of cocoa polyphenols [3, 24, 25].

A total of 11 different cocoa powder products available in the Spanish market presented a mean content of total flavonoids equivalent to 2.65 mg/g, with a high prevalence of (−)-epicatechin. The alkalinization process of cocoa powder resulted in 60% loss of the flavonoid content. Among flavanols, (−)-epicatechin presented a larger decline (67%) than (+)-catechin (38%), probably because of its epimerization into (−)-catechin, a less bioavailable form of catechin. Among flavonols, quercetin suffered the highest loss (86%), whereas quercetin-3-glucuronide, quercetin-3-arabinoside, and isoquercitrin showed a similar decrease (58, 62, and 61%, respectively). Di-, tri-, and tetrameric procyanidins also declined, 69% for dimer B2, 67% for trimer C1, and 31% for tetramer D [26]. Todorovic et al. [27] also reported a 1.8 times lower polyphenol content in alkalized cocoa samples than in natural ones with a decrease in epicatechin/catechin ratio (2.21 in natural and 1.45 in alkalized cocoa powders).

Processing also affects the bioavailability of cocoa polyphenols. The comparison of an unfermented, nonroasted, and blanch-treated cocoa powder with a conventional one showed four times more procyanidins, and eight times more epicatechin and procyanidin B2. A crossover intervention with healthy volunteers consuming cocoa milk drinks prepared with these powders showed that the content of epicatechin glucuronide, the main metabolite detected in plasma, was five-fold higher upon consumption of the low-processed as compared with the conventionally processed cocoa powder [28].

7 Health Benefits Associated to Cocoa and Cupuassu Ingestion

Dietary consumption of cocoa and dark chocolate has been associated to beneficial effects on health, mainly related to polyphenols and their antioxidant and anti-inflammatory activities affecting some important signaling pathways, such as Toll-like receptor 4 (TLR4)/nuclear factor kappa B (NF-κB) /signal transducer and activator of transcription. Vasodilation and cardioprotective effects of cocoa polyphenols are related to inducing release of nitric oxide (NO) through activation of endothelial NO synthase. The effects of chocolate, cocoa, and flavan-3-ols on major cardiovascular risk factors were systematically reviewed by Hooper et al. [29] who observed significant improvement of insulin resistance and flow-mediated dilatation (FMD) and reductions in diastolic blood pressure and mean arterial pressure. Also, cocoa polyphenols also modulate intestinal microbiota, leading to the growth of bacteria that trigger a tolerogenic anti-inflammatory pathway in the host [30].

However, commercial cocoa and chocolate products present a high variability in polyphenols content [26], and as a consequence, it is very difficult to generalize any associated health benefits of their consumption and/or deriving from cocoa polyphenols. Below we examine specifically the reported effects of cocoa and cupuassu polyphenols on obesity and glucose metabolism.

8 Effects of Cocoa and Cupuassu on Obesity

Although the scientific research on the beneficial properties of cocoa or dark chocolate has increased, clinical studies determining their effects on weight regulation have rarely been conducted. Many studies were related to cardiovascular diseases and body fat is only assessed as part of routine measurement of the research [31–33]. So far, there are no studies with humans evaluating the effects of *Theobroma grandiflorum* (cupuassu) consumption on weight control.

Interventional and observational studies that relate cocoa consumption with antiobesity effects have presented many controversial results until now. Some studies found an inverse correlation between body mass index (BMI) and regular cocoa consumption at small portions [31, 34, 35, 36]. However, other studies did not detect significant changes in weight following the regular consumption of dark chocolate [37–41], even when offered along with a restrictive diet [42]. In contrast, Desch et al. [43] found a slight increase in body weight of patients with hypertension that consumed 25 g of dark chocolate daily for 3 months. Greemberg et al. [44] found that there was an increase in body weight with chocolate consumption by healthy individuals in a dose-response manner, concluding that the hypothesis that chocolate intake was associated with lower body weight did not apply to participants without preexisting illness.

Therefore, this led to more focused animal studies on the effects of cocoa on weight control. Some studies have been conducted to examine whether cocoa may reduce weight gain, hyperlipidemia and inflammation and, thus, improve weight

control and other outcomes related to obesity [33, 45–49]. Some animal studies have also reported the effects of cupuassu on weight control [50, 51]. These studies are summarized in Table 3 and potential mechanisms by which cocoa could exert antiobesity effects are discussed below.

Almost all studies listed in Table 3 demonstrated reduced weight gain in high fat mice induced obesity by cocoa ingestion, while cupuassu demonstrated no changes or only attenuated weight gain. However, supplementation with both fruits promoted improvements in lipid-related outcomes as dyslipidemia and hepatic steatosis, glucose-related outcomes as hyperinsulinemia and hyperglycemia, inflammation and oxidative stress. Different outcomes may be explained by the wide range of doses used, as well as the variety of intervention methods (gavage, in diet), different types of products utilized (powder, extract, liquor, pure compounds) and the role of intervention (preventive or treatment).

Among treatments for obesity, one of the most promising strategies to decrease energy intake without altering central mechanisms is the development of nutrients digestion inhibitors [52]. Thus, interactions of polyphenols with digestive enzymes altering their activity may represent a significant caloric restriction and contribute to improve the energy metabolism regulation [53]. Cocoa extracts and mainly procyanidins with higher degree of polymerization have demonstrated inhibition of pancreatic lipase and secreted phospholipase A2 activities in a dose-dependent manner in vitro [54]. In this sense, a lower weight gain and adipogenesis observed in mice fed a high-fat diet supplemented with cocoa was suggested to be in part due to cocoa's inhibitory effect on pancreatic lipase activity [46, 47, 55]. Cocoa supplementation resulted in reduced fat absorption from the gut as indicated by the presence of a high fecal fat content in mice fed a high fat diet [47]. Cupuassu phenolic extract also showed inhibition of pancreatic lipase in vitro and increased lipid fecal excretion in mice fed high-fat high-sucrose diet, followed by reduction of liver triacylglycerol contents and cholesterol levels in plasma [50]. However, cocoa flavanols and cupuassu polyphenols have not been evaluated for inhibition of lipid digestion and absorption in humans yet.

In addition to the reduction of fat absorption, several studies have shown that cocoa decreases diet induced lipogenesis and hyperlipidemia by enhancing fat oxidation and inhibiting the syntheses of fatty acids in liver and adipose tissue of mice fed a high-fat diet [49, 56, 57]. Ali et al. [56] demonstrated that cocoa polyphenols treatment down-regulated the expression of PPAR-γ, SREBP-1c, Acaca and Mcat genes in white adipose tissue and Fasn and Scd1 genes in the liver, the key enzymes of fatty acid synthesis. Conversely, the expression of genes involved in fatty acid oxidation PPAR-α, CPT1, and Prkaa1 was significantly higher in liver and in adipose tissue of mice treated with cocoa [56, 58]. In accordance with these results, Matsui et al. [48] found that cocoa ingestion decreased the expression of genes for fatty acid synthesis in the liver, however without activating the expression of genes for fatty acid oxidation in this tissue. In adipose tissue, cocoa ingestion decreased the expression of genes for fatty acid transport molecules and their transcription factor PPAR-γ with concurrent activation of thermogenesis. In contrast, other study [33] showed that cocoa and their polyphenolic derivatives were

Table 3 Animal studies related to the effects of cocoa and cupuassu polyphenols on obesity[a]

Vehicle	Dose	Duration of intervention	Model	Animals	Biomarkers affected	References
Crude polyphenolic extract by gastric gavage	600 mg/kg bw/day	4 weeks	Obesity induced by high-fat diet (treatment)	Sprague Dawley rats	↓ body weight gain, liver and MESWAT weight ↓ liver lipid contents ↓ FFA and insulin serum levels ↓lipogenesis related genes in liver ↑β-oxidation related genes in liver	[58]
Crude polyphenolic extract by gastric gavage	600 mg/kg bw/day	4 weeks	Obesity induced by high-fat diet (treatment)	Sprague Dawley rats	↓ body weight gain, WAT and liver weight ↓lipogenesis related genes in liver and visceral fats ↓ TG, TC, FFA and insulin serum levels ↑β-hydroxybutirate serum levels ↓ liver lipid contents ↓lipogenesis related genes in liver and visceral fats ↑β-oxidation related genes in liver and visceral fats	[56]
Cupuassu phenolic extract by oral gavage	2.25 and 4.5 mg EC/kg bw/day	8 weeks	Obesity induced by high-fat high sucrose diet (prevention)	Male C57BL/6 J mice	Attenuated body weight gain ↓ liver weight ↑fecal TG content ↓ ALT, AST, LDL and VLDL serum levels ↓ liver peroxidation and TG contents Improved fasting glucose and glucose tolerance	[50]
Cocoa flavanol extract or a flavanol fraction enriched with procyanidins in diet	25 mg/kg bw/day	12 weeks	Obesity induced by high-fat diet (prevention)	Male C57BL/6 J mice	Attenuated body weight gain and adipose tissue gain ↓insulin serum levels ↓endotoxin plasmatic levels	[46]

(*continued*)

Table 3 (continued)

Vehicle	Dose	Duration of intervention	Model	Animals	Biomarkers affected	References
Cocoa powder in diet	10% of diet	4 weeks	Obesity induced by high-fat diet (prevention)	Swiss male mice	↓ body weight gain ↓ liver lipid contents ↓ oxidative stress	[57]
Cocoa powder in diet	8% (w/w) of diet	18 weeks	Obesity-related Inflammation induced by high-fat diet (prevention)	Male C57BL/6 J mice	No body changes ↓ inflammatory genes expression in adipose tissue ↓ AA contents, eicosanoid-generating enzymes and NF-κB in WAT ↓ inflammation related genes in WAT ↓ endotoxin plasmatic levels ↑GLP-2 plasmatic levels	[47]
Cocoa powder in diet	8% (w/w) of diet	10 weeks	Obesity-related Inflammation induced by high-fat diet (treatment)	Male C57BL/6 J mice	↓ body weight gain and WAT weight ↑fecal lipid content ↑ adiponectin plasmatic levels ↓ insulin, ALT and pro-inflammatory mediators plasmatic levels ↓ lipid contents in liver ↓ inflammation related genes in WAT	[59]
Cocoa powder in diet	12.5% (w/w) of diet	3 weeks	Obesity induced by high-fat diet (prevention)	Male Wistar rats	↓ body weight gain and MES-WAT weight ↓TG serum levels ↑TC and HDL serum levels ↓gene expression of the FA synthesis in the liver ↓gene expression of the FA synthesis and transport and ↑thermogenesis in the adipose tissue	[48]

Cocoa polyphenol extract dissolved in sodium carboxytmethylcellulose	40 and 200 mg/kg of bw	5 weeks	Obesity induced by high-fat diet (prevention)	Male C57BL/6 N Mice	↓ body weight gain, liver and epididymal weight ↓ food intake ↓TG plasmatic levels	[55]
Cupuassu and cocoa liquor phenolic-rich extracts by oral gavage	2.1 and 7.2 g/kg bw	4 weeks	Obesity induced by high-fat diet (treatment)	Male Wistar rats	↑antioxidant capacity in plasma and liver ↓ alt, AST ↓ glucose intolerance and insulin resistance (only cupuassu)	[51]
Cocoa powder, cocoa extract and its flavanols (Epicatechin, Catechin and Procyanidin B2) By gastric gavage	1 g/ kg, 100 mg/Kg and 10 mg/kg bw/day	8 weeks	Obesity induced by high-fat high sucrose diet (prevention)	Male Wistar rats	↓body weight gain, attenuated fat mass accumulation ↓ food intake ↓TG, TC and LDLc serum levels ↓ fasting glucose and insulin serum levels ↓ adipocytokine levels in retroperitoneal adipose ↑ genes involved in FA uptake and β-oxidation	[33]
Cacao liquor procyanidin extract in diet	0.5 or 2% (w/w) of diet	13 weeks	Obesity induced by high-fat diet (prevention)	Male C57BL/6 mice	↓ body weight gain ↓ hyperglycemia and hyperinsulinemia ↓ TC and leptin plasmatic levels ↑ adiponectin plasmatic levels ↑ translocation of GLUT-4, UCP expression and AMPK phosphorylation	[49]

[a]The arrow indicates an increase (↑) or decrease (↓) in the levels or activity of the different parameters analyzed. *AA* Arachidonic acid, *ALT* alanine aminotransferase, *AMPK* AMP-activated protein kinase, *AST* aspartate aminotransferase, *FA* fatty acid, *FFA* free fatty acids, *GLP* Glucagon-like peptide, *GLUT* glucose transporter, *HDLc* high-density lipoprotein, *LDLc* low-density lipoprotein, *MES-WAT* mesenteric white adipose tissue, *NF-κB* factor nuclear kappa B, *TC* total cholesterol, *TG* triacylglycerol, *UCP* uncoupling protein, *VLDLc* very low-density lipoprotein

associated with the upregulation of the expression of genes involved in fatty acid uptake (PPAR-γ and FAT/CD36) suggesting an improvement in adipose tissue function with a better capacity to store nutritional overload and reduce ectopic lipid deposition in other tissues and lipoproteins levels. However, fat oxidation in adipose tissue was also enhanced by the upregulation and activation of PPARα, PGC1α and SIRT1. In addition, some studies also related the antiobesity effect of cocoa with the increase of the gene and protein expressions of the UCPs in the adipose tissues and skeletal muscle, which are involved in the reduction of weight, lipid contents in tissues and lipoprotein levels [33, 48, 49]. Although the intake of cupuassu polyphenols did not demonstrate an effective reduction in weight gain, improvements related to lipotoxicity could be observed. Supplementation with cupuassu phenolic extract significantly reduced the plasma concentrations of VLDL and LDL cholesterol, and the accumulation of triglycerides in the liver [50, 51]. This suggests that the presence of flavones, which are not found in cocoa, in combination with flavanols, may also help to alleviate the metabolic changes triggered by a diet rich in fats and sugars. However, further studies are needed to elucidate the mechanisms associated with these effects.

Evermore, obesity chronic inflammation seems to be a key mediator of other pathologies including insulin resistances and fatty liver disease. Cocoa has been found to ameliorate obesity related inflammation, insulin resistance, and fatty liver disease in HF-fed mice, possibly by reducing pro-inflammatory gene expression and adipokines in white adipose tissue and modulating eicosanoid metabolism, gut barrier function and metabolic endotoxemia [46, 47, 59]. Cocoa supplementation also significantly increased plasmatic adiponectin levels in mice compared to HF-fed controls, which may contribute to the decreased triglyceride accumulation in liver and protect from inflammation and fibrosis [33, 49, 59].

In fact, adiponectin has been associated with maintaining glucose homeostasis through activation of AMPK. Cocoa has the potential to prevent insulin resistance and obesity by activating the adiponectin–AMPK pathway. Interestingly, cocoa also reduced insulin plasmatic level without having effect on fasting glycaemia in high-fat fed induced obesity studies. However, cupuassu improved fasting glycaemia and glucose tolerance, which was associated with reduced lipotoxicity and oxidative stress [50, 51]. The effects of *Theobroma* on glucose metabolism will be discussed in the next section.

Until now, these antiobesity effects of cocoa can be explained by the activation of AMPK and PPARα signaling, molecules responsible for suppressing lipogenesis (fatty acid synthesis) and promoting lipolysis (fatty acid oxidation). These effects also appear to be due in part to the modulation of dietary fat absorption, modulation of gut microbiota and the down-regulation of NF-κB target gene expression, reducing inflammation. However, other genes and/or transcription factors may play a role in beneficial effects. Although research to date has not specifically addressed the mechanisms that *Theobroma* can act on obesity, there is reason to believe that consumption of cocoa and cupuassu may induce beneficial metabolic changes through the effects described above.

9 Effects of Cocoa and Cupuassu on Glucose Metabolism

As antidiabetic, cocoa polyphenols have been found to attenuate postprandial glycemic responses while improving acute insulin secretion and insulin sensitivity in animal models and in a limited number of human studies [46, 60–64]. In fact, cocoa ingestion has been shown to protect β-cells by reducing oxidative stress and decreasing levels of blood glucose, limiting the risk factors for diabetes and other chronic diseases [63, 65, 66].

Currently, animal studies continue to evaluate the physiological effects of *Theobroma*'s intake on different diabetic models. These studies are summarized in Table 4 and potential mechanisms by which cocoa could exert antidiabetic effects are discussed below.

The possible mechanisms of *Theobroma*'s action include inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic *β*-cells, modulation of glucose release from the liver, activation of insulin receptors and glucose uptake in the insulin-sensitive tissues, and modulation of intracellular signaling pathways and gene expression. These positive effects on glucose homeostasis are also supported by epidemiological evidence on polyphenol-rich diets [67–69].

Experimental data from in vitro studies have suggested that cocoa and its main phenolic compounds can act as potential antidiabetic agents by inhibition of carbohydrate digestion [54, 70] and glucose absorption in the intestinal lumen [71, 72]. Cocoa extract demonstrated an in vitro inhibition of α-amylase activity in a dose-dependent manner, which was enhanced by the degree of polymerization of cocoa procyanidins [54, 70]. Cupuassu extract rich in flavonoids also demonstrated a potent inhibition in vitro of α-amylase activity when compared to other Brazilian fruit extracts [73]. However, these results were not related to procyanidins contents because the extracts were obtained through a solid phase extraction in a polyamide SC6 column, which eliminates procyanidins, sugars, vitamins and minerals. In addition, in this study, flavanol contents of other fruit extracts seemed to be related to inhibitory activity of α-amylase. Interestedly, in contrast with other studies [6, 50, 51], flavanol contents were not detected in cupuassu in this study, what suggests that flavones may also exert effects on α-amylase activity. In another study [50], cupuassu phenolic extract also inhibited α-glucosidase activity in a dose-dependent manner and was more effective than acarbose in inhibiting α-glucosidase in vitro.

Chronic hyperglycemia and hyperlipidemia exposure increases intracellular reactive oxygen species (ROS) generation, disturbs mitochondrial function and can exert deleterious effects on β-cell function impairing insulin secretion [69, 74, 75]. Cocoa polyphenols have been suggested to protect pancreatic β-cells from oxidative stress and improve insulin secretion, thus alleviating hallmarks of T2D [63, 65, 66].

Studies in vitro demonstrated that cocoa polyphenols could protect INS-1 cells against oxidative stress and modulate insulin secretion [65, 66]. Supporting these findings, a study using diabetic fatty rats showed that cacao rich diet ingestion increased β-cell mass and function by preventing beta cell apoptosis and improving antioxidant defenses in pancreas [66]. In accordance with these data, ob/ob rats fed

Table 4 Animal studies related to the effects of dietary cocoa and cupuassu polyphenols on glucose metabolism[a]

Vehicule	Dose	Duration	Model	Animals	Outcomes	References
Cocoa powder	Cocoa rich diet (10%)	9 weeks	Type 2 diabetes (pre-diabetic stage)	Male Zucker diabetic fatty rats (mutation in the leptin receptor)	↓ body weight gain and liver weight ↓ glucose and insulin plasmatic levels Improved glucose tolerance and insulin resistance Liver: ↓ p-(Ser)-IRS-1, p-GS and PEPCK ↑ p-GSK3, GK and GLUT-2 ↓ p-JNK and p-p38	[60]
Cocoa powder	Cocoa rich diet (10%)	9 weeks	Type 2 diabetes (pre-diabetic stage)	Male Zucker diabetic fatty rats	↓ body weight gain ↓ glucose and insulin plasmatic levels ↑ insulin sensitivity Liver: ↓ ROS, carbonyl groups, HO-1, p-Nrf2, p65-NFκB ↑ sod	[61]
Cocoa powder	Cocoa rich diet (10%)	9 weeks	Type 2 diabetes (pre-diabetic stage)	Male Zucker diabetic fatty rats	↓ body weight gain ↓ glucose and insulin plasmatic levels ↓ HOMA-B, ↓ HOMA-IR Pancreas: ↑cell mass, ↑ Bcl-xL, ↑ GPx, ↑ GR,, ↓ carbonyl groups, ↓ Bax, ↓caspase-3 activity, ↓ TBARS	[66]

(*continued*)

Table 4 (continued)

Vehicule	Dose	Duration	Model	Animals	Outcomes	References
Cocoa extract by oral gavage	600 mg/kg bw in diet	4 weeks	High-fat diet STZ injection	Male Sprague-Dawley rats	↓ FFA, 8-isoprostane plasmatic levels	[76]
Cupuassu and cocoa liquors	3.6 and 7.2 g/kg bw	40 days	Streptozotocin (STZ)-induced diabetic rats	Male Wistar rats	↓TG plasmatic levels Plasma HDLc Plasma antioxidant capacity ↓ lipid peroxidation in plasma and tissues	[78]
Cacao liquor Proanthocyanidin	0.5%, 1.0% (w/w) in diet	3 weeks		Female, db/db Mice (obese, diabetic)	↓blood glucose in a dose dependent manner	[64]
Cacao liquor Procyanidin	0, 0·5 or 1% (w/w) 50 or 250 mg/kg bw	Acute and 7 days		Male ICR and C57BL/6 mice	↓ glucose and insulin plasmatic levels ↑ GLUT4 translocation in skeletal muscle	[77]

[a]The arrow indicates an increase (↑) or decrease (↓) in the levels or activity of the different parameters analyzed. *FFA* free fatty acids, *GK* glucokinase, *GLUT* glucose transporter, *GS* glycogen synthase, *GSK3* glycogen synthase kinase 3, *GPx* glutathione peroxidase, *GR* glutathione reductase, *HDLc* high-density lipoprotein, *HO-1* heme oxygenase-1, *HOMA-B* homeostatic model assessment of cell function, *IRS* insulin receptor substrate, *JNK* Jun N-terminal kinase, *NFkB* nuclear factor-kappa B, *Nrf2* nuclear factor erythroid 2-related factor, *PEPCK* phosphoenolpyruvate carboxykinase, *ROS* reactive oxygen species, *SOD* superoxide dismutase, *TBARS* thiobarbituric acid reactive substances, *TG* triacylglycerol

cocoa extracts demonstrated decreased oxidative stress and enhanced glucose-stimulated insulin secretion, thereby enhancing functional β-cell mass [76].

In addition, cocoa might improve insulin secretion by inhibiting lipid accumulation in the cells. Considering this hypothesis, the supplementation of cupuassu polyphenols in mice fed a diet high fat high sucrose diet reestablished the phosphorylation of AKT and therefore insulin signaling in liver. These results were associated with a reduction of triacylglycerol and oxidative stress in liver, as evidenced by the reduction of MDA production and plasma transaminase activities [50]. However, further studies are needed to identify the exact mechanisms by which these effects are induced.

Liver is a key organ in the maintenance of blood glucose levels in tight cooperation with peripheral tissues. In this sense, Cordero-Herrera et al. [60] demonstrated that cocoa ameliorates hyperglycemia through its ability to preserve the hepatic functionality by preserving the levels of GLUT-2 and glycogen content and to

modulate gluconeogenic and glycolytic enzymes, as it decreases hepatic PEPCK and enhances hepatic GK. Although enzymes involved in hepatic production have not been evaluated in the cupuassu study, the increase of AKT phosphorylation suggests inhibition of hepatic glucose production through the improved insulin signaling in this tissue and reduced glucose plasmatic levels [50].

Cocoa polyphenols may also exert important antidiabetic effects by improving glucose uptake in muscles and adipocytes, through the action of GLUT4 [77]. Since cupuassu administration did not demonstrate a significant reduction in adipose tissue and body weight in mice fed a high fat high sucrose diet, the decrease in glycolipotoxicity demonstrated could be explained by an increase in GLUT4 activity in adipose tissue. However, this hypothesis was not evaluated for cupuassu yet.

10 Conclusions

Considering the evidences presented, cocoa may be useful in ameliorating insulin resistance, body weight gain and lipid alterations, thus reducing metabolic damage associated to diabetes and obesity. Numerous studies on the effect of cocoa on endothelial function also point to a possible effect on insulin sensitivity. However, additional studies to confirm these effects in humans are needed, mainly establishing a dose-response relationship between cocoa polyphenols and their overall effect. Cupuassu, another *Theobroma*, but little studied, also deserves attention as a potential polyphenolic source to act against metabolic alterations caused by diabetes and obesity.

References

1. Rusconi M, Conti A (2010) *Theobroma cacao* L., the food of the gods: a scientific approach beyond myths and claims. Pharmacol Res 61:5–13
2. Nazaré RFR, Barbosa WC, Viégas RMF (1990) Processamento das sementes de cupuaçu para obtenção de cupulate, 1st edition; Boletim de Pesquisa EMBRAPA, n.108, EMBRAPA – CPATU (Empresa Brasileira de Pesquisa Agropecuária – Centro de Pesquisa Agropecuária do Trópico Úmido), Belém, 38p
3. Wollgast J, Anklam E (2000) Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Res Int 33(6):423–447
4. Caprioli G, Fiorini D, Maggi F, Nicoletti M, Ricciutelli M, Toniolo C, Prosper B, Vittori S, Sagratini G (2016) Nutritional composition, bioactive compounds and volatile profile of cocoa beans from different regions of Cameroon. Int J Food Sci Nutr 67(4):422–430
5. Torres-Moreno M, Torrescasana E, Salas-Salvadò J, Blanch C (2015) Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions. Food Chem 166:125–132
6. Pugliese AG, Tomas-Barberan FA, Truchado P, Genovese MI (2013) Flavonoids, proanthocyanidins, vitamin C, and antioxidant activity of *Theobroma grandiflorum* (Cupuassu) pulp and seeds. J Agric Food Chem 61(11):2720–2728
7. Pugliese AG (2010). Compostos fenólicos do cupuaçu (*Theobroma grandiflorum*) e do cupulate: composição e possíveis benefícios. Dissertation, University of São Paulo
8. Lannes SCS, Medeiros ML, Gioielli LA (2004) Rheological properties of cupuassu and cocoa fats. Grasas Aceites 55(2):115–121

9. Lannes SCS, Medeiros ML, Amaral RL (2002) Formulação de "chocolate" de cupuaçu e reologia do produto líquido. Braz J Pharm Sci 38:463–467
10. Lucas V (2001) Fracionamento térmico e obtenção de gorduras de cupuaçu alternativas à manteiga de cacau para uso na fabricação de chocolate. 195 p. Phd thesis – Faculdade de Engenharia Química, UNICAMP, Campinas. http://www.bibliotecadigital.unicamp.br/document/?code=vtls000235715. Accessed 3 July 2017
11. Bezerra CV, Rodrigues AMD, de Oliveira PD, da Silva DA, da Silva LHM (2017) Technological properties of amazonian oils and fats and their applications in the food industry. Food Chem 221:1466–1473. https://doi.org/10.1016/j.foodchem.2016.11.004
12. Salgado JM, Rodrigues BS, Donado-Pestana CM, Dias CTD, Morzelle MC (2011) Cupuassu (*Theobroma grandiflorum*) peel as potential source of dietary Fiber and phytochemicals in whole-bread preparations. Plant Foods Hum Nutr 66(4):384–390
13. Belscak A, Komes D, Horzic D, Ganic KK, Karlovic D (2009) Comparative study of commercially available cocoa products in terms of their bioactive composition. Food Res Int 42 (5–6):707–716
14. Petyaev IM, Bashmakov YK (2016) Cocobiota: implications for human health. J Nutr Metab:7906927. 3 pages
15. Franco R, Oñatibia-Astibia A, Martínez-Pinilla E (2013) Health benefits of Methylxanthines in cacao and chocolate. Forum Nutr 5(10):4159–4173
16. Carrillo LC, Londoño-Londoño J, Gil A (2014) Comparison of polyphenol, methylxanthines and antioxidant activity in *Theobroma cacao* beans from different cocoa-growing areas in Colombia. Food Res Int 60:273–280
17. Peláez PP, Bardón I, Camasca P (2016) Methylxanthine and catechin content of fresh and fermented cocoa beans, dried cocoa beans, and cocoa liquor. Sci Agropecu 7(4):355–365
18. Trognitz B, Cros E, Assemat S, Davrieux F, Forestier-Chiron N, Ayestas E, Kuant A, Scheldeman X, Hermann M (2013) Diversity of cacao trees in Waslala, Nicaragua: associations between genotype spectra, product quality and yield potential. PLoS One 8(1):e54079
19. Camu N, De Winter T, Addo SK, Takrama JS, Bernaert H, De Vuyst L (2008) Fermentation of cocoa beans: influence of microbial activities and polyphenol concentrations on the flavour of chocolate. J Sci Food Agr 88(13):2288–2297
20. Lo Coco F, Lanuzza F, Micali G, Cappellano G (2007) Determination of theobromine, theophylline, and caffeine in by-products of Cupuacu and cacao seeds by high-performance liquid chromatography. J Chromatogr Sci 45:273–275
21. Bruna C, Eichholz I, Rohn S, Kroh LW, Huyskens-Keil S (2009) Bioactive compounds and antioxidant activity of cocoa hulls (*Theobroma cacao* L.) from different origins. J App Bot Food Qual 83(1):9–13
22. Yang H, Protiva P, Cui B, Ma C, Baggett S, Hequet V, Mori S, Weinstein IB, Kennelly EJ (2003) New bioactive polyphenols from *Theobroma grandiflorum* ("cupuaçu"). J Nat Prod 66:1501–1504
23. Kuskoski EM, Asuero AG, Troncoso AM, Mancini-Filho J, Fett R (2005) Aplicación de diversos métodos químicos para determinar actividad antioxidante em pulpa de frutos. Ciênc Tecnol Aliment 25:726–732
24. McShea A, Ramiro-Puig E, Munro SB, Casadesus G, Castell M, Smith MA (2008) Clinical benefit and preservation of flavonols in dark chocolate manufacturing. Nutr Rev 66(11):630–641
25. Oracz J, Zyzelewicz D, Nebesny E (2015) The content of polyphenolic compounds in cocoa beans (*Theobroma cacao* L.), depending on variety, growing region, and processing operations: a review. Crit Rev Food Sci Nutr 55(9):1176–1192
26. Andres-Lacueva C, Monagas M, Khan N, Izquierdo-Pulido M, Urpi-Sarda M, Permanyer J, Lamuela-Raventos RM (2008) Flavanol and flavonol contents of cocoa powder products: influence of the manufacturing process. J Agric Food Chem 56(9):3111–3117
27. Todorovic V, Milenkovic M, Vidovic B, Todorovic Z, Sobajic S (2017) Correlation between antimicrobial, antioxidant activity, and polyphenols of alkalized/nonalkalized cocoa powders. J Food Sci 82(4):1020–1027
28. Tomas-Barberan FA, Cienfuegos-Jovellanos E, Marin A, Muguerza B, Gil-Izquierdo A, Cerda B, Zafrilla P, Morillas J, Mulero J, Ibarra A, Pasamar MA, Ramon D, Espin JC (2007) A new

process to develop a cocoa powder with higher flavonoid monomer content and enhanced bioavailability in healthy humans. J Agric Food Chem 55(10):3926–3935
29. Hooper L, Kay C, Abdelhamid A (2012) Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. Am J Clin Nutr 95(3):740–775
30. Magrone T, Russo MA, Jirillo E (2017) Cocoa and dark chocolate polyphenols: from biology to clinical applications. Front Immunol 8:677
31. Bohannon J, Koch D, Homm P, Driehaus A (2015) Chocolate with high cocoa content as a weight-loss accelerator. Int Arch Med 8(55):1–8
32. Farhat G, Drummond S, Fyfe L, Al-Dujaili EAS (2014) Dark chocolate: an obesity paradox or a culprit for weight gain? Phytother Res 28(6):791–797
33. Rabadan-Chávez G, Quevedo-Corona L, Garcia AM, Reyes-Maldonado E, Jaramillo-Flores ME (2016) Cocoa powder, cocoa extract and epicatechin attenuate hypercaloric diet-induced obesity through enhanced β-oxidation and energy expenditure in white adipose tissue. J Funct Foods 20:54–67
34. Cuenca-García M, Ruiz JR, Ortega FB, Castillo MJ (2014) HELENA study group association between chocolate consumption and fatness in European adolescents. Nutr 30:236–239
35. Golomb BA, Koperski S, White HL (2012) Association between more frequent chocolate consumption and lower body mass index. Arch Int Med 172(6):519–521
36. Strandberg TE, Strandberg AY, Pitkälä K, Salomaa VV, Tilvis RS et al (2008) Chocolate, well-being and health among elderly men. Eur J Clin Nutr 62:247–253
37. Davison K, Coates AM, Buckley JD, Howe PRC (2008) Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects. Int J Obes 32(8):1289–1296
38. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, Ferri C (2005) Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. Hypertension 46(2):398–405
39. Shrime MG, Bauer SR, McDonald AC, Chowdhury NH, Coltart CEM, Ding EL (2011) Flavonoid-rich cocoa consumption affects multiple cardiovascular risk factors in a meta-analysis of short-term studies. J Nutr 141(11):1982–1988
40. Yeh M, Platkin C, Estrella P, Allinger D, Elbaum R, Brumaru B, Wyka K (2016) Chocolate consumption and health beliefs and its relation to BMI in college students. J Obes Weight Loss 2:1–7
41. Taubert D, Roesen R. Lehmann C, Jung N, Schomig E (2007) Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide. JAMA. 298:49–60
42. Nickols-Richardson SM, Piehowski KE, Metzgar CJ, Miller, DL, Preston AG (2014) Changes in body weight, blood pressure and selected metabolic biomarkers with an energy-restricted diet including twice daily sweet snacks and once daily sugar-free beverage. Nutrition Research and Practice 8(6):695–704
43. Desch S, Kobler D, Schmidt J, Sonnabend M, Adams V, Sareban M, Thiele H (2010) Low vs. Higher-Dose Dark Chocolate and Blood Pressure in Cardiovascular High-Risk Patients. Am J Hypertens 23(6):694–700
44. Greenberg JA, Buijsse B (2013) Habitual Chocolate Consumption May Increase Body Weight in a Dose-Response Manner. PLoS ONE 8(8)
45. Ali F, Ismail A, Esa NM, Pei CP, Kersten S (2015) Hepatic genome-wide expression of lipid metabolism in diet-induced obesity rats treated with cocoa polyphenols. J Funct Foods 17: 969–978
46. Dorenkott MR, Griffin LE, Goodrich KM, Thompson-Witrick KA, Fundaro G, Ye L, Neilson AP (2014) Oligomeric cocoa procyanidins possess enhanced bioactivity compared to monomeric and polymeric cocoa procyanidins for preventing the development of obesity, insulin resistance, and impaired glucose tolerance during high-fat feeding. J Agric Food Chem 62(10): 2216–2227
47. Gu Y, Yu S, Park JY, Harvatine K, Lambert JD (2014) Dietary cocoa reduces metabolic endotoxemia and adipose tissue inflammation in high-fat fed mice. J Nutr Biochem 25(4): 439–445

48. Matsui N, Ito R, Nishimura E, Yoshikawa M, Kato M, Kamei M, Hashizume S (2005) Ingested cocoa can prevent high-fat diet-induced obesity by regulating the expression of genes for fatty acid metabolism. Nutr 21(5):594–601
49. Yamashita Y, Okabe M, Natsume M, Ashida H (2012) Prevention mechanisms of glucose intolerance and obesity by cacao liquor procyanidin extract in high-fat diet-fed C57BL/6 mice. Arch Biochem Bioph 527(2):1–10
50. Barros, HRM. (2016) Effects of camu camu and cupuassu phenolic compounds on obesity and type 2 diabetes mellitus development. Tesis. http://www.teses.usp.br/teses/disponiveis/9/9131/tde-19022016-151536/pt-br.php. Acessed 20 july 2017
51. Oliveira TB, Rogero MM, Genovese MI (2015) Poliphenolic-rich extracts from cocoa (Theobroma cacao L.) and cupuassu (Theobroma grandiflorum Willd. Ex Spreng. K. Shum) liquors: A comparison of metabolic effects in high-fat fed rats. PharmaNutrition 3(2):20–28
52. Yun JW (2010) Possible anti-obesity therapeutics from nature–a review. Phytochemistry 71(14–15):1625–41
53. Garcia-Conesa MT (2015) Dietary Polyphenols against Metabolic Disorders: How Far Have We Progressed in the Understanding of the Molecular Mechanisms of Action of These Compounds? Crit Rev Food Sci Nutr
54. Gu Y, Hurst WJ, Stuart D, Lambert JD (2011) Inhibition of key digestive enzymes by cocoa extracts and procyanidins. J Agric Food Chem 59(10):5305–5311
55. Min SY, Yang H, Seo SG, Shin SH, Chung M-Y, Kim J, Lee KW (2013) Cocoa polyphenols suppress adipogenesis in vitro and obesity in vivo by targeting insulin receptor. Internat J Obes 37(4):584–92
56. Ali F, Ismail A, Esa NM, Pei C (2016) Cocoa polyphenols treatment ameliorates visceral obesity by reduction lipogenesis and promoting fatty acid oxidation genes in obese rats through interfering with AMPK pathway. Eur J Lipid Sci Technol 118(4):564–575
57. Fidaleo M, Fracassi A, Zuorro A, Lavecchia R, Moreno S, Sartori C (2014) Cocoa protective effects against abnormal fat storage and oxidative stress induced by a high-fat diet involve PPAR alpha signalling activation. Food Funct 5(11):2931–2939
58. Ali F, Ismail A, Esa NM, Pei CP, Kersten S (2015) Hepatic genome-wide expression of lipid metabolism in diet-induced obesity rats treated with cocoa polyphenols. J Funct Foods 17:969–978
59. Gu Y, Yu S, Lambert JD (2014) Dietary cocoa ameliorates obesity-related inflammation in high fat-fed mice. Eur J Nutr 53(1):149–158
60. Cordero-Herrera I, Martin MA, Escriva F, Alvarez C et al (2015) Cocoa-rich diet ameliorates hepatic insulin resistance by modulating insulin signaling and glucose homeostasis in Zucker diabetic fatty rats. J. Nutr. Biochem 26:704–712
61. Cordero-Herrera I, Martin MA, Goya L, Ramos S (2015) Cocoa intake ameliorates hepatic oxidative stress in young Zucker diabetic fatty rats. Food Res Int 69:194–201
62. Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, Blumberg JB, Ferri C (2008) Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. J Nutr 138:1671–1676
63. Martin MA, Goya L, Ramos S (2016) Antidiabetic actions of cocoa flavanols. Mol Nutr Food Res 60(8):1756–1769
64. Tomaru M, Takano H, Osakabe N, Yasuda A, Inoue K-I, Yanagisawa R, et al (2007) Dietary supplementation with cacao liquor proanthocyanidins prevents elevation of blood glucose levels in diabetic obese mice. Nutr 23:351–5
65. Fernández-Millán E, Ramos S, Alvarez C, Bravo L, Goya L, Martín MÁ (2014) Microbial phenolic metabolites improve glucose-stimulated insulin secretion and protect pancreatic beta cells against tert-butyl hydroperoxide-induced toxicity via ERKs and PKC pathways. Food Chem Toxicol 66:245–253
66. Fernandez-Millan E, Cordero-Herrera I, Ramos S, Escriva F, Alvarez C, Goya L Martin MA (2015) Cocoa-rich diet attenuates beta cell mass loss and function in young Zucker diabetic fatty rats by

preventing oxidative stress and beta cell apoptosis. Molecular Nutrition & Food Research 59(4):820–824
67. Andújar I, Recio M C, Giner RM, Ríos JL (2012) Cocoa polyphenols and their potential benefits for human health. Oxidative Medicine and Cellular Longevity
68. Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, et al (2010) Impact of dietary polyphenols on carbohydratemetabolism. Int J Mol Sci 11:1365–402
69. Anhê FF, Desjardins Y, Pilon G, Dudonné S, Genovese M I, Lajolo FM, Marette A (2013) Polyphenols and type 2 diabetes: A prospective review. PharmaNutrition 1(4):105–114
70. Barret A, Ndou T, Hughey CA, Straut C, Howel A, Dai Z, Kaletunc G (2013) Inhibition of α-amylase and glucoamylase by tannins extracted from cocoa, pomegranates, cranberries, and grapes. J Agric Food Chem 61(7):1477–86
71. Johnston K, Sharp P, Clifford M, Morgan L (2005) Dietary polyphenols decrease glucose uptake by human intestinal Caco-2 cells. FEBS Lett 579(7):1653–7
72. Katz DL, Doughty K, Ali A (2011) Cocoa and Chocolate in Human Health and Disease. Antioxid Redox Signal 15(10):2779–2811
73. Gonçalves AESS, Lajolo FM, Genovese MI (2010) Chemical composition and antioxidant/ antidiabetic potential of brazilian native fruits and commercial frozen pulps. J Agric Food Chem 58(8):4666–4674
74. Kim Y, Keogh JB, Clifton PM (2016) Polyphenols and Glycemic Control. Nutrients 8(1):17
75. Strat KM, Rowley TJ, Smithson AT, Tessem JS, Hulver MW, Liu D, Davy BM, Davy KP, Neilson AP (2016) Mechanisms by which cocoa flavanols improve metabolic syndrome and related disorders. J Nutr Biochem 35:1–21
76. Jalil A-M-M, Ismail A, Pei C-P, Hamid M, Kamaruddin S-H-S (2008) Effects of cocoa extract on glucometabolism, oxidative stress, and antioxidant enzymes in obese- diabetic (Ob-db) rats. J Agric Food Chem 56:7877–84
77. Yamashita Y, Okabe M, Natsume M, Ashida H (2012a) Cacao liquor procyanidin extract improves glucose tolerance by enhancing GLUT4 translocation and glucose uptake in skeletal muscle. J Nutr Sci, 1, e 2
78. Oliveira TB, Genovese MI (2013) Chemical composition of cupuassu (Theobroma grandiflorum) and cocoa (Theobroma cacao) liquors and their effects on streptozotocin-induced diabetic rats. Food Res Internatl 51(2):929–935

Olive Oil and Health Effects

37

Álvaro Hernáez, Julieta Valussi, Alejandra Pérez-Vega, Olga Castañer, and Montserrat Fitó

Contents

Abstract

Beneficial health effects of olive oil and its phenolics are presented in light of the Mediterranean diet (MD), which is characterized by (1) a high intake of cereals, vegetables including leafy greens, legumes, nuts, and fruit; (2) a moderate intake

Olga Castañer and Montserrat Fitó have contributed equally to this work.

Á. Hernáez · J. Valussi · A. Pérez-Vega · O. Castañer · M. Fitó (✉)
Cardiovascular Risk and Nutrition Research Group (CARIN), Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain

CIBER of the Physiopathology of Obesity and Nutrition (CIBEROBN), Institut Hospital del Mar d'Investigacions Mediques (IMIM), Barcelona, Spain
e-mail: mfito@imim.es

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_33

of poultry, fish, eggs, milk and dairy products, as well as a regular but moderate ethanol consumption (generally in the form of wine during meals); and (3) a low intake of red and processed meat and industrial confectionary. Olive oil (OO) is the main fat used in all preparations of the MD. The health benefits of consuming OO have been known since antiquity and were traditionally attributed to its high MUFA content, mainly oleic acid. A large number of epidemiological and laboratory studies suggest beneficial and protective effects of OO in reduced risk of suffering cardiovascular disease (CVD) and cerebrovascular diseases, diabetes mellitus, metabolic syndrome, certain cancers, and neurodegenerative diseases. Potential effects of OO on various diseases-related parameters are discussed in relation to OO and its phenolics.

Keywords
Olive oil · Mediterranean diet · Phenolic compounds · Virgin olive oil · PUFA · MUFA

Abbreviations

CRP	C-reactive protein
EFSA	European Food Safety Agency
GcMAF	Gc protein-derived macrophage activating factor
HDL	High-density lipoprotein
ICAM-1	Intercellular adhesion molecule-1
IL	Interleukin
LDL	Low-density lipoprotein
MD	Mediterranean diet
MUFA	Monounsaturated fatty acid
OO	Olive oil
OOPC	Olive oil phenolic compound
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
VCAM-1	Vascular cell adhesion molecule-1
VOO	Virgin olive oil

1 Introduction

In a number of studies, the Mediterranean diet (MD) has been connected with longevity and a reduced risk of morbidity and mortality. Life-style factors, such as regular physical activity, a healthy diet, and the existing social cohesion in southern European countries have been recognized as candidate protective elements which may explain the Mediterranean paradox. The term MD was coined in the 1960s by Ancel Keys within the framework of the Seven Countries Study. This epidemiological study with more than 12,000 individuals reported that Italian and Greek populations had lower mortality rates, and a reduced incidence of cancer and cardiovascular disease (CVD), compared to those from other European countries,

America, and Asia [1]. Such findings led to an exponential increase from 1999 of original articles regarding the MD [2]. High adherence to this diet pattern has been associated with a reduction in the risk of suffering CVD and cerebrovascular diseases, diabetes mellitus, metabolic syndrome, certain cancers, and neurodegenerative diseases [3]. The link between adherence to the MD and a reduction in total mortality has also been confirmed [4, 5].

In general, the MD is characterized by (1) a high intake of cereals, vegetables including leafy greens, legumes, nuts, and fruit; (2) a moderate intake of poultry, fish, eggs, milk and dairy products, as well as a regular but moderate ethanol consumption (generally in the form of wine during meals); and (3) a low intake of red and processed meat and industrial confectionary [4–7]. Along with some other traits of the Mediterranean diet, the use of olive oil (OO), as the main source of fat (especially dressings), is common in southern European countries. A relatively high fat consumption (up to 40% of total energy intake), mostly from monounsaturated fatty acids (MUFAs) (up to 20% of total energy consumption) is characteristic of this diet [8]. In the Mediterranean area, it is estimated that subjects consume between 25 and 50 mL of OO per day (raw and that used for cooking). Nevertheless, its consumption varies around the Mediterranean countries.

The health benefits of consuming OO have been known since antiquity and were traditionally attributed to its high MUFA content, mainly oleic acid. In this regard, a recent meta-analysis of 32 cohort studies with the aim of studying MUFA (of both plant and animal origin), oleic acid, MUFA/saturated fatty acid (SFA) ratio, and OO intake, indicated that, when comparing the upper to the lower tertile of consumption, OO, but not MUFA, was associated with a reduced risk of all-cause mortality, CVD events, and stroke [9]. Thus, the extra constituents of OO may also have a protective potential [10–12].

2 Cancer

Cancer is a multifactorial disease in which several aberrant processes are involved (deregulation of cell cycle, abnormal expression of pro-oncogenes, deregulated angiogenesis, excessive oxidative stress, chronic inflammatory responses, etc.). Several lifestyle factors such as smoking, unhealthy eating, and sedentary habit can increase the predisposition to develop cancer. Thirty to 40% of all cancers could be prevented by appropriate diets, physical activity practice, and maintenance of appropriate body weight [13]. Traditionally, a high consumption of fruit and vegetables has been inversely related to chronic degenerative diseases such as cancer [13, 14]. In the EPIC study, fiber intake from cereals, fruit, and vegetables showed a protective effect on colorectal cancer, and fruit consumption was also associated with lower rates of lung cancer [15]. Among the Mediterranean countries, meta-analyses of ecological and cohort studies found that cancer morbidity and mortality are lower than in other ones [16]. A high adherence to the MD, in which OO is the main source of fat, has been associated with reduced mortality for all type of cancers in prospective studies [5]. Around 25% of colorectal cancer incidence, 15% of breast

cancer, and 10% of prostate, pancreas, and endometrial cancer could be prevented by a TMD in the Western countries [17]. Recently, a 4.8-year adherence to a traditional MD (supplemented with virgin olive oil (VOO) or nuts) was found to protect against breast cancer development in an elderly women [18]. In contrast, in Mediterranean cohorts within the framework of the EPIC Study, OO consumption was not linked to lower breast cancer risk in postmenopausal women. Nevertheless, an inverse association between OO intake and the levels of estrogens and progesterone receptor-negative tumors was suggested [19]. Also within the EPIC Study, the adherence to an MD was associated with a decrease in the incidence of gastric adenocarcinoma [20]. In this respect, the antimicrobial activity conferred by OO against *Helicobacter pylori* (a microorganism related to gastric ulcers and subsequent carcinomas) could play a role in such an observation [21]. Finally, in Caucasian populations in the Mediterranean basin, prostate cancer incidence is lower than that in Caucasian males from other areas. The richness of MUFAs versus SFAs in the MD pattern has been postulated as a possible explanation [22].

With reference to OO, an inverse association between its intake and the appearance of different types of cancers has been described mainly from case-control studies [23]. In this respect, a meta-analysis of case-control studies assessing the effects of OO and MUFA intake was performed ($N = 19$, 13,800 cancer patients and 23,340 controls). Subjects in the group with the highest OO consumption presented lower odds of suffering any type of cancer (logOR $= -0.41$; CI: $[-0.53, -0.29]$). Considering different cancer origins, the meta-analysis showed that high OO intake is linked to a lesser probability of having cancer of the digestive tract (logOR $= -0.36$; CI: $[-0.50, -0.21]$) and breast cancer (logOR $= -0.45$; CI: $[-0.78, -0.12]$) [24].

In a clinical trial, patients with advanced cancer received a dietary intervention rich in oleic acid and Gc protein-derived macrophage activating factor (GcMAF) which can inhibit cancer cell proliferation. The diet, which was low in carbohydrates, rich in proteins, fermented milk products (which contains naturally-produced GcMAF), Vitamin D3, and omega-3 fatty acids, enhanced the immune system, and a 25% reduction in tumor volume was observed [25].

3 Neurodegenerative Diseases

Chronic inflammatory status, together with oxidative stress, affects neuron functionality. Oxidation and inflammation processes promote the deposition of a number of proteins inside neurons, consequently, the correct function of the mitochondria becomes impaired. When these processes are perpetuated over a long period of time, the development of several neurodegenerative diseases can occur. In particular, Alzheimer's disease, which is one of the greatest challenges of any national health system, given the population aging. As there is no curative treatment to date, preventive strategies based on a healthy aging are being promoted. A number of epidemiological studies suggests that several nutrients (such as antioxidants, E and B-vitamins, and polyunsaturated fatty acids) [26], and foods (such as fish,

vegetables, fruit, and wine) [27] may decline cognitive impairment, including Alzheimer's disease [28]. As oxidative stress can play a role in the development of neurodegenerative diseases [29], the intake of phenolic compounds (PCs), with a considerable spectra of bioactivities in vitro and in vivo, has been proposed for their management [30].

Given the association between cardiovascular risk factors and neurodegenerative ones, the cardiovascular health benefits of the MD can also confer protection against neurodegenerative disorders [31]. While diets rich in saturated fats and simple carbohydrates are linked to neurodegenerative diseases [32], the MD is able to enhance cognitive function. An association between MD adherence and delay in cognitive decline was observed in elderly subjects in a prospective cohort study in France [6]. Moreover, in elderly individuals at high cardiovascular risk following the MD, better cognitive efficiency [28] and performance were reported [33]. The majority of these studies have been performed in the Mediterranean areas; nevertheless, other projects have been conducted in non-Mediterranean countries which suggest that the healthy benefits of an MD can be transferred to other populations. In this respect, greater adherence to the MD pattern was linked to a reduced risk of Alzheimer's disease with approximately 2000 subjects in New York [7].

The reduced vascular comorbidities observed with an MD, together with its richness in compounds with antioxidant and anti-inflammatory effects, can play a role in central nervous system benefits [34–36]. In this regard, VOO, one of the key foods of the MD pattern, has been linked to improved cognitive function due to its relevant bioactive compounds [28]. Hydroxytyrosol, oleuropein, and especially oleuropein aglycon have been shown to inhibit Tau aggregation in vitro [37].

4 Diabetes Mellitus

4.1 Type-II Diabetes and Impaired Glucose Tolerance

Impaired glucose tolerance is defined as high blood glucose levels after eating, whereas impaired fasting glucose is defined as high blood glucose after a period of fasting. People with impaired glucose tolerance are at high risk of developing DM2. Unsurprisingly, impaired glucose tolerance shares many characteristics with DM2 and is associated with obesity, advancing age, and the inability of the body to use the insulin it produces. Not everyone with impaired glucose tolerance goes on to develop DM2 [38]. DM2 is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels [39]. Some 382 million people worldwide, or 8.3% of adults, are estimated to have diabetes. According to International Diabetes Federation, the causes are still unclear but people with overweight, poor diet, lack of physical activity, and family story of diabetes are at high risk of DM2 [38].

Several studies have found a link between MD and glucose metabolism. The ATTICA study observed that adherence to MD was related to better homoeostasis factors related to fasting glucose (fasting plasma glucose, insulin levels and the insulin resistance index: HOMA) in normoglycemic people [40]. Moreover, PREDIMED study found that a long-term intervention with a high-quality dietary pattern based on traditional MD and rich in EVOO could reduce the incidence of DM2 in older people at high cardiovascular risk. This beneficial effect was mainly due to the overall composition of the dietary pattern and not to calorie restriction, increased physical activity, or weight loss [41]. A couple of meta-analysis [42, 43] also found a significant association between adherence to dietary patterns and decreased risk of DM2. One of the previous meta-analysis specifically concluded that adherence to a MD is associated with a decreased risk of becoming diabetic at a reasonable magnitude (19%) [43]. Other systematic review of eight meta-analyses and five randomized controlled trials studied the effect of MD on the treatment of DM2 and prediabetic states. This review indicated that in DM2 patients, the adherence to MD was associated with lower glycated hemoglobin (HbA1c) levels and improved cardiovascular risk factors, as compared with control diets, mainly lower fat diets [44]. Thus, it is suggested that the Mediterranean diet is not only suitable for prevention but it is also an appropriate dietary pattern for the management of DM2.

Another meta-analysis of prospective cohort studies to assess the association between different diets and prevention of DM2 found that although the diets associated with prevention of DM2 may vary in their composition, nonetheless they shared several common components, including whole grains, fruit, vegetables, nuts, legumes, protein sources such as white meat and seafood, little or moderate alcohol, and reduced intake of red and processed meats and sugar-sweetened beverages, and noteworthy healthy table oils (i.e., olive oil) [45]. MD covers all of the above being one of its emblematic characteristics is the use of olive oil.

One of olive oil's characteristics is its high content in monounsaturated fatty acids (MUFA). Evidence suggests that diets enriched in MUFAS from OO have a positive effect in glycemic control. A study examining intakes of MUFA through the increased consumption of olive oil found an association with lower fasting plasma glucose concentration [46, 47]. Another cohort study of women found a similar result after a 22 years follow-up. The published results showed that higher olive oil intake was associated with modestly lower risk of DM2 and that hypothetically substituting other types of fats and salad dressings (stick margarine, butter, and mayonnaise) with olive oil was inversely associated with the onset of DM2 [48]. In another cross-sectional study in Spain (Pizarra study), it was found that insulin resistance was significantly lower in people who consumed OO than in those who consumed sunflower oil or a mixture [167].

Furthermore, a randomized trial with two groups of DM2 patients, following a MD using OO or a control low-fat diet presented that the MD with OO can improve endothelial dysfunction, inflammation, and oxidative in DM2 patients compared to a control diet [49]. The PREDIMED trial also provides strong evidence that long-term adherence to a MD supplemented with EVOO, which is high in MUFAS and

bioactive polyphenols, results in a substantial reduction in the risk for DM2 among older people with high cardiovascular risk [41].

Oleic acid (cis C18:1 n-9), the predominant MUFA on OO, has been related to a lower insulin resistance as well. A study addressing [50] the relationship between changes in membrane fatty acid composition and glucose transport as an index of insulin sensitivity found a reduction in insulin resistance when a polyunsaturated (linoleic acid; C18:2 n-6) rich diet was changed to an oleic acid (C18:1 n-9) rich diet. This improvement was related to the change in membrane fluidity, as an oleic acid-rich membrane would be less fluid to its linoleic acid-rich equivalent [46]. Similar results were found in another study [51] where changes in membrane fatty acids and signaling proteins induced by VOO consumption in elderly persons with DM2 compared to a control group were analyzed. The long-term consumption of VOO induced changes in the fatty acid content of erythrocyte membranes from elderly DM2 participants. These changes were due to an increase amount of oleic acid in the membranes and in consequence biochemical changes in the amount of signaling proteins (G proteins and protein kinase C). The above could explain the mechanisms of glycemic homeostasis after consumption of olive oil.

In addition to MUFA, extra virgin olive oil contains also other bioactive components, some of them are called the phenolic compounds such as oleuropein and hydroxytyrosol, flavonoids, specially flavones, and lignans. Apart from PREDIMED, other clinical trials have evaluated the effect of EVOO rich in polyphenols on glycemic biomarkers [52]. Daily consumption of polyphenol-rich EVOO (25 mL/day, 577 mg of phenolic compounds/kg) for 8 weeks significantly reduced fasting plasma glucose and HbA1c, as well as other circulating inflammatory adipokines (visfatin), in overweight patients with DM2 [53], in a crossover randomized trial.

Mediterranean-type dietary patterns are known to improve several parameters of the postprandial state with high atherogenic potential, such as the glycemic load and the secretion and clearance of chylomicrons [54]. The consumption of OOPCs has been able to decrease oxidative stress (as observed in several biomarkers related to oxidative modifications of plasma lipids, proteins, and DNA) in healthy volunteers after meals [10] and in long-term interventions [11]. Concretely, a single dose of 25 mL of OO does not promote postprandial oxidative stress whereas doses equal or superior to 40 mL do [10, 55]. In addition, phenolic compounds from OO have been able to modulate postprandial oxidative stress in healthy volunteers [10]. The glycemic load of a meal can depend on the bioavailability of carbohydrates and food preparation. In this regard, in a study with 12 women with obesity and insulin resistance, food fried in VOO improved both insulin and C peptide responses after a meal [56]. VOO may additionally contribute, within the context of a traditional MD, to the long-term improvement of the glycemic load and the dietary glycemic index [57].

In conclusion, there is already widespread experience of the benefits of olive oil in glucose metabolism, both in a medium- and long-term period and in the postprandial phase, in the context of dietary patterns, of olive oil by itself, and the bioactive compounds of olive oil.

5 Cardiovascular Diseases

The development of an atherosclerotic plaque in the endothelium of blood vessels is common to CVDs. The plaque is formed by the accumulation of cholesterol inside the macrophages and other cells, such as the smooth muscle ones, located in the intima media. This generates an inflammatory chronic response that eventually can trigger acute thrombotic vascular disease, including myocardial infarction, stroke, and sudden cardiac death [58]. Atherosclerotic plaques are linked to the coexistence of several processes related to inflammation, lipid oxidation, dysfunction of the vascular endothelium, exacerbated activation of immune cells, and migration of vascular smooth muscle cells, among others [59].

Since Ancel Keys presented the MD as a health protecting one [1], there have been many studies supporting this link [5, 7, 16, 60]. The most impressive benefits of this diet, however, have been related to reductions in cardiovascular morbidity and mortality [61]. In general, countries from southern Europe present the lowest values of accumulated incidence and mortality rate of coronary heart disease [62, 63]. The paradox of the Mediterranean countries with a low incidence rate of cardiovascular disease [64–66], in spite of a marked prevalence of classical cardiovascular risk factors [67, 68], is attributed in part to a high degree of adherence to the MD. Nevertheless, most studies were observational so that any causal inference was hindered by residual confounding among other biases. Thus, large-scale randomized trials using dietary patterns, and assessing clinical end-points, are needed to provide a high level of scientific evidence. In this regard, very few randomized trials have been performed. On one hand, the Lyon Diet Heart Study, a secondary prevention trial, showed a large reduction in rates of coronary heart disease events with a modified MD enriched with alpha-linolenic acid [69]. On the other hand, the PREDIMED study, a multicenter, randomization, intervention trial with the MD, reported the protection of the traditional Mediterranean Diet (TMD) on incident CVD in high cardiovascular risk individuals, in primary prevention [70]. In addition, the PREDIMED Study has also provided evidence of the efficacy of the TMD on primary prevention for stroke [70], atrial fibrillation [71], type-2 diabetes (DM2) [72], and peripheral vascular disease [73].

Nowadays, the relevance of overall high-quality diet patterns, rather than focusing on single nutrients and foods, has highlighted the need to address the complexity of dietary exposures. Nevertheless, it is well-known that OO plays a pivotal role in this diet pattern. Data from EPIC cohorts showed an inverse relationship between OO consumption and coronary heart disease mortality and incidence [74–76]. Also, results from the Three-City Study reported an inverse relationship between OO consumption and stroke risk in women [77]. Finally, results of the PREDIMED Study showed that consumption of OO, specifically the extra-virgin variety, within the framework of the Mediterranean diet, reduces the risk of CVD and mortality in elderly high cardiovascular risk individuals [78].

It is increasingly accepted that chronic degenerative diseases, such as CVD, cancer, and neurodegenerative ones, share common risk factors. Besides the classical

cardiovascular risk factors (diabetes, hypertension, lipid profile, tobacco, and obesity), perpetuated molecular dysregulation with respect to oxidation, low-grade inflammation, LDL atherogenicity, HDL function, and endothelial dysfunction, among others, may be behind their onset.

5.1 Arterial Hypertension and Endothelial Dysfunction

OO has also been linked to an improvement of endothelial dysfunction and blood pressure state. In a meta-analysis of randomized controlled assays ($N = 12$, with a duration of 6 months or longer) high- versus low-MUFA intake (low MUFA intake was considered a MUFA consumption below 12% of total daily energy consumption) was compared, and MUFA-rich diets decreased systolic (mean effect: −2.26 mmHg; CI: [−4.28, −0.25]; $p = 0.03$) and diastolic blood pressure (mean effect: −1.15 mmHg; CI: [−1.96, −0.34]; $p = 0.005$]. In addition, an improvement of the fat mass −1.94 kg, CI: [−3.72, −0.17], p = 0.03] was additionally reported [79]. MUFA intake also has effects on doses of antihypertensive drugs prescribed to patients. A MUFA-rich diet (17.2% of total daily energy intake by MUFAs, 3.8% by PUFAs) was able to decrease blood pressure relative to a polyunsaturated fatty acid (PUFA)-rich one (10.5% of total daily energy intake, 10.5% by PUFAs) and, moreover, decreased the daily dosage of hypertensive agents [80]. A decrease of diastolic blood pressure in hypertensive women was observed after an MD intervention, enriched with VOO or nuts, within the context of the PREDIMED Study [81]. Nitric oxide and endothelin-1 levels, together with endothelin-1 receptor gene expression, could play a role in blood pressure improvement [82].

Regarding OOPCs, in a meta-analysis with 13 studies concerning the effects of high phenolic OO oil on cardiovascular risk factors, medium effects for lowering systolic blood pressure (n = 69; mean effect: −0.52; CI: [−0.77, −0.27]; $p<0.01$) were described; however, no effects for improving diastolic blood pressure were reported (mean effect: −0.20; CI: [−1.01, 0.62]; $p = 0.64$) [83].

Endothelial function improved in hypercholesterolemic patients at postprandial state with phenol-rich OOs and OOPC-enriched functional OO intake [84, 85], after a 4-month intervention of a daily intake of VOO in patients with incipient atherosclerosis [86], and after 2 months in women with high-normal blood pressure or stage-1 essential hypertension [87].

Endothelial homeostasis can be disturbed by oxidative stress and chronic inflammatory processes and finally lead to endothelial dysfunction [58]. An increase in nitric oxide metabolites was observed after a phenol-rich OO postprandial intake versus a low-phenolic OO [84]. Also, a decrease in systolic blood pressure after the intake of VOO was described, with a decrease in lipid oxidation markers, in hypertensive stable patients [88]. Finally, in two randomized crossover studies, a 50 mL intake of phenol-rich OO decreased the postprandial leukotriene B4 (a pro-inflammatory eicosanoid) and thromboxane B2 (a vasoconstrictor eicosanoid) levels in comparison to refined OO in healthy [169] and mildly dyslipidemic subjects [89].

5.2 Haemostasis and Platelet Aggregation

Olive oil has been shown to improve the production of coagulation factors and biomarkers linked to platelet aggregation, thus improving the thrombogenetic profile [90].

With regard to monounsaturated fats, a MUFA-rich diet such as the MD decreases postprandial levels of coagulation factor VIIc [91]. In addition, oleic acid (75% of OO fatty acids) attenuates the prothrombotic state in the postprandial phase [91–93].

Regarding OOPCs, the consumption of phenol-rich OOs improved the postprandial prothrombotic state (activated coagulation factor VII, tissue factor, tissue plasminogen activator, plasminogen activator inhibitor type-1, and fibrinogen) in a number of randomized controlled trials in both healthy [94] and hypercholesterolemic subjects [90]. In long-term interventions, a decrease in plasma fibrinogen in women with high baseline fibrinogen concentrations has been reported after OO consumption, in a randomized crossover trial [95].

5.3 Lipid Profile

Improved dietary fat quality, achieved through a replacement of SFA by unsaturated fat, enhances the lipid profile by reducing low density lipoprotein (LDL) cholesterol and increasing high-density lipoprotein (HDL) cholesterol [96]. Olive oil intake assures an increase of MUFA, without a significant rise of SFA, and guarantees an appropriate intake of (PUFA). As a consequence, the MUFA/SFA ratio is much higher in areas where an MD is followed [97]. An updated review suggested a small, but potentially relevant, reduction in cardiovascular risk with low saturated fat intake [98]. The replacement of energy from saturated fat with polyunsaturated fat provides more healthy benefits than with carbohydrate. Regarding the substitution of SFAs for MUFAs, although the benefits are more modest, some positive effects on lipid profile have been observed. The reduction of dietary saturated fat, and partial replacement by unsaturated fats, has been proposed to achieve maximum health benefits although the ideal type of unsaturated fat is still unclear [98].

Schwingshackl et al. published a meta-analysis including 32 studies with overweight and obese patients. On one hand, decreases in total cholesterol (weighted mean difference −0.12 mmol/L, 95% CI: [−0.28 to −0.03]; P = 0.01) and LDL cholesterol (−0.08 mmol/L, 95% CI: [−0.12 to −0.04]; P<0.0001) were more marked after low-fat diets. On the other hand, a rise in HDL cholesterol (0.06 mmol/L, 95% CI: [0.03, 0.09]; P<0.0001) and a reduction in triglyceride (−0.095 mmol/L, 95% CI: [−0.15, −0.04]; P = 0.001) were more pronounced after the high-fat diet groups [99].

Regarding OO, in a recent meta-analysis about the effects of PC-rich OO on cardiovascular risk factors, no significant effects in improving lipid profile were observed (total cholesterol $N = 400$; mean effect: −0.05; CI: [−0.16, 0.05]; $p = 0.33$); HDL cholesterol ($N = 400$; mean effect: −0.03; CI: [−0.14, 0.08]; $p = 0.62$); LDL cholesterol ($N = 400$; mean effect: −0.03; CI: [−0.15, 0.09]; $p = 0.61$); and triglycerides ($N = 360$; mean effect: 0.02; CI: [−0.22, 0.25];

$p = 0.90$). Nevertheless, the small number of studies ($N = 8$) is a limitation [83]. Specifically, the beneficial effects of OO polyphenols on blood HDL cholesterol concentrations were evaluated by the European Food Safety Authority (EFSA) and they concluded that evidence was insufficient to establish a cause-effect relationship [100]. A recent review indicated that the intake of PC-rich OO induced no significant increases in HDL cholesterol levels [83], while several high-quality randomized controlled trials have pointed to a dose-dependent increment in HDL cholesterol after the consumption of OOPCs [11, 12, 101].

5.4 Lipid Oxidation

In November 2004, the USA Federal Drug Administration [102] permitted a claim on OO labels regarding "the benefits on the risk of coronary heart disease of taking 2 tablespoons (23 grams) of OO daily, due to monounsaturated fat." However, if the effect of OO is only attributed to its MUFA content, any type of MUFA-rich food (such as rapeseed oil, canola oil, or MUFA-enriched fats) would provide the same beneficial effects for health. The minor components of OO (1–2% of the total content) are classified in two groups: (1) the unsaponifiable: squalene and other triterpenes, sterols, tocopherol, carotenoids, and pigments and (2) the soluble fraction which includes the PCs [168]. In this respect, the EFSA released a health claim concerning the protection of OOPC (5 mg/day) against LDL oxidation [103]. Based on well-designed intervention trials, a cause-effect relationship was established by the EFSA between the consumption of VOO and the protection of LDL particles from oxidative damage [103].

Not only changes in LDL cholesterol are involved in cardiovascular risk, oxidation modifications of the LDL particle may play a major role in atherosclerosis [58, 104, 105]. Several OO components contribute to decreasing LDL oxidizability. On the one hand, MUFAs are less prone to becoming oxidized when compared to other unsaturated fats [106]. On the other hand, OO antioxidants (vitamin E, carotenoids, and OOPCs) can bind to the LDL particle and protect it from oxidative modifications.

A recent meta-analysis ($N = 13$) reported that oxidized LDL levels decrease significantly after the consumption of high-phenolic OO ($N = 300$; mean effect: -0.25; CI: $[-0.50, 0.00]$; $p = 0.05$) [83]. In the EUROLIVE Study, 200 volunteers were given 25 mL/day of raw OO with high (366 mg/kg), medium (164 mg/kg), and low (3 mg/kg) phenolic content in a randomized, crossover, and controlled trial [11]. Covas et al. reported a decrease of the in vivo lipid oxidative damage (concretely in oxidized LDL, uninduced conjugated dienes, and hydroxy fatty acids) in a linear manner with the phenolic content of the OO administered [11]. As potential mechanisms to explain this benefit, we could consider an increase in the content of vitamin E and OOPC in LDL that may counteract locally oxidative modifications [107, 108]. In addition, the reductive effect of the OOPC on oxidized LDL could be due to the generation of antibodies [109]. Vázquez-Velasco et al. observed, in healthy volunteers, a decrease in the concentration of oxidized LDL when

hydroxytyrosol-enriched sunflower oil (45–50 mg/d) was administered during 3 weeks [110]. In 40 males suffering from stable coronary heart disease, VOO was able to decrease the oxidized LDL in plasma compared with another olive oil with a smaller amount of PC in a randomized crossover trial [88]. Finally, LDL oxidizability has also been evaluated in vitro, and it decreased after the consumption of MUFAs [111, 112] and phenol-rich OOs [11, 101, 113, 114].

Finally, Fitó et al. reported an improvement of circulating oxidized LDL after a 3-month MD intervention in high cardiovascular risk patients [115].

5.5 HDL Functionality

Although low levels of HDL cholesterol are considered an independent cardiovascular risk factor [116], it has been recently observed that presenting high HDL cholesterol levels does not always lead to a decrease in cardiovascular risk [117–119]. An increase in HDL cholesterol is one of the goals of clinical management of cardiovascular diseases [117]. In this regard, recent studies have shown that the functionality of HDL can be of greater relevance than its amount [120]. Decreased values of the most relevant HDL function have been reported to be related to a high incidence of subclinical atherosclerosis [121] and coronary events [122].

The functionality of the HDL particle involves the promotion of cholesterol efflux from macrophages and peripheral cells, which constitutes part of the so-called "reverse cholesterol transport" [123]. Furthermore, HDLs play a crucial role in inhibiting the oxidation of plasma lipids (mainly, the ones in LDLs) and also present anti-inflammatory and vasoprotective capacities [124]. Oxidative modifications of the HDL can additionally affect the lipid and/or protein of the lipoprotein and alter its physiological properties [120, 124–126] and, in this regard, antioxidants linked to the particle could be able, direct or indirectly, to counteract such oxidation. An increment in HDL fluidity, enhanced HDL composition, and better HDL size distribution could also mediate improvements in HDL function [127–129].

Regarding OO, the consumption of a MUFA-rich diet improved the cholesterol efflux capacity of HDLs in a linear trial [130]. This functional improvement could be due to a lower degree of oxidative modifications of the lipoprotein after increasing its MUFA content [131]. Regarding OOPCs, Hernáez et al. reported for the first time an increase in cholesterol efflux after the daily intake of 25 mL of VOO (366 mg/kg) in healthy volunteers within the framework of the EUROLIVE Study [132]. In parallel, biological metabolites of OOPC bound to the HDLs (hydroxytyrosol sulfate, and homovanillic acid sulfate, and glucuronate) were determined. The improvement of HDL fluidity and a triglyceride-poor core can also result in a more functional HDL particle [132]. In this respect, functional VOO supplemented with olive and thyme phenols versus a VOO intervention produced an increase in the lecithin-cholesterol acyltransferase concentration which esterifies free cholesterol and mediates its migration into the particle core [133]. The consumption of OOPCs has also been shown to be able to increase the levels of the main HDL antioxidant enzyme, paraoxonase-1, as well as HDL anti-inflammatory ability, in a noncontrolled trial [134]. Besides the direct

antioxidant effect of OOPCs on HDL particles, these compounds have also been observed to be able to improve the gene expression related to HDL function [135].

Finally, a TMD, especially when supplemented with real-life doses VOO, was able to improve HDL functionality [136]. Concretely, a 1-year intervention with an MD increased cholesterol efflux capacity and, in particular, the VOO-rich MD enhanced nitric oxide synthesis by endothelial cells promoted by HDLs, decreased cholesteryl ester transfer protein activity, and increased HDL ability to esterify cholesterol and paraoxonase-1 arylesterase activity [136].

5.6 Inflammatory Processes and Systemic Oxidative Status

Oxidation and inflammation are intertwined processes which, when sustained for a long period, may induce the onset of a number of chronic degenerative diseases, such as CVD, diabetes, neurodegenerative diseases, and cancer. The increased circulating concentrations of pro-inflammatory analytes (tumor necrosis factor-α, monocyte chemotactic protein-1, soluble vascular cell adhesion molecule 1, and soluble intercellular adhesion molecule-1) perpetuate the inflammatory response in the subendothelial space and establish endothelial dysfunction.

Traditionally, the health benefits of the MD have been attributed to its richness in antioxidants. Among the approximately 230 chemical compounds of OO, the main antioxidants are carotenes and phenolic compounds, including lipophilic and hydrophilic phenols [137]. Current evidence points to oxidative damage as a promoter of pathophysiological processes in oxidative stress-related diseases such as coronary heart disease, cancer, neurodegenerative pathologies, and, in addition, aging [138–140]. Although phenolic compounds are good antioxidants in vitro, their in vivo effects can be indirectly mediated through the activation of several nutrigenomic pathways and not only by their intrinsic antioxidant activity [141, 170]. Benefits of olive oil on lipid oxidation have been extensively explained in the Sect. 5.4 apart.

Several benefits on the levels of pro-inflammatory biomarkers have been proven in human trials with OO or MUFA-rich diets. A daily intake of real-life doses of OO has been shown to produce a decrease of the C-reactive protein (mean effect: −0.64 mg/L; CI: [−0.96, −0.31]; $p<0.0001$) and IL-6 (mean effect: −0.29; CI: [−0.70, −0.02]; $p<0.04$) in a recent systematic review [43]. Nevertheless, the heterogeneity of design among studies makes further research necessary. With regard to other MUFA-rich diets, a randomized controlled trial with 28 hypertriglyceridemic and 14 healthy males who followed a diet rich in refined OO (high-oleic acid diet) or a diet rich in high-palmitic sunflower oil, revealed a postprandial decrease of the soluble adhesion molecules (VCAM-1, ICAM-1) after the high-oleic intervention [142]. And in another randomized controlled trial, a 2-month MUFA-rich diet decreased the expression by peripheral blood mononuclear cells of ICAM-1 in healthy males [143]. In contrast, a randomized crossover trial in healthy subjects regarding the effect of three Malaysian diets: palm olein, coconut oil, and OO (the fat source providing approximately 20% of total energy intake in

each case), the postprandial and 2-week fasting circulating concentrations of a number of inflammatory biomarkers (tumor necrosis factor-α, IL-1β, IL-6, IL-8, CRP, and interferon-γ) were not affected [144].

The consumption of OO, which provides oleic acid as the main fatty acid, also yields a moderate intake of PUFA (mainly omega-6 linoleic acid and omega-3 alpha-linolenic acid) without a noticeable increase in the intake of SFA. Omega-6 fatty acids, in which PUFAs are predominant (especially in the Western diets), are precursors of pro-inflammatory eicosanoids (2-prostaglandins, tromboxanes, and 4-leukotrienes) [145]. In this regard, omega-3 fatty acids in OO may play a role in the inhibition of the inflammatory process boosted by omega-6 fatty acids [146]. It has been reported that a low incidence of coronary heart disease is related to a diet rich in omega-3 fatty acids [147].

The OOPCs have also shown effects on pro-inflammatory biomarkers. Surprisingly, Beauchamp et al. described oleocanthal anti-inflammatory properties that were similar to those of ibuprofen (cyclooxygenase-2 inhibition and nuclear factor kappa beta counteract) [148]. Moreover, the intake of phenol-enriched OO (50 mL/day) decreased C-reactive protein (CRP) levels at postprandial state relative to refined olive or corn oils in healthy individuals, in a randomized controlled trial. In this regard, a regular intake of OO at long term can contribute to controlling the postprandial inflammatory burden [149]. Also, a combined consumption of white wine (2–3 glasses/daily) and extra VOO (*ad libitum*) for a 2-week period decreased the levels of CRP and IL-6 in both patients with chronic kidney disease and healthy subjects [150]. Finally, a raw real-life daily dose of 50 mL of VOO for 3 weeks decreased the IL-6 and CRP concentrations (versus refined OO) in a randomized controlled trial with stable coronary heart disease patients [151].

Within the context of a traditional MD, supplementation with VOO or nuts decreased the systemic levels of CRP, IL-6, sVCAM1, and sICAM1 in high cardiovascular risk volunteers within the framework of the PREDIMED Study [81]. In addition, the traditional MD (supplemented with VOO or nuts) was able after 3 months of intervention to decrease the expression of CD49d (an adhesion molecule) and CD40 (a pro-inflammatory ligand) in monocytes isolated from the blood of high cardiovascular risk volunteers [152].

The MD participants had lower plasma concentrations of atherosclerosis-related inflammatory markers (IL-6, IL-8, MCP-1, and MIP-1β) after 3 and 5 years of an MD intervention versus a low-fat diet control group. In addition, IL-1β, IL-5, IL-7, IL-12p70, IL-18, TNF-α, IFN-γ, GCSF, GMCSF, and ENA78 decreased especially after an MD supplemented with VOO [153]. Finally, the reduction in CD49d and CD40 expressions in T lymphocytes and monocytes at 3 years were greater in the MD group than in the low-fat diet control one [154].

5.7 Immune System

Regarding MUFAs, they have been reported to modulate a number of biological pathways of immune competent cells [155]. In this regard, in a randomized

controlled trial with healthy males, there was a significant decrease in the expression of intercellular adhesion molecule 1 by peripheral blood mononuclear cells after MUFA-rich 2 month-diet. Nevertheless, natural killer cell activity and the proliferation of mitogen-stimulated leukocytes were not affected [143].

The consumption of a functional VOO showed an increase in the proportion of IgA-coated bacteria, which indicates a local stimulation of the intestinal mucosal immunity [156]. Based on the effects of OO intake on the immune system, it has been suggested that OO consumption may have benefits on rheumatoid arthritis [155]. In contrast, other authors have published that OO-rich diets do not alter host resistance to infection [157, 158]. Given that data are scarce, more studies are required to establish the possible immunostimulation of OO in vivo.

5.8 Microbiome

Gut microbiota, a complex and dynamic ecosystem, is an emergent factor of a number of diseases including obesity and type-II diabetes [159]. PCs can selectively stimulate growth bacteria, such as Lactobacillus [160], which can play a role in lowering cholesterol levels [161]. Intestinal lactobacilli produce bile-salt hydrolase, which deconjugates bile acids, prevents their reabsorption, and therefore promotes their fecal excretion [162]. This can be one of the mechanisms involved in the decrease of systemic cholesterol after PC consumption [163]. PCs may also be related to the growth of other bacterial populations (such as Bifidobacterium) which might be linked to a lesser development of the atherosclerotic plaque [164].

Since most PCs are not fully absorbed into the upper gastrointestinal tract and reach the large intestine, they can be metabolized by gut microflora [165]. A 3-week intake of a phenol-enriched VOO (with OO and thyme PCs) increased populations of Bifidobacteria and the levels of microbial metabolites of antioxidant PCs, such as protocatechuic acid and hydroxytyrosol, in hypercholesterolemic individuals [166].

6 Conclusion

In summary, current evidence indicates the potential benefits of olive oil, within the framework of a healthy diet such as the Mediterranean one, on the prevention of chronic degenerative diseases including cardiovascular and degenerative ones. To date, the majority of these effects have been demonstrated from the perspectives of atherosclerosis and cardiovascular risk research. Nevertheless, there is increasing evidence that such beneficial properties also display a protection towards other diseases including neurodegenerative ones and cancer.

The daily diet represents the consumption of a mixture of foods in which the type of cooking plays an indisputable role in the availability and properties of nutrients. Furthermore, synergism among nutrients and foods, and their cumulative effects, must also be taken into consideration. With respect to the

Mediterranean diet, olive oil as the main source of fat is determinant with respect to the properties attributed to this dietary pattern.

Olive oil intake facilitates more vegetable consumption and thus benefits on health can be maximized. Nutrient-specific biases and type of olive oil should be considered in order to disentangle the benefits of MUFA and/or other minor components. Further well-designed, large-scale cohort studies in Mediterranean and non-Mediterranean areas are needed to reaffirm the therapeutic properties of olive oil and establish in which subjects and under which conditions benefits can be more easily achieved.

Acknowledgments This work was supported by: *Agència de Gestió d'Ajuts Universitaris i de Recerca* (2014-SGR-240), *Instituto de Salud Carlos III* (CB06/03/0028, JR14/00008, JR17/00022, CD17/00122, and CES12/025), and the Spanish Ministry of Education, Culture and Sport (FPU12/01318). The *CIBER de Fisiopatología de la Obesidad y Nutrición* (CIBEROBN) is an initiative of the *Instituto de Salud Carlos III*.

References

1. Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, Djordjevic BS, Dontas AS, Fidanza F, Keys MH (1986) The diet and 15-year death rate in the seven countries study. Am J Epidemiol 124:903–915
2. Serra-Majem L, Roman B, Estruch R (2006) Scientific evidence of interventions using the Mediterranean diet: a systematic review. Nutr Rev 64:S27–S47
3. Sofi F, Macchi C, Abbate R, Gensini GF, Casini A (2013) Mediterranean diet and health. Biofactors 39:335–342
4. Trichopoulou A (2004) Traditional Mediterranean diet and longevity in the elderly: a review. Public Health Nutr 7:943–947
5. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D (2003) Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 348:2599–2608
6. Féart C, Samieri C, Rondeau V, Amieva H, Portet F, Dartigues J-F, Scarmeas N, Barberger-Gateau P (2009) Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 302:638–648
7. Scarmeas N, Stern Y, Tang M-X, Mayeux R, Luchsinger JA (2006) Mediterranean diet and risk for Alzheimer's disease. Ann Neurol 59:912–921
8. Urpi-Sarda M, Casas R, Chiva-Blanch G, Romero-Mamani ES, Valderas-Martínez P, Arranz S, Andres-Lacueva C, Llorach R, Medina-Remón A, Lamuela-Raventos RM, Estruch R (2012) Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomakers related to atherosclerosis. Pharmacol Res 65:577–583
9. Schwingshackl L, Hoffmann G (2014) Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. Lipids Health Dis 13:154
10. Covas M-I, de la Torre K, Farré-Albaladejo M, Kaikkonen J, Fitó M, López-Sabater C, Pujadas-Bastardes MA, Joglar J, Weinbrenner T, Lamuela-Raventós RM, de la Torre R (2006a) Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. Free Radic Biol Med 40:608–616
11. Covas M-I, Nyyssönen K, Poulsen HE, Kaikkonen J, Zunft HF, Kiesewetter H, Gaddi A, de la Torre R, Mursu J, Bäumler H, Nascetti S, Salonen JT, Fitó M, Virtanen J, Marrugat J (2006b) The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. Ann Intern Med 145:333–341
12. Weinbrenner T, Fitó M, de la Torre R, Saez GT, Rijken P, Tormos C, Coolen S, Albaladejo MF, Abanades S, Schroder H, Marrugat J, Covas M-I (2004a) Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. J Nutr 134:2314–2321

13. Donaldson MS (2004) Nutrition and cancer: a review of the evidence for an anti-cancer diet. Nutr J 3:19
14. Block G, Patterson B, Subar A (1992) Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 18:1–29
15. Bradbury KE, Appleby PN, Key TJ (2014) Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). Am J Clin Nutr 100:394S–398S
16. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A (2008) Adherence to Mediterranean diet and health status: meta-analysis. BMJ 337:a1344
17. Trichopoulou A, Lagiou P, Kuper H, Trichopoulos D (2000) Cancer and Mediterranean dietary traditions. Cancer Epidemiol Biomarkers Prev 9:869–873
18. Toledo E, Salas-Salvadó J, Donat-Vargas C, Buil-Cosiales P, Estruch R, Ros E, Corella D, Fitó M, Hu FB, Arós F, Gómez-Gracia E, Romaguera D, Ortega-Calvo M, Serra-Majem L, Pintó X, Schröder H, Basora J, Sorlí JV, Bulló M, Serra-Mir M, Martínez-González MA (2015) Mediterranean diet and invasive breast cancer risk among women at high cardiovascular risk in the PREDIMED trial: a randomized clinical trial. JAMA Intern Med 175:1752–1760
19. Buckland G, Travier N, Agudo A, Fonseca-Nunes A, Navarro C, Lagiou P, Demetriou C, Amiano P, Dorronsoro M, Chirlaque MD, Huerta JM, Molina E, Pérez MJS, Ardanaz E, Moreno-Iribas C, Quirós JR, Naska A, Trichopoulos D, Giurdanella MC, Tumino R, Agnoli C, Grioni S, Panico S, Mattiello A, Masala G, Sacerdote C, Polidoro S, Palli D, Trichopoulou A, González CA (2012b) Olive oil intake and breast cancer risk in the Mediterranean countries of the European Prospective Investigation into Cancer and Nutrition study. Int J Cancer 131:2465–2469
20. Buckland G, Agudo A, Luján L, Jakszyn P, Bueno-de-Mesquita HB, Palli D, Boeing H, Carneiro F, Krogh V, Sacerdote C, Tumino R, Panico S, Nesi G, Manjer J, Regnér S, Johansson I, Stenling R, Sanchez M-J, Dorronsoro M, Barricarte A, Navarro C, Quirós JR, Allen NE, Key TJ, Bingham S, Kaaks R, Overvad K, Jensen M, Olsen A, Tjønneland A, Peeters PHM, Numans ME, Ocké MC, Clavel-Chapelon F, Morois S, Boutron-Ruault M-C, Trichopoulou A, Lagiou P, Trichopoulos D, Lund E, Couto E, Boffeta P, Jenab M, Riboli E, Romaguera D, Mouw T, González CA (2010) Adherence to a Mediterranean diet and risk of gastric adenocarcinoma within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study. Am J Clin Nutr 91:381–390
21. Castro M, Romero C, de Castro A, Vargas J, Medina E, Millán R, Brenes M (2012) Assessment of Helicobacter pylori eradication by virgin olive oil. Helicobacter 17:305–311
22. Stamatiou K, Delakas D, Sofras F (2007) Mediterranean diet, monounsaturated: saturated fat ratio and low prostate cancer risk. A myth or a reality? Minerva Urol Nefrol 59:59–66
23. Martin-Moreno JM (2000) The role of olive oil in lowering cancer risk: is this real gold or simply pinchbeck? J Epidemiol Community Health 54:726–727
24. Psaltopoulou T, Kosti RI, Haidopoulos D, Dimopoulos M, Panagiotakos DB (2011) Olive oil intake is inversely related to cancer prevalence: a systematic review and a meta-analysis of 13,800 patients and 23,340 controls in 19 observational studies. Lipids Health Dis 10:127
25. Ruggiero M, Ward E, Smith R, Branca JJV, Noakes D, Morucci G, Taubmann M, Thyer L, Pacini S (2014) Oleic Acid, deglycosylated vitamin D-binding protein, nitric oxide: a molecular triad made lethal to cancer. Anticancer Res 34:3569–3578
26. Roberts RO, Cerhan JR, Geda YE, Knopman DS, Cha RH, Christianson TJH, Pankratz VS, Ivnik RJ, O'Connor HM, Petersen RC (2010) Polyunsaturated fatty acids and reduced odds of MCI: the Mayo Clinic Study of Aging. J Alzheimers Dis 21:853–865
27. Polidori MC, Praticó D, Mangialasche F, Mariani E, Aust O, Anlasik T, Mang N, Pientka L, Stahl W, Sies H, Mecocci P, Nelles G (2009) High fruit and vegetable intake is positively correlated with antioxidant status and cognitive performance in healthy subjects. J Alzheimers Dis 17:921–927
28. Valls-Pedret C, Lamuela-Raventós RM, Medina-Remón A, Quintana M, Corella D, Pintó X, Martínez-González MÁ, Estruch R, Ros E (2012) Polyphenol-rich foods in the Mediterranean diet are associated with better cognitive function in elderly subjects at high cardiovascular risk. J Alzheimers Dis 29:773–782

29. Gandhi S, Abramov AY (2012) Mechanism of oxidative stress in neurodegeneration. Oxid Med Cell Longev 2012:428010
30. Ramassamy C (2006) Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. Eur J Pharmacol 545:51–64
31. Williams RJ, Spencer JPE (2012) Flavonoids, cognition, and dementia: actions, mechanisms, and potential therapeutic utility for Alzheimer disease. Free Radic Biol Med 52:35–45
32. Kanoski SE, Davidson TL (2011) Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. Physiol Behav 103:59–68
33. Martínez-Lapiscina EH, Clavero P, Toledo E, San Julián B, Sanchez-Tainta A, Corella D, Lamuela-Raventós RM, Martínez JA, Martínez-Gonzalez MÁ (2013) Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. J Nutr Health Aging 17:544–552
34. Larsson SC (2014) Coffee, tea, and cocoa and risk of stroke. Stroke 45:309–314
35. Rodríguez-Campello A, Jiménez-Conde J, Ois Á, Cuadrado-Godia E, Giralt-Steinhauer E, Schroeder H, Romeral G, Llop M, Soriano-Tárraga C, Garralda-Anaya M, Roquer J (2014) Dietary habits in patients with ischemic stroke: a case-control study. PLoS One 9:e114716
36. Wang Z-M, Zhao D, Nie Z-L, Zhao H, Zhou B, Gao W, Wang L-S, Yang Z-J (2014) Flavonol intake and stroke risk: a meta-analysis of cohort studies. Nutrition 30:518–523
37. Daccache A, Lion C, Sibille N, Gerard M, Slomianny C, Lippens G, Cotelle P (2011) Oleuropein and derivatives from olives as Tau aggregation inhibitors. Neurochem Int 58:700–707
38. International Diabetes Ferderation (2013) IDF diabetes atlas, 6th edn [Internet]. Available from: https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/19-atlas-6th-edition.html
39. WHO (2017) Diabetes – fact sheet. http://www.who.int/mediacentre/factsheets/fs312/en
40. Panagiotakos DB, Tzima N, Pitsavos C, Chrysohoou C, Zampelas A, Toussoulis D et al (2007) The association between adherence to the mediterranean diet and fasting indices of glucose homoeostasis: the ATTICA Study. J Am Coll Nutr 26(1):32–38
41. Salas-Salvadó J, Bulló M, Estruch R, Ros E, Covas MI, Ibarrola-Jurado N et al (2014) Prevention of diabetes with mediterranean diets: a subgroup analysis of randomized trial. Ann Intern Med 160(1):1–11
42. Koloverou E, Esposito K, Giugliano D, Panagiotakos D (2014) The effect of Mediterranean diet on the development of type 2 diabetes mellitus: a meta-analysis of 10 prospective studies and 136,846 participants. Metabolism 63(7):903–911. https://doi.org/10.1016/j.metabol.2014.04.010
43. Schwingshackl L, Missbach B, König J, Hoffmann G (2015) Adherence to a Mediterranean diet and risk of diabetes: a systematic review and meta-analysis. Public Health Nutr 18 (7):1292–1299
44. Esposito K, Maiorino MI, Bellastella G, Chiodini P, Panagiotakos D, Giugliano D (2015) A journey into a Mediterranean diet and type 2 diabetes: a systematic review with meta-analyses. BMJ Open 5(8):e008222
45. Esposito K, Chiodini P, Maiorino MI, Bellastella G, Panagiotakos D, Giugliano D (2014) Which diet for prevention of type 2 diabetes? A meta-analysis of prospective studies. Endocrine 47(1):107–116
46. Tierney AC, Roche HM (2007) The potential role of olive oil-derived MUFA in insulin sensitivity. Mol Nutr Food Res 51(10):1235–1248
47. Trevisan M, Krogh V, Freudenheim J, Blake A, Muti P, Panico S et al (1990) Consumption of olive oil, butter and vegetable oils and coronary heart disease risk factors. The Research Group ATS-RF2 of the Italian National Research Council. J Am Med Assoc 26:688–692
48. Guasch-Ferre M, Hruby A, Salas-salvado J, Martinez-Gonzalez MA, Sun Q, Willett WC et al (2015) Olive oil consumption and risk of type 2 diabetes in US women. Am J Clin Nutr 102 (1):479–486
49. Ceriello A, Esposito K, La Sala L, Pujadas G, De Nigris V, Testa R et al (2014) The protective effect of the Mediterranean diet on endothelial resistance to GLP-1 in type 2 diabetes: a preliminary report. Cardiovasc Diabetol 13(1):1–9

50. Ryan M (2000) Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. QJM [Internet] 93(2):85–91 Available from: https://academic.oup.com/qjmed/article-lookup/doi/10.1093/qjmed/93.2.85
51. Perona JS, Vögler O, Sánchez-Domínguez JM, Montero E, Escribá PV, Ruiz-Gutierrez V (2007) Consumption of virgin olive oil influences membrane lipid composition and regulates intracellular signaling in elderly adults with type 2 diabetes mellitus. Journals Gerontol – Ser A Biol Sci Med Sci 62(3):256–263
52. Guasch-Ferre M, Merino J, Sun Q, Fito M, Salas-Salvado J (2017) Dietary polyphenols, Mediterranean diet, prediabetes, and type 2 diabetes: a narrative review of the evidence. Oxid Med Cell Longev 2017:6723931
53. Santangelo C, Filesi C, Varì R, Scazzocchio B, Filardi T, Fogliano V et al (2016) Consumption of extra-virgin olive oil rich in phenolic compounds improves metabolic control in patients with type 2 diabetes mellitus: a possible involvement of reduced levels of circulating visfatin. J Endocrinol Invest 39(11):1295–1301
54. Lairon D (2008) Macronutrient intake and modulation on chylomicron production and clearance. Atheroscler Suppl 9:45–48
55. Weinbrenner T, Fitó M, Farré Albaladejo M, Saez GT, Rijken P, Tormos C, Coolen S, De La Torre R, Covas MI (2004b) Bioavailability of phenolic compounds from olive oil and oxidative/antioxidant status at postprandial state in healthy humans. Drugs Exp Clin Res 30:207–212
56. Farnetti S, Malandrino N, Luciani D, Gasbarrini G, Capristo E (2011) Food fried in extra-virgin olive oil improves postprandial insulin response in obese, insulin-resistant women. J Med Food 14:316–321
57. Rodríguez-Rejón AI, Castro-Quezada I, Ruano-Rodríguez C, Ruiz-López MD, Sánchez-Villegas A, Toledo E, Artacho R, Estruch R, Salas-Salvadó J, Covas MI, Corella D, Gómez-Gracia E, Lapetra J, Pintó X, Arós F, Fiol M, Lamuela-Raventós RM, Ruiz-Gutierrez V, Schröder H, Ros E, Martínez-González MÁ, Serra-Majem L (2014) Effect of a Mediterranean diet intervention on dietary glycemic load and dietary glycemic index: the PREDIMED Study. J Nutr Metab 2014:985373
58. Ross R (1999) Atherosclerosis – an inflammatory disease. N Engl J Med 340:115–126
59. Libby P, Ridker PM, Hansson GK (2009) Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol 54:2129–2138
60. Benetou V, Trichopoulou A, Orfanos P, Naska A, Lagiou P, Boffetta P, Trichopoulos D, Greek EPIC cohort (2008) Conformity to traditional Mediterranean diet and cancer incidence: the Greek EPIC cohort. Br J Cancer 99:191–195
61. Parikh P, McDaniel MC, Ashen MD, Miller JI, Sorrentino M, Chan V, Blumenthal RS, Sperling LS (2005) Diets and cardiovascular disease: an evidence-based assessment. J Am Coll Cardiol 45:1379–1387
62. Dégano IR, Elosua R, Marrugat J (2013) Epidemiology of acute coronary syndromes in Spain: estimation of the number of cases and trends from 2005 to 2049. Rev española Cardiol (English ed) 66:472–481
63. Tunstall-Pedoe H, Kuulasmaa K, Mähönen M, Tolonen H, Ruokokoski E, Amouyel P (1999) Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. Lancet (London, England) 353:1547–1557
64. Gjonça A, Bobak M (1997) Albanian paradox, another example of protective effect of Mediterranean lifestyle? Lancet (London, England) 350:1815–1817
65. Masiá R, Pena A, Marrugat J, Sala J, Vila J, Pavesi M, Covas M, Aubó C, Elosua R (1998) High prevalence of cardiovascular risk factors in Gerona, Spain, a province with low myocardial infarction incidence. REGICOR Investigators. J Epidemiol Community Health 52:707–715
66. Renaud S, de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet (London, England) 339:1523–1526

67. Aravanis C, Corcondilas A, Dontas AS, Lekos D, Keys A (1970) Coronary heart disease in seven countries. IX. The Greek islands of Crete and Corfu. Circulation 41:88–100
68. McGovern PG, Pankow JS, Shahar E, Doliszny KM, Folsom AR, Blackburn H, Luepker RV (1996) Recent trends in acute coronary heart disease – mortality, morbidity, medical care, and risk factors. The Minnesota Heart Survey Investigators. N Engl J Med 334:884–890
69. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation 99:779–785
70. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez JA, Martínez-González MA (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med 368:1279–1290
71. Martínez-González MÁ, Toledo E, Arós F, Fiol M, Corella D, Salas-Salvadó J, Ros E, Covas MI, Fernández-Crehuet J, Lapetra J, Muñoz MA, Fitó M, Serra-Majem L, Pintó X, Lamuela-Raventós RM, Sorlí JV, Babio N, Buil-Cosiales P, Ruiz-Gutierrez V, Estruch R, Alonso A, PREDIMED Investigators (2014) Extravirgin olive oil consumption reduces risk of atrial fibrillation: the PREDIMED (Prevención con Dieta Mediterránea) trial. Circulation 130:18–26
72. Salas-Salvadó J, Bulló M, Babio N, Martínez-González MÁ, Ibarrola-Jurado N, Basora J, Estruch R, Covas MI, Corella D, Arós F, Ruiz-Gutiérrez V, Ros E, Study Investigators PREDIMED (2011) Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. Diabetes Care 34:14–19
73. Ruiz-Canela M, Estruch R, Corella D, Salas-Salvadó J, Martínez-González MA (2014) Association of Mediterranean diet with peripheral artery disease. JAMA 311:415
74. Bendinelli B, Masala G, Saieva C, Salvini S, Calonico C, Sacerdote C, Agnoli C, Grioni S, Frasca G, Mattiello A, Chiodini P, Tumino R, Vineis P, Palli D, Panico S (2011) Fruit, vegetables, and olive oil and risk of coronary heart disease in Italian women: the EPICOR Study. Am J Clin Nutr 93:275–283
75. Buckland G, Mayén AL, Agudo A, Travier N, Navarro C, Huerta JM, Chirlaque MD, Barricarte A, Ardanaz E, Moreno-Iribas C, Marin P, Quirós JR, Redondo M-L, Amiano P, Dorronsoro M, Arriola L, Molina E, Sanchez M-J, Gonzalez CA (2012a) Olive oil intake and mortality within the Spanish population (EPIC-Spain). Am J Clin Nutr 96:142–149
76. Buckland G, Travier N, Barricarte A, Ardanaz E, Moreno-Iribas C, Sánchez M-J, Molina-Montes E, Chirlaque MD, Huerta JM, Navarro C, Redondo ML, Amiano P, Dorronsoro M, Larrañaga N, Gonzalez CA (2012c) Olive oil intake and CHD in the European Prospective Investigation into Cancer and Nutrition Spanish cohort. Br J Nutr 108:2075–2082
77. Samieri C, Féart C, Proust-Lima C, Peuchant E, Tzourio C, Stapf C, Berr C, Barberger-Gateau P (2011) Olive oil consumption, plasma oleic acid, and stroke incidence: the Three-City Study. Neurology 77:418–425
78. Guasch-Ferré M, Hu FB, Martínez-González MA, Fitó M, Bulló M, Estruch R, Ros E, Corella D, Recondo J, Gómez-Gracia E, Fiol M, Lapetra J, Serra-Majem L, Muñoz MA, Pintó X, Lamuela-Raventós RM, Basora J, Buil-Cosiales P, Sorlí JV, Ruiz-Gutiérrez V, Martínez JA, Salas-Salvadó J (2014) Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. BMC Med 12:78
79. Schwingshackl L, Strasser B, Hoffmann G (2011) Effects of monounsaturated fatty acids on glycaemic control in patients with abnormal glucose metabolism: a systematic review and meta-analysis. Ann Nutr Metab 58:290–296
80. Ferrara LA, Raimondi AS, d'Episcopo L, Guida L, Dello Russo A, Marotta T (2000) Olive oil and reduced need for antihypertensive medications. Arch Intern Med 160:837–842
81. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, Fiol M, Gómez-Gracia E, López-Sabater MC, Vinyoles E, Arós F, Conde M, Lahoz C, Lapetra J, Sáez G, Ros E (2006) Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med 145:1–11

82. Storniolo CE, Casillas R, Bulló M, Castañer O, Ros E, Sáez GT, Toledo E, Estruch R, Ruiz-Gutiérrez V, Fitó M, Martínez-González MA, Salas-Salvadó J, Mitjavila MT, Moreno JJ (2017) A Mediterranean diet supplemented with extra virgin olive oil or nuts improves endothelial markers involved in blood pressure control in hypertensive women. Eur J Nutr 56(1):89–97
83. Hohmann CDD, Cramer H, Michalsen A, Kessler C, Steckhan N, Choi K, Dobos G (2015) Effects of high phenolic olive oil on cardiovascular risk factors: a systematic review and meta-analysis. Phytomedicine 22:631–640
84. Ruano J, Lopez-Miranda J, Fuentes F, Moreno JA, Bellido C, Perez-Martinez P, Lozano A, Gómez P, Jiménez Y, Pérez Jiménez F (2005) Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. J Am Coll Cardiol 46:1864–1868
85. Valls R-M, Farràs M, Suárez M, Fernández-Castillejo S, Fitó M, Konstantinidou V, Fuentes F, López-Miranda J, Giralt M, Covas M-I, Motilva M-J, Solà R (2015) Effects of functional olive oil enriched with its own phenolic compounds on endothelial function in hypertensive patients. A randomised controlled trial. Food Chem 167:30–35
86. Widmer RJ, Freund MA, Flammer AJ, Sexton J, Lennon R, Romani A, Mulinacci N, Vinceri FF, Lerman LO, Lerman A (2013) Beneficial effects of polyphenol-rich olive oil in patients with early atherosclerosis. Eur J Nutr 52:1223–1231
87. Moreno-Luna R, Muñoz-Hernandez R, Miranda ML, Costa AF, Jimenez-Jimenez L, Vallejo-Vaz AJ, Muriana FJGG, Villar J, Stiefel P (2012) Olive oil polyphenols decrease blood pressure and improve endothelial function in young women with mild hypertension. Am J Hypertens 25:1299–1304
88. Fitó M, Cladellas M, de la Torre R, Martí J, Alcántara M, Pujadas-Bastardes M, Marrugat J, Bruguera J, López-Sabater MC, Vila J, Covas MI, Members of the SOLOS Investigators (2005) Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. Atherosclerosis 181:149–158
89. Visioli F, Caruso D, Grande S, Bosisio R, Villa M, Galli G, Sirtori C, Galli C (2005) Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. Eur J Nutr 44:121–127
90. Ruano J, López-Miranda J, de la Torre R, Delgado-Lista J, Fernández J, Caballero J, Covas MI, Jiménez Y, Pérez-Martínez P, Marín C, Fuentes F, Pérez-Jiménez F (2007) Intake of phenol-rich virgin olive oil improves the postprandial prothrombotic profile in hypercholesterolemic patients. Am J Clin Nutr 86:341–346
91. Capurso C, Massaro M, Scoditti E, Vendemiale G, Capurso A (2014) Vascular effects of the Mediterranean diet part I: anti-hypertensive and anti-thrombotic effects. Vascul Pharmacol 63:118–126
92. Delgado-Lista J, Garcia-Rios A, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F (2011) Olive oil and haemostasis: platelet function, thrombogenesis and fibrinolysis. Curr Pharm Des 17:778–785
93. Fernández de la Puebla RA, Pérez-Martínez P, Carmona J, López-Miranda Carmen Marín J, Paniagua JA, Fuentes F, Pérez-Jiménez F (2007) Factor VII polymorphisms influence the plasma response to diets with different fat content, in a healthy Caucasian population. Mol Nutr Food Res 51:618–624
94. Pacheco YM, López S, Bermúdez B, Abia R, Muriana FJG (2006) Extra-virgin vs. refined olive oil on postprandial hemostatic markers in healthy subjects. J Thromb Haemost 4:1421–1422
95. Oosthuizen W, Vorster HH, Jerling JC, Barnard HC, Smuts CM, Silvis N, Kruger A, Venter CS (1994) Both fish oil and olive oil lowered plasma fibrinogen in women with high baseline fibrinogen levels. Thromb Haemost 72:557–562
96. Harland JI (2012) Food combinations for cholesterol lowering. Nutr Res Rev 25:249–266
97. Trichopoulou A, Lagiou P (1997) Worldwide patterns of dietary lipids intake and health implications. Am J Clin Nutr 66:961S–964S

98. Hooper L, Martin N, Abdelhamid A, and Davey Smith G (2015) Reduction in saturated fat intake for cardiovascular disease. Cochrane database Syst Rev CD011737
99. Schwingshackl L, Hoffmann G (2013) Comparison of effects of long-term low-fat vs high-fat diets on blood lipid levels in overweight or obese patients: a systematic review and meta-analysis. J Acad Nutr Diet 113(12):1640–1661
100. EFSA Panel on Dietetic Products N and A (NDA) (2012) Scientific opinion on the substantiation of a health claim related to polyphenols in olive and maintenance of normal blood HDL cholesterol concentrations (ID 1639, further assessment) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J 10:2848
101. Marrugat J, Covas M-I, Fitó M, Schröder H, Miró-Casas E, Gimeno E, López-Sabater MC, de la Torre R, Farré M, SOLOS Investigators (2004) Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation – a randomized controlled trial. Eur J Nutr 43:140–147
102. US Food and Drug Administration (2004) Press release P04-100, 1 Nov 2004
103. EFSA Panel on Dietetic Products N and A (NDA) (2011) Scientific opinion on the substantiation of health claims related to polyphenols in olive oil and protection of LDL particles from oxidative damage. EFSA J 2011:9
104. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, Collen D, Muls E, Van de Werf F (2001) Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol 21:844–848
105. Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W (2005) Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. Circulation 112:651–657
106. Navab M, Ananthramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fonarow GC, Vahabzadeh K, Hama S, Hough G, Kamranpour N, JA B, Lusis AJ, Fogelman AM (2004) The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. J Lipid Res 45:993–1007
107. Gimeno E, de la Torre-Carbot K, Lamuela-Raventós RM, Castellote AI, Fitó M, de la Torre R, Covas M-I, MC L-S (2007) Changes in the phenolic content of low density lipoprotein after olive oil consumption in men. A randomized crossover controlled trial. Br J Nutr 98:1243–1250
108. Gimeno E, Fitó M, Lamuela-Raventós RM, Castellote AI, Covas M, Farré M, de La Torre-Boronat MC, López-Sabater MC (2002) Effect of ingestion of virgin olive oil on human low-density lipoprotein composition. Eur J Clin Nutr 56:114–120
109. Castañer O, Fitó M, López-Sabater MC, Poulsen HE, Nyyssönen K, Schröder H, Salonen JT, De la Torre-Carbot K, Zunft H-F, De la Torre R, Bäumler H, Gaddi AV, Saez GT, Tomás M, Covas M-I, EUROLIVE Study Group (2011) The effect of olive oil polyphenols on antibodies against oxidized LDL. A randomized clinical trial. Clin Nutr 30:490–493
110. Vázquez-Velasco M, Esperanza Díaz L, Lucas R, Gómez-Martínez S, Bastida S, Marcos A, Sánchez-Muniz FJ (2011) Effects of hydroxytyrosol-enriched sunflower oil consumption on CVD risk factors. Br J Nutr 105:1448–1452
111. Ashton EL, Best JD, Ball MJ (2001) Effects of monounsaturated enriched sunflower oil on CHD risk factors including LDL size and copper-induced LDL oxidation. J Am Coll Nutr 20:320–326
112. Nicolaïew N, Lemort N, Adorni L, Berra B, Montorfano G, Rapelli S, Cortesi N, Jacotot B (1998) Comparison between extra virgin olive oil and oleic acid rich sunflower oil: effects on postprandial lipemia and LDL susceptibility to oxidation. Ann Nutr Metab 42:251–260
113. Hernáez Á, Remaley AT, Farràs M, Fernández-Castillejo S, Subirana I, Schröder H, Fernández-Mampel M, Muñoz-Aguayo D, Sampson M, Solà R, Farré M, de la Torre R, López-Sabater M-C, Nyyssönen K, Zunft H-JF, Covas M-I, Fitó M (2015) Olive oil polyphenols decrease LDL concentrations and LDL atherogenicity in men in a randomized controlled trial. J Nutr 145:1692–1697

114. Svegliati Baroni S, Amelio M, Fiorito A, Gaddi A, Littarru G, Battino M (1999) Monounsaturated diet lowers LDL oxidisability in type IIb and type IV dyslipidemia without affecting coenzyme Q10 and vitamin E contents. Biofactors 9:325–330
115. Fitó M, Guxens M, Corella D, Sáez G, Estruch R, de la Torre R, Francés F, Cabezas C, López-Sabater Mdel C, Marrugat J, García-Arellano A, Arós F, Ruiz-Gutierrez V, Ros E, Salas-Salvadó J, Fiol M, Solá R, Covas MI (2007) Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. Arch Intern Med 167(11):1195–1203
116. Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB (1986) Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 256:2835–2838
117. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJP, Komajda M, Lopez-Sendon J, Mosca L, Tardif J-C, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B (2007) Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 357:2109–2122
118. Keene D, Price C, Shun-Shin MJ, Francis DP (2014) Effect on cardiovascular risk of high density lipoprotein targeted drug treatments niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117,411 patients. BMJ 349:g4379
119. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Hólm H, Ding EL, Johnson T, Schunkert H, Samani NJ, Clarke R, Hopewell JC, Thompson JF, Li M, Thorleifsson G, Newton-Cheh C, Musunuru K, Pirruccello JP, Saleheen D, Chen L, Stewart AFR, Schillert A, Thorsteinsdottir U, Thorgeirsson G, Anand S, Engert JC, Morgan T, Spertus J, Stoll M, Berger K, Martinelli N, Girelli D, McKeown PP, Patterson CC, Epstein SE, Devaney J, Burnett M-S, Mooser V, Ripatti S, Surakka I, Nieminen MS, Sinisalo J, Lokki M-L, Perola M, Havulinna A, de Faire U, Gigante B, Ingelsson E, Zeller T, Wild P, de Bakker PIW, Klungel OH, Maitland-van der Zee A-H, Peters BJM, de Boer A, Grobbee DE, Kamphuisen PW, Deneer VHM, Elbers CC, Onland-Moret NC, Hofker MH, Wijmenga C, Verschuren WMM, Boer JMA, van der Schouw YT, Rasheed A, Frossard P, Demissie S, Willer C, Do R, Ordovas JM, Abecasis GR, Boehnke M, Mohlke KL, Daly MJ, Guiducci C, Burtt NP, Surti A, Gonzalez E, Purcell S, Gabriel S, Marrugat J, Peden J, Erdmann J, Diemert P, Willenborg C, König IR, Fischer M, Hengstenberg C, Ziegler A, Buysschaert I, Lambrechts D, Van de Werf F, Fox KA, El Mokhtari NE, Rubin D et al (2012) Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet 380:572–580
120. Rosenson RS, Brewer HB, Ansell BJ, Barter P, Chapman MJ, Heinecke JW, Kontush A, Tall AR, Webb NR (2016) Dysfunctional HDL and atherosclerotic cardiovascular disease. Nat Rev Cardiol 13:48–60
121. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ (2011) Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med 364:127–135
122. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, Shaul PW (2014) HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med 371:2383–2393
123. Rosenson RS, Brewer HB, Davidson WS, Fayad ZA, Fuster V, Goldstein J, Hellerstein M, Jiang X-C, Phillips MC, Rader DJ, Remaley AT, Rothblat GH, Tall AR, Yvan-Charvet L (2012) Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. Circulation 125:1905–1919
124. Besler C, Lüscher TF, Landmesser U (2012) Molecular mechanisms of vascular effects of high-density lipoprotein: alterations in cardiovascular disease. EMBO Mol Med 4:251–268
125. Norata GD, Pirillo A, Catapano AL (2006) Modified HDL: biological and physiopathological consequences. Nutr Metab Cardiovasc Dis 16:371–386
126. Shao B (2012) Site-specific oxidation of apolipoprotein A-I impairs cholesterol export by ABCA1, a key cardioprotective function of HDL. Biochim Biophys Acta 1821:490–501

127. Bonnefont-Rousselot D, Motta C, Khalil AO, Sola R, La Ville AE, Delattre J, Gardès-Albert M (1995) Physicochemical changes in human high-density lipoproteins (HDL) oxidized by gamma radiolysis-generated oxyradicals. Effect on their cholesterol effluxing capacity. Biochim Biophys Acta 1255:23–30
128. Kontush A, Chapman MJ (2006) Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. Pharmacol Rev 58:342–374
129. Pirillo A, Norata GD, Catapano AL (2013) High-density lipoprotein subfractions – what the clinicians need to know. Cardiology 124:116–125
130. Sola R, Motta C, Maille M, Bargallo MT, Boisnier C, Richard JL, Jacotot B (1993) Dietary monounsaturated fatty acids enhance cholesterol efflux from human fibroblasts. Relation to fluidity, phospholipid fatty acid composition, overall composition, and size of HDL3. Arterioscler Thromb 13:958–966
131. Solà R, La Ville AE, Richard JL, Motta C, Bargalló MT, Girona J, Masana L, Jacotot B (1997) Oleic acid rich diet protects against the oxidative modification of high density lipoprotein. Free Radic Biol Med 22:1037–1045
132. Hernáez Á, Fernández-Castillejo S, Farràs M, Catalán Ú, Subirana I, Montes R, Solà R, Muñoz-Aguayo D, Gelabert-Gorgues A, Díaz-Gil Ó, Nyyssönen K, Zunft H-JF, de la Torre R, Martín-Peláez S, Pedret A, Remaley AT, Covas M-I, Fitó M (2014) Olive oil polyphenols enhance high-density lipoprotein function in humans: a randomized controlled trial. Arterioscler Thromb Vasc Biol 34:2115–2119
133. Farràs M, Castañer O, Martín-Peláez S, Hernáez Á, Schröder H, Subirana I, Muñoz-Aguayo D, Gaixas S, de la TR, Farré M, Rubió L, Díaz Ó, Fernández-Castillejo S, Solà R, Motilva MJ, Fitó M (2015) Complementary phenol-enriched olive oil improves HDL characteristics in hypercholesterolemic subjects. A randomized, double-blind, crossover, controlled trial. The VOHF study. Mol Nutr Food Res 59:1758–1770
134. Loued S, Berrougui H, Componova P, Ikhlef S, Helal O, Khalil A (2013) Extra-virgin olive oil consumption reduces the age-related decrease in HDL and paraoxonase 1 anti-inflammatory activities. Br J Nutr 110:1272–1284
135. Farràs M, Valls RM, Fernández-Castillejo S, Giralt M, Solà R, Subirana I, Motilva M-J, Konstantinidou V, Covas M-I, Fitó M (2013) Olive oil polyphenols enhance the expression of cholesterol efflux related genes in vivo in humans. A randomized controlled trial. J Nutr Biochem 24:1334–1339
136. Hernáez Á, Castañer O, Elosua R, Pintó X, Estruch R, Salas-Salvadó J, Corella D, Arós F, Serra-Majem L, Fiol M, Ortega-Calvo M, Ros E, Martínez-González MÁ, de la Torre R, López-Sabater MC, Fitó M (2017) Mediterranean diet improves high-density lipoprotein function in high-cardiovascular-risk individuals: A randomized controlled trial. Circulation 135(7):633–643
137. Servili M, Montedoro G (2002) Contribution of phenolic compounds to virgin olive oil quality. Eur J Lipid Sci Technol 104:602–613
138. Li L, Ishdorj G, Gibson SB (2012) Reactive oxygen species regulation of autophagy in cancer: implications for cancer treatment. Free Radic Biol Med 53:1399–1410
139. Lönn ME, Dennis JM, Stocker R (2012) Actions of "antioxidants" in the protection against atherosclerosis. Free Radic Biol Med 53:863–884
140. Wang JC, Bennett M (2012) Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. Circ Res 111:245–259
141. Konstantinidou V, Covas M-I, Sola R, Fitó M (2013) Up-to date knowledge on the in vivo transcriptomic effect of the Mediterranean diet in humans. Mol Nutr Food Res 57:772–783
142. Pacheco YM, López S, Bermúdez B, Abia R, Villar J, Muriana FJG (2008) A meal rich in oleic acid beneficially modulates postprandial sICAM-1 and sVCAM-1 in normotensive and hypertensive hypertriglyceridemic subjects. J Nutr Biochem 19:200–205
143. Yaqoob P, Knapper JA, Webb DH, Williams CM, Newsholme E a, Calder PC (1998) Effect of olive oil on immune function in middle-aged men. Am J Clin Nutr 67:129–135

144. Voon PT, Ng TKW, Lee VKM, Nesaretnam K (2011) Diets high in palmitic acid (16:0), lauric and myristic acids (12:0 + 14:0), or oleic acid (18:1) do not alter postprandial or fasting plasma homocysteine and inflammatory markers in healthy Malaysian adults. Am J Clin Nutr 94:1451–1457
145. Dennis EA, Norris PC (2015) Eicosanoid storm in infection and inflammation. Nat Rev Immunol 15:511–523
146. Wardhana, Surachmanto ES, EA D (2011) The role of omega-3 fatty acids contained in olive oil on chronic inflammation. Acta Med Indones 43:138–143
147. Capron L (1993) Marine oils and prevention of cardiovascular diseases. Rev Prat 43:164–170
148. Beauchamp GK, Keast RSJ, Morel D, Lin J, Pika J, Han Q, Lee C-H, Smith AB, Breslin PAS (2005) Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. Nature 437:45–46
149. Lucas L, Russell A, Keast R (2011) Molecular mechanisms of inflammation. Anti-inflammatory benefits of virgin olive oil and the phenolic compound oleocanthal. Curr Pharm Des 17:754–768
150. Migliori M, Panichi V, de la Torre R, Fitó M, Covas M, Bertelli A, Muñoz-Aguayo D, Scatena A, Paoletti S, Ronco C (2015) Anti-inflammatory effect of white wine in CKD patients and healthy volunteers. Blood Purif 39:218–223
151. Fitó M, Cladellas M, de la Torre R, Martí J, Muñoz D, Schröder H, Alcántara M, Pujadas-Bastardes M, Marrugat J, López-Sabater MC, Bruguera J, Covas MI, SOLOS Investigators (2008) Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. Eur J Clin Nutr 62:570–574
152. Mena M-P, Sacanella E, Vazquez-Agell M, Morales M, Fitó M, Escoda R, Serrano-Martínez M, Salas-Salvadó J, Benages N, Casas R, Lamuela-Raventós RM, Masanes F, Ros E, Estruch R (2009) Inhibition of circulating immune cell activation: a molecular antiinflammatory effect of the Mediterranean diet. Am J Clin Nutr 89:248–256
153. Casas R, Urpi-Sardà M, Sacanella E, Arranz S, Corella D, Castañer O, Lamuela-Raventós RM, Salas-Salvadó J, Lapetra J, Portillo MP, Estruch R (2017) Anti-inflammatory effects of the Mediterranean diet in the early and late stages of atheroma plaque development. Mediators Inflamm 2017:3674390
154. Casas R, Sacanella E, Urpí-Sardà M, Corella D, Castañer O, Lamuela-Raventos RM, Salas-Salvadó J, Martínez-González MA, Ros E, Estruch R (2016) Long-term immunomodulatory effects of a Mediterranean diet in adults at high risk of cardiovascular disease in the PREvención con DIeta MEDiterránea (PREDIMED) randomized controlled trial. J Nutr 146(9):1684–1693
155. Carrillo C, Cavia Mdel M, Alonso-Torre S (2012) Role of oleic acid in immune system; mechanism of action; a review. Nutr Hosp 27:978–990
156. Martín-Peláez S, Castañer O, Solà R, Motilva MJ, Castell M, Pérez-Cano FJ, Fitó M (2016) Influence of phenol-enriched olive oils on human intestinal immune function. Nutrients 8:213
157. Puertollano MA, Puertollano E, Alvarez de Cienfuegos G, de Pablo Martínez MA (2010) Olive oil, immune system and infection. Nutr Hosp 25:1–8
158. Puertollano MA, Puertollano E, Alvarez de Cienfuegos G, de Pablo MA (2007) Significance of olive oil in the host immune resistance to infection. Br J Nutr 98(Suppl 1):S54–S58
159. Baothman OA, Zamzami MA, Taher I, Abubaker J, Abu-Farha M (2016) The role of gut microbiota in the development of obesity and Diabetes. Lipids Health Dis 15:108
160. Landete JM, Curiel JA, Rodríguez H, de las Rivas B, Muñoz R (2008) Study of the inhibitory activity of phenolic compounds found in olive products and their degradation by Lactobacillus plantarum strains. Food Chem 107:320–326
161. Martínez I, Wallace G, Zhang C, Legge R, Benson AK, Carr TP, Moriyama EN, Walter J (2009) Diet-induced metabolic improvements in a hamster model of hypercholesterolemia are strongly linked to alterations of the gut microbiota. Appl Environ Microbiol 75:4175–4184
162. De Smet I, Van Hoorde L, De Saeyer N, Woestyne MV, Verstraete W (1994) In vitro study of bile salt hydrolase (BSH) activity of BSH isogenic Lactobacillus plantarum 80 strains and estimation of cholesterol lowering through enhanced BSH activity. Microb Ecol Health Dis 7:315–329

163. Brown MS, Goldstein JL (1983) Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. Annu Rev Biochem 52:223–261
164. Leahy SC, Higgins DG, Fitzgerald GF, van Sinderen D (2005) Getting better with bifidobacteria. J Appl Microbiol 98:1303–1315
165. Corona G, Tzounis X, Assunta Dessì M, Deiana M, Debnam ES, Visioli F, Spencer JPE (2006) The fate of olive oil polyphenols in the gastrointestinal tract: implications of gastric and colonic microflora-dependent biotransformation. Free Radic Res 40:647–658
166. Martín-Peláez S, Mosele JI, Pizarro N, Farràs M, de la Torre R, Subirana I, Pérez-Cano FJ, Castañer O, Solà R, Fernandez-Castillejo S, Heredia S, Farré M, Motilva MJ, and Fitó M (2015) Effect of virgin olive oil and thyme phenolic compounds on blood lipid profile: implications of human gut microbiota. Eur J Nutr. https://doi.org/10.1007/s00394-015-1063-2
167. Soriguer F, Esteva I, Rojo-Martinez G, Ruiz de Adana MS, Dobarganes MC, García-Almeida JM, Tinahones F, Beltrán M, González-Romero S, Olveira G, Gómez-Zumaquero JM (2004) Oleic acid from cooking oils is associated with lower insulin resistance in the general population (Pizarra study). Eur J Endocrinol 150(1):33–39
168. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, Fiol M, Gómez-Gracia E, López-Sabater MC, Vinyoles E, Arós F, Conde M, Lahoz C, Lapetra J, Sáez G, Ros E (2006) PREDIMED Study Investigators. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med 145(1):1–11
169. Bogani P, Galli C, Villa M, Visioli F (2007) Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis 190(1):181–6
170. Konstantinidou V, Covas MI, Muñoz-Aguayo D, Khymenets O, de la Torre R, Saez G, Tormos Mdel C, Toledo E, Marti A, Ruiz-Gutiérrez V, Ruiz Mendez MV, Fito M (2010) In vivo nutrigenomic effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. FASEB J 24(7):2546–57

Anthocyanins: Nutrition and Health

38

Iva Fernandes, Cláudia Marques, Ana Évora, Ana Faria, Conceição Calhau, Nuno Mateus, and Victor de Freitas

Contents

I. Fernandes · A. Évora · N. Mateus · V. de Freitas (✉)
REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal
e-mail: iva.fernandes@fc.up.pt; anasfiaevora@hotmail.com; nbmateus@fc.up.pt; vfreitas@fc.up.pt

C. Marques
Nutrição e Metabolismo, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal

ProNutri - Clinical Nutrition and Disease Programming, CINTESIS - Center for Research in Health Technologies and Information Systems, Porto, Portugal

Faculty of Medicine, University of Porto, Porto, Portugal
e-mail: clau.marques_88@hotmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_79

Abstract

After consumption of anthocyanin-rich foods, there is a long journey before these bioactives may exert a health-promoting property. They must pass through the oral cavity, the gastrointestinal tract, undergo metabolism events, pass cellular barriers, and eventually trigger a biological event.

Hence, before looking at the health effects of anthocyanins, some topics related to their food bioaccessibility, interaction with biomolecules (proteins, biomembranes, and DNA), and bioavailability/metabolism will be described as possible interferers to their bioactive effects.

There are several reports on the health preventing properties of anthocyanins on several diseases like cardiovascular diseases, some types of cancers, diseases, diabetes, allergies and osteoporosis. In this chapter some of the less revisited ones giving emphasis to obesity/metabolic syndrome, microbiota modulation, neurodegenerative diseases and skin health will be reviewed.

Keywords

Anthocyanins · Bioactives · Disease prevention · Food

1 Introduction

1.1 Chemistry, Occurrence, and Stability

Anthocyanins constitute the largest group of water-soluble pigments widespread in the plant kingdom arising from plant secondary metabolism, being responsible for the colors displayed by many flowers, fruits, and leaves of angiosperms (Fig. 1). Chemically, anthocyanins are flavonoids, hence based on a C15 skeleton with a chromane ring bearing a second aromatic ring B in position 2 (C6–C3–C6). In nature, anthocyanidins are usually glycosylated in one or more positions of the basic flavanic nucleus by different sugars (Fig. 2). According to the literature,

A. Faria
REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Nutrição e Metabolismo, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal

ProNutri - Clinical Nutrition and Disease Programming, CINTESIS - Center for Research in Health Technologies and Information Systems, Porto, Portugal
e-mail: anafaria@med.up.pt

C. Calhau
Nutrição e Metabolismo, NOVA Medical School, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal

ProNutri - Clinical Nutrition and Disease Programming, CINTESIS - Center for Research in Health Technologies and Information Systems, Porto, Portugal
e-mail: ccalhau@med.up.pt

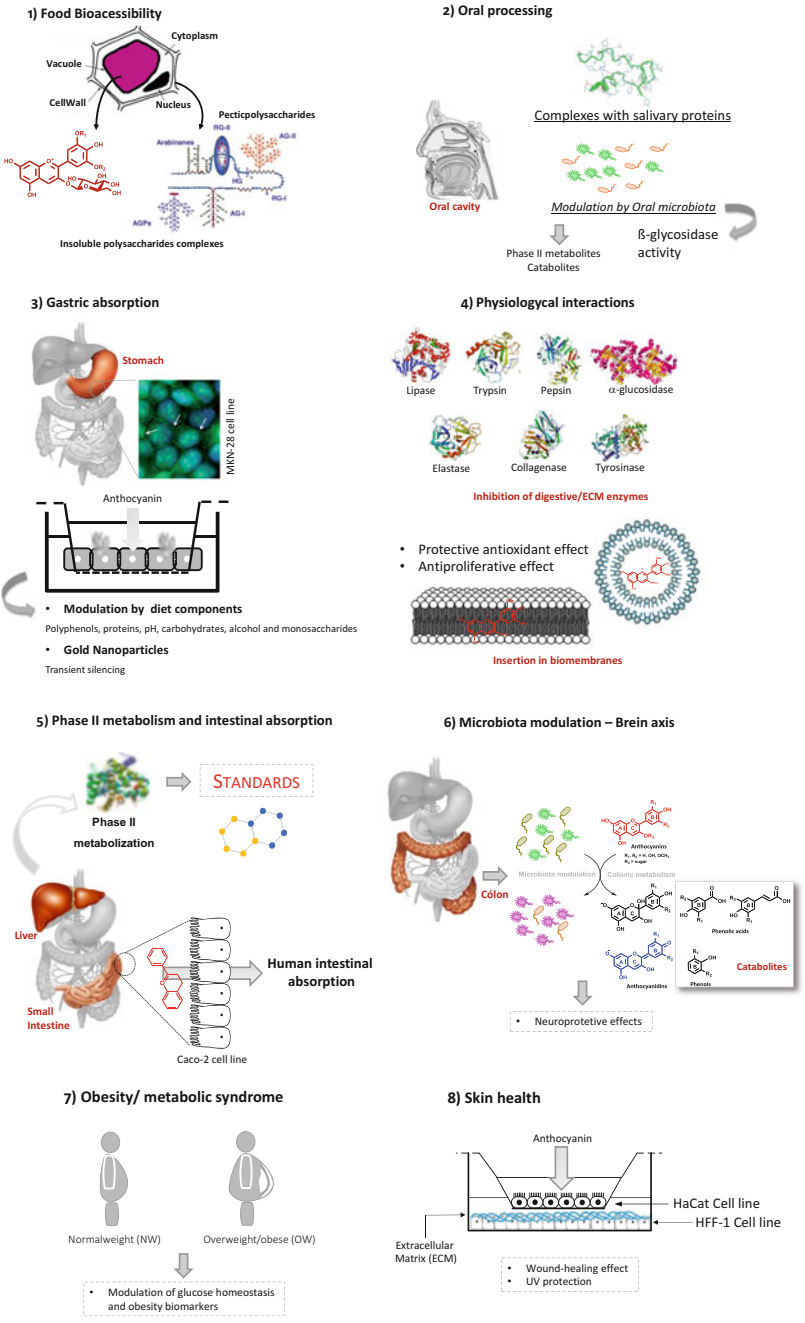


Fig. 1 Schematic representation of anthocyanin long journey after consumption and some of their biological targets

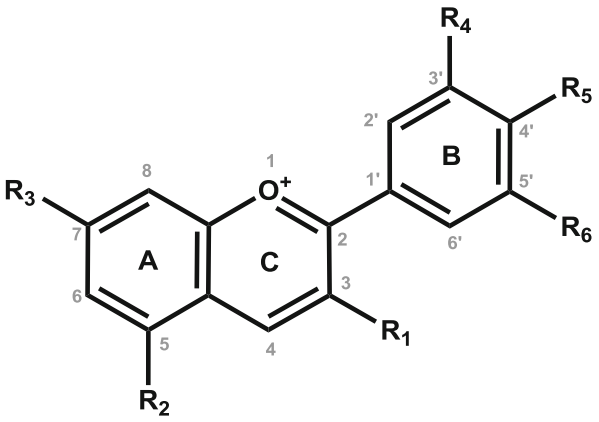


Fig. 2 Basic structure of anthocyanidin pigments (flavylium form) in which R could be H, OH, or OCH_3. The nomenclature for numbering carbons is indicated in the structure

Table 1 Anthocyanidins found in nature [3]

Name	Position of substitution
Substituted with a hydroxyl group	
Apigeninidin	5,7,4′
Aurantinidin	3,5,6,7,4′
Cyanidin	3,5,7,3′,4′
Delphinidin	3,5,7,3′,4′,5′
6-Hydroxycyanidin	3,5,6,7,3′,4′
Luteolinidin	5,7,3′,4′
Pelargonidin	3,5,7,4′
Triacetidin	5,7,3′,4′,5′
Substituted with a methyl ether group	
Apensinidin	5,3′,5′
Europenidin	5,3′
Hirsutidin	7,3′,5′
Malvidin	3,5′
5-Methylcyanidin	5
Poenidin	3′
Petunidin	3′
Pulchellidin	5
Rosinidin	7,3′

there are 17 anthocyanidins with differences in the number and position of hydroxyl groups and/or methyl ether groups, but six of them are the most common anthocyanidin in the plant kingdom (Table 1, in bold). From these 17 structures, the ones with at least one sugar molecule give origin to anthocyanin compounds. The range of anthocyanin is greatly increased by the sugar diversity and all the possible structural positions of glycosylation (glucose, rhamnose, xylose, galactose, arabinose, and fructose). Additionally, many acylated anthocyanins show in their

structures ester bonds between sugars and organic acids such as coumaric, caffeic, ferulic, *p*-hydroxybenzoic, synaptic, malonic, acetic, succinic, oxalic, and malic.

While acylated anthocyanins in general have increased stability due to co-pigmentation effects, this effect may also decrease the absorption of these compounds during digestion [1]. In fact, in human studies have found that non-acylated anthocyanins are more bioavailable than acylated anthocyanins [2].

The degree of complexity of those structures in aqueous solution increase because they are in different equilibrium forms depending on the pH [4].

In very acidic aqueous solution (pH $<$ 1), these pigments are present as red flavylium cations. The increase of pH leads to a reduction of the intensity of the color due to a decrease of the concentration of the flavylium cation that is converted into its colorless hemiketal form through nucleophilic attack of water. The hemiketal further undergoes a tautomerization reaction to give the pale yellow *cis*-chalcone (Cc), which isomerizes to *trans*-chalcone (Ct). At low acidic, neutral, and basic pH values, deprotonation of the flavylium cation also occurs, giving rise to the violet/blue quinoidal forms (Fig. 3) [4]. Indeed, anthocyanins GI absorption is highly affect by this pH dependence (Fig. 3).

Anthocyanins are responsible for many attractive colors, from scarlet to blue, of flowers, fruits, leaves, and storage organs. Anthocyanins display several functions in plants: antioxidant, photoprotection, defense mechanism, as well as other reproductive functions such as pollination and seed dispersal. The type of anthocyanins in plants is so variable that some plants present only one type of anthocyanin, whereas others have mixtures. In the same trend, some fruits are a source of one anthocyanin: cyanidin in apple, cherry, fig, and peach and delphinidin in eggplant and pomegranate; some fruits have two main anthocyanins such as cherry sweet and cranberry (cyanidin and peonidin), while others have several anthocyanins (grape, myrtillus). In general, the anthocyanin concentration in most of the fruits and vegetables goes from 0.1 up to 1% dry weight [3].

Other anthocyanin derivatives may be found in processed foodstuff and in fermented and unfermented fruit juices including pyranoanthocyanins and polymeric anthocyanins (Fig. 4) derived from these common anthocyanins by the reaction with a reaction partner that may include hydroxycinnamic acids, vinylphenols, vinylflavanols, pyruvic acid, acetaldehyde, acetone, or the genuine pigment [4].

Since anthocyanins are the most important coloring compounds between flavonoids, they provide characteristic colors to fruits, vegetables, and food beverages having a huge impact on this key quality parameter that influences consumer acceptance.

From a nutritional perspective, anthocyanins are consumed not only in fresh fruits and vegetables but also in many foodstuffs that are thermally processed prior to consumption, and this process can greatly influence anthocyanin content in the final product. Heating at lower temperatures (~40 °C) for up to 3 days may not be detrimental, but heating at higher temperatures (60–125 °C) for more than 8 h results in considerable loss of anthocyanins [6]. Bleaching by the effect of heat occurs because the anthocyanin equilibrium is moved toward the uncolored forms, as shown in Fig. 3. The flavonoid structure opens to form a chalcone, which is

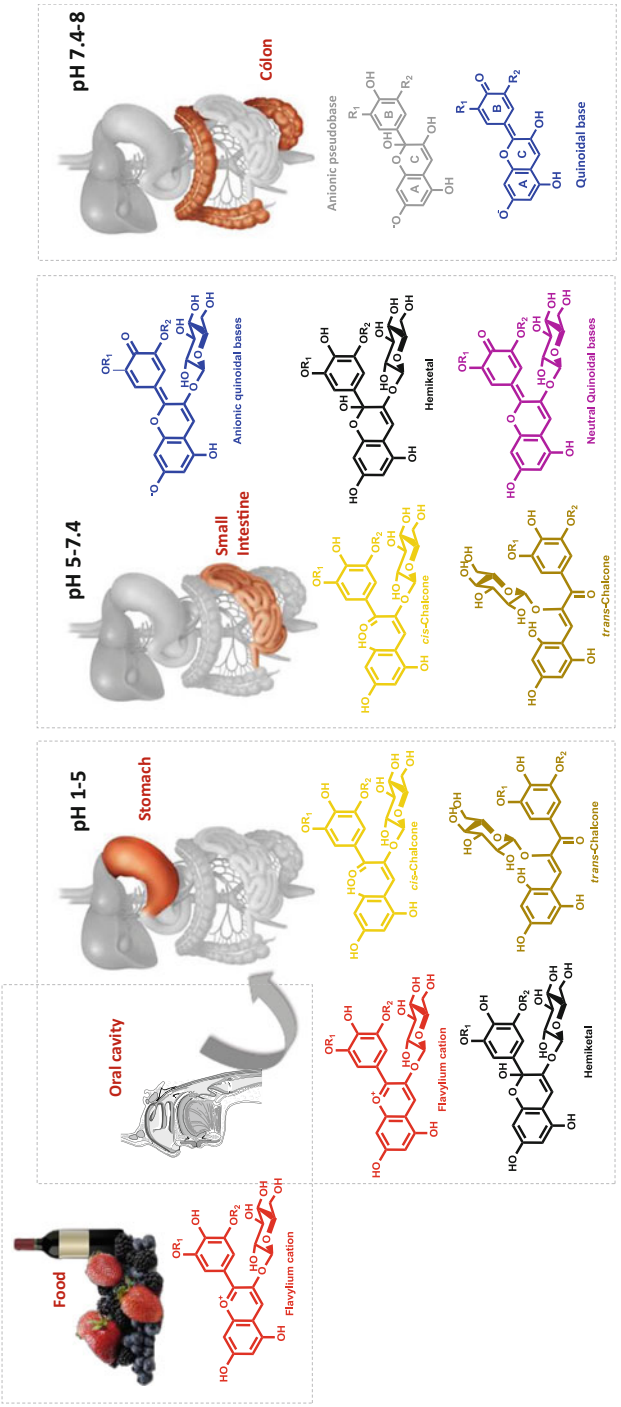


Fig. 3 Anthocyanin equilibrium forms according to the GI tract pH. (Adapted from [5])

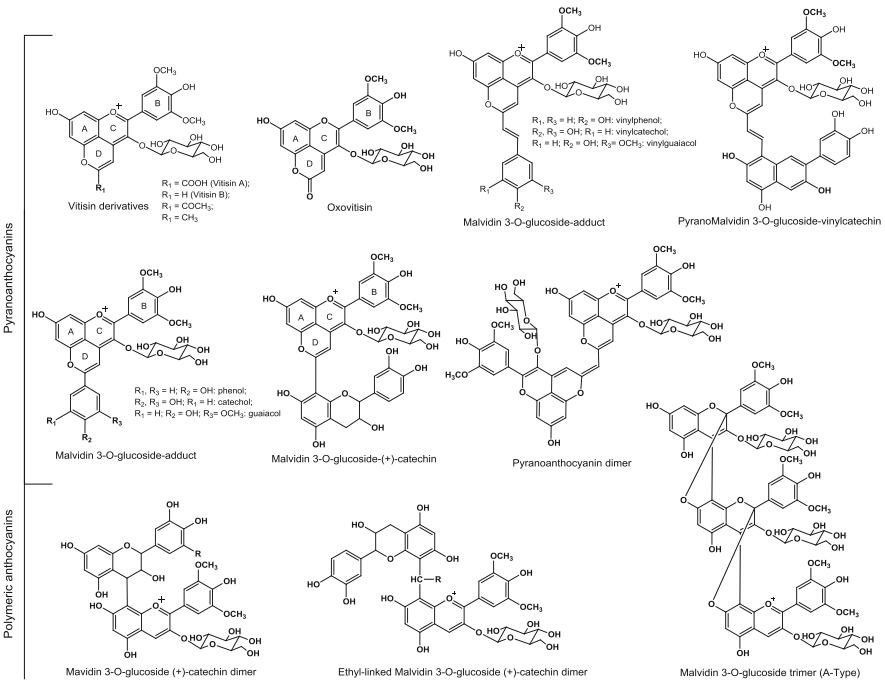


Fig. 4 Pyranoanthocyanins and polymeric anthocyanins identified in processed foodstuffs and in fermented and unfermented fruit juices

further degraded to brown products. In slightly alkaline solutions (pH 8 to 10), highly colored ionized bases are formed [7].

This thermal degradation can have a dramatic impact on color and may also affect nutritional properties. Still, the thermal degradation of anthocyanins could be accompanied by the formation of products which may also possess antioxidant properties. Therefore, it is not clear whether the formation of these components results in an overall reduction in antioxidant activity, besides affecting other food features [8].

Besides, anthocyanin stability is not merely dependent on the final processing temperature but is in turn influenced by the intrinsic properties of the product and the process such as pH, storage temperature, chemical structure and concentration of anthocyanins, light, oxygen, the presence of enzymes, proteins, metallic ions, and carbohydrates.

1.2 Anthocyanin Food Bioaccessibility

The structural diversity and physical-chemical properties of anthocyanins greatly interferes with their absorption and digestion and also with their final excretion during metabolic processes [1]. Before the endogenous factors such as pH, the presence of food, digestive enzymes, bile acids, microbiota, and the motility and permeability of the gastrointestinal (GI) tract may exert their actions, food bioaccessibility is one major impairment of anthocyanins absorption.

In order for anthocyanins in fruits and vegetables to become available for absorption within the human GI tract, these compounds must be released from the vacuole of plant cells [9]. When cells are ruptured during processing or oral mastication, anthocyanin and plant cell wall component (cellulose and pectin) complexes come into contact for the first time with other in vivo components [9, 10]. In processed foodstuffs, interactions between different anthocyanins and the food matrix (carbohydrates, proteins, fibers, other polyphenols) are likely to affect their bioaccessibility (i.e., the amount of anthocyanins released from the food matrix prior to absorption) of anthocyanins. This could potentially have an impact on the nutritional content and functional potential of diets [11]. Besides the initial mechanic and enzymatic action in the oral cavity (further discussed), foodstuffs are ingested as small pieces, so these types of interaction may be kept until the gastric environment.

In a recent work, it was shown that the flavylium cation (at pH 1) and hemiketal forms (at pH 3–5) of two anthocyanins (cyanidin-3-glucoside and delphinidin-3-glucoside) interact with ionic carbohydrates (pectin) [12]. This is indicative that this type of complexes may be stable at the gastric environment and prevent absorption and metabolization. In addition, the more polar anthocyanins revealed a stronger binding to pectin [13].

Anthocyanin uptake in the small intestine appears to be very likely considering the increase in their plasmatic levels [14], but their release from complexes may not be easily achieved, and their maintenance in the intestine/gut in that form may resemble a prebiotic effect.

The co-ingestion of anthocyanins with other meal components is also detrimental for the absorption of anthocyanins [15, 16]. Normally, to reduce the biological samples complexity, animal and human studies are performed with purified anthocyanins or extracts dissolved in water or even red fruit consumed after overnight fasting, thereby not taking into account possible interactions of anthocyanins with other dietary constituents. These are highly reactive pigments, and their possible reaction with other polyphenols and interaction with carbohydrates or proteins may greatly alter their pharmacokinetics.

Likewise, Xiao and coworkers concluded that milk did not influence the oral relative bioavailability of pelargonidin anthocyanins under meal conditions; however, the oral relative bioavailability of pelargonidin anthocyanins was reduced by ~50% by milk under meal conditions ($p < 0.05$) [15]. The simultaneous intake of a food source reduces anthocyanins absorption and urinary excretion [16]. However, the additional food matrix does not appear to have an effect on anthocyanin metabolism [16].

2 Interactions of Anthocyanins with Biomolecules

2.1 Proteins

During oral consumption, a variety of molecular interactions can occur, namely, interaction with food proteins resulting from the mastication processes or with salivary proteins, and later in the digestive process, the released anthocyanins may exert their effect in the modulation of the activity of digestive enzymes [17].

Only some local effects in the oral epithelium may be considered. Anthocyanins are not usually directly linked to astringency sensation, although they may contribute to the bitterness of some fruits and drinks [18]. The bitter taste perceived after the ingestion of some foodstuffs, for example, red or port wine, may be attributed to the activation of some bitter receptors [19], or anthocyanins could form complexes with saliva proteins [18].

Moreover, anthocyanins may display inhibitory effects on carbohydrate digestion and glucose absorption. Suppression of the activity of pancreatic lipase, α-glucosidase, and α-amylase is positively associated with the reduction of fat and sugar absorption from the gastrointestinal tract [20]. This ability may be explored as part of a strategy to reduce postprandial hyperglycemia by optimizing the functionality of foods with anthocyanin extracts that would strengthen efforts to reduce the risk of T2D.

Inhibition of pancreatic α-amylase and α-glucosidase activity was correlated with anthocyanin ingestion resulting in a reduction of blood glucose levels after starch-rich meals [20, 21]. A range of berry polyphenols (e.g., flavonols, anthocyanidins, ellagitannins, and proanthocyanidins) can inhibit protease activities at levels which could affect protein digestion in the gastrointestinal tract [21, 22].

In addition, anthocyanidins with vicinal hydroxyl groups in ring B (cyanidin and delphinidin) were found to inhibit glycogen phosphorylase activity being able to affect glucose/glycogen homeostasis [23].

This in vitro data is supported by several human trials reporting the regular consumption of berries in association with a reduction in the risk of T2D. Growing evidence from randomized controlled trials suggests that berry extracts, purees, and nectars acutely inhibit postprandial glycemia and insulinemia following oral carbohydrate loads [24]. Consumption of black currant extract in amounts roughly equivalent to 100 g black currants reduced postprandial glycemia, insulinemia, and incretin secretion, which suggests that inclusion of black currant polyphenols in foods may provide cardio-metabolic health benefits [24, 25].

Berry extracts and anthocyanins inhibit the activities of pancreatic α-amylase and α-glucosidase in the gut lumen and interact with intestinal sugar transporters, sodium-dependent glucose transporter 1, and GLUT2, hence reducing the rate of glucose uptake into the circulation. This topic is further explored in the bioavailability section.

In a fundamental work, the affinity of Cy3glc for human serum albumin (HSA) was studied showing to be stronger at pH 7, thereby underlining its potential in the transport and distribution of anthocyanins in the organism [26]. Hydroxyl substituents and glycosylation of anthocyanins decreased the affinity for binding to HSA at lower pH (especially pH 4) but increased the strength of binding at pH 7.4. Conversely, methylation of a hydroxyl group enhanced the binding at acidic pH, while this substitution reduced the strength of binding at pH 7.4. According to these findings, binding of anthocyanins to HSA is influenced not only by their structure but also by the pH of the medium [27].

Some questions still remain as to how far such interactions may improve the stability of anthocyanins and how far such interactions may influence their bioavailability.

2.2 Biomembranes

In a recent work of Trouillas and collaborators, the capacity of various anthocyanin derivatives to insert lipid bilayer membrane was evaluated [28]. Malvidin-3-O-glucoside was studied in its different charge forms (flavylium cation, neutral and anionic quinonoidal bases) as well as its deglycosylated, hydrated, and conjugated derivatives. Most of the derivatives were theoretically predicted to insert rather deep in the membrane, i.e., embedded in between lipid chains, therefore being prone to scavenge both the initiation and propagation stages of lipid peroxidation.

Besides this protective antioxidant effect, in a recent in vitro study, the effect of anthocyanins (pelargonidin-3-O-glucoside chloride and cyanidin-3-O-galactoside chloride) on erythrocytes morphology showed that their incorporation into the outer region of the erythrocyte membrane affects its shape and lipid packing order, which is reflected in the increasing number of echinocytes (abnormal red blood cell membrane) [29].

This involvement of anthocyanins in cellular morphologic alterations through membrane interactions may be associated with the antiproliferative effect of anthocyanins present in several foodstuffs [30]. Similar to what was observed by other authors, anthocyanidins were shown to interact with the deeper regions of the lipid bilayers in a structure-dependent manner and to decrease their fluidity. Greater membrane interacting potency was associated with a 3-hydroxyl group, 3′,4′-dihydroxyl groups of ring B, and 5,7-dihydroxyl groups of ring a, and anthocyanidins meet these structural requirements.

The amphiphilic properties of flavonoids allow them to interact not only hydrophobically with phospholipid acyl chains but also electrostatically with phospholipid polar heads. In addition to hydrophobic interaction and hydrogen bonding, steric configuration also participates in the interaction between anthocyanins and membranes. The presence of polyhydroxyl groups, the heterocyclic ring C (pyran or pyrone), and structural hydrophobicity are important for flavonoids to interact with biomimetic membranes. The lower membrane interactivities are attributable to the more hydrophobic anthocyanins, like delphinidin.

2.3 DNA

Nucleic acids such as DNA and RNA have also been proved to act as highly reactive ligands for anthocyanins. The interaction of anthocyanins with calf thymus DNA (ctDNA) was reported [31]. The anthocyanin-DNA complexes might be a possible defense mechanism against oxidative damage to DNA and might have normal physiological functions in vivo. This interaction is thought to occur between the positively charged flavylium cation and the PO_2 group; the ctDNA complex led to phosphate charge neutralization and helix stabilization [31]. In vivo, this type of interaction is not likely to occur since the nuclear pH is around 7.4, and at this value, the positively charged fraction of the anthocyanin equilibrium forms is almost absent.

On the other hand, the DNA triplex stabilization property of seven natural anthocyanins (five monoglucosides and two diglucosides) has been determined by triplex denaturation experiments [32]. It was assumed that the difference between the diglucosides and monoglucosides could be due to the supplementary sugar moiety at position 5 for the diglucosylated compounds, which would make anthocyanins too crowded and close together to allow their interactions with the triplex. Furthermore, the most active compounds among the monoglucoside series were the only anthocyanins that bear a catechol B-ring in their structure, which could be important for such an interaction.

This ability of anthocyanins may be a possible mechanism for the antiproliferative against cancer cell lines described for this class of pigments.

3 Bioavailability and Metabolism of Anthocyanins

Considering the wide distribution of anthocyanins in the diet, it is reasonable to assume that humans are very likely to consume these compounds in large amounts. In a survey with Italian subjects, anthocyanin daily intake was in the range 25–215 mg/person, depending on gender and age, and this intake is largely enough to induce pharmacological effects [3]. Nonetheless, among all polyphenol classes, anthocyanins are the ones considered to have the lowest bioavailability. However, recent reports suggest an underevaluation of the real bioavailability of these compounds.

The early appearance of anthocyanins in stated blood and urine after consumption of anthocyanin-rich foods is not compatible with an exclusive absorption at the intestinal level, as initially accepted [33]. Furthermore, when considering the metabolites of anthocyanins resulting from the action of gastrointestinal enzymes and flora, their bioavailability increases substantially [33].

3.1 Oral Cavity

The oral cavity is the first environment for the absorption and digestion of anthocyanins in humans. In chokeberry juice, among the several anthocyanins present in the original juice, Cy3glc was the one preferentially accumulated in epithelium cells [17]. This result suggests that anthocyanin structure affects oral cell uptake and therefore the potential anthocyanin type and concentration available for further absorption. Due to the short residence time of food in this compartment and the transient passage of drinks, its contribution to the oral uptake of anthocyanins is still in discussion, although their possible adhesion to the surface of the oral cavity cells should be explored, together with the effect of chronic exposure (dairy intake). This adhesion could be prompted by interaction of anthocyanins with membrane proteins.

Besides the physiological conditions (pH and temperature) impact, the dental plaque and saliva oral microorganisms may contribute to anthocyanin degradation during oral intake [17, 34]. Some reports point for the oral degradation of

anthocyanins after 5–60 min of residence in in vitro or ex vivo simulations or even in human intervention studies [17, 34]. These may be attributed to enzymatic action of oral microbiota, high oral temperatures, and pH and to binding of anthocyanin to salivary proteins [18, 35]. In fact, in the oral cavity (pH ~6.8), the chalcone form of cyanidin 3-O-glucoside may account for as much as 30% of the original amount of standard cyanidin 3-O-glucoside incubated with ex vivo saliva [35].

Besides pH interconversion, deglycosylation of anthocyanins due to oral native or microbiota-related glycosidases is one of the main products identified in the oral cavity [35, 36]. In this latter study, Cy3glc microbiota metabolite, protocatechuic acid, was also detected in saliva. The enzymatic machinery for phase II metabolism and enteric recycling of anthocyanin was identified in the oral cavity of humans [36]. Furthermore, saliva samples (collected at 5, 60, 120, and 240 min) contain glucuronidated anthocyanin conjugates and glucuronidated metabolites, consistent with intracellular uptake and phase II conversion of anthocyanins [36]. Besides, as subtracts of phase II enzymes, anthocyanins seem also to contribute to their activation [36].

3.2 Stomach

Based on the short residence time of anthocyanins in the oral cavity, their bulk portion should reach the stomach in their ingested native form.

Within the stomach and due to its quite acidic pH, anthocyanins are in their most stable medium. Besides being the main breakdown site in the digestion system of the human body for other food ingredients, in the case of anthocyanins, it may also have an important role in their absorption and metabolism. In fact, previous studies indicated that significant amounts of anthocyanins could be absorbed quickly and efficiently in the stomach in their native forms [37, 38]. In vitro studies using the human MKN-28 and NCI-N87 as gastric barrier models also indicated that the stomach could be an important site for anthocyanin absorption [39, 40].

By using in vitro cell barrier models, the gastric absorption mechanism was described, and the influence of different parameters was determined. The transport efficiency of anthocyanins is dependent on incubation time, pH, and anthocyanin polarity and size and independent of the presence of ethanol [39, 41].

The presence of D-(+)-glucose was found to decrease anthocyanins gastric uptake suggesting the involvement of glucose transporters [39]. In addition, gastric cells pretreated with anthocyanins were found to increase anthocyanin transport, possibly by inducing the expression of glucose transporters.

Using gene silencing technology in the same in vitro gastric model, it was possible to attribute to glucose transporters the partial implication on the transport of anthocyanins independent on the position of the glucose residue in the anthocyanin moiety. The fact that these compounds can reach an equilibrium in different structural forms at a specific pH value (Fig. 3) suggests that anthocyanin transport in vivo is dependent on the molar fraction of each equilibrium form. This highlights for the fact that this particular class of polyphenols can be absorbed in different forms by

different transporters that contribute to the net transport efficiency determined. This ability of anthocyanins to share the transport mechanism with glucose may give to these compounds the capacity to modulate glucose absorption, which could be important for several pathologies.

In addition, anthocyanins can be absorbed in fermented drinks like red wine in the form of derivative pigments like carboxy-pyranoanthocyanins (type A vitisins) and methyl-pyranoanthocyanins [42]. The gastric absorption of pyranoanthocyanin derivatives is slightly reduced in comparison with the parental anthocyanins possibly due to some steric interferences resulting from the higher size of these molecules. The slightly higher gastric absorption value observed for oxovitisin among other pyranoanthocyanins may be related to the absence of charge on this molecule [42]. The importance of pH on the anthocyanin transport efficiency that may be related not only with the main anthocyanin forms present but also with the influence on the possible transporters involved in their transport was already reported [39]. Besides the proven gastric absorption of these pigments, also the intestinal compartment may contribute to the circulatory appearance of anthocyanin derivative pigments [43]. Few in vivo studies have already proven the rapid detection of anthocyanin derivatives in rat plasma after oral administration of malvidin-3-glucoside-pyruvic acid adduct [44].

Considering studies with human volunteers, additional information may be obtained from the pharmacokinetic parameters and from the chromatographic profiles of the biological samples. Moreover, besides extracts or purified anthocyanins, different foods are consumed as anthocyanin sources, which will give a more realistic scenario of the matrix effect, especially in the crossover studies.

Previous studies evaluating the bioavailability of red wine anthocyanins have only looked for the main native anthocyanin (Mv3glc) in plasma and urine, underestimating the total anthocyanin content in biological samples [45, 46]. Increases in plasma of malvidin-3-*O*-glucoside (Mv3glc) concentrations were not significantly different after the consumption of either red wine or dealcoholized red wine [45]. In the work of Garcia-Alonso et al. where volunteers consumed 180 mg of a grape anthocyanin extract in a sugar-sweetened yogurt, the main pigment detected in plasma was native Mv3glc followed by peonidin-3-*O*-glucoside (Pn3glc), but the authors were already able to identify some anthocyanin metabolites in plasma including glucuronyl conjugates of malvidin and peonidin [47]. Similarly, after anthocyanin-rich grape juice consumption, the most abundant native anthocyanins found in plasma and urine were malvidin and peonidin and glucuronidated metabolites [48].

In another work, several anthocyanin conjugates (methylated, glucuronidated, and sulfated) were detected both in plasma and urine after ingestion of blackberry purees, with or without ethanol, by two groups of volunteers with different body mass indexes [14]. Altogether these works highlight for the possible implication of the gastric epithelium on the metabolization of these pigments. In fact, previous in vitro works had already detected methylated anthocyanins after incubation with total protein extract of gastric cells [33]. The majority of these compounds may be produced in the liver and kidneys since the plasma concentration of total

anthocyanin conjugates continues to increase after 60 min [14]. Moreover, this study indicated for the first time that ethanol enhances cyanidin-3-*O*-glucoside (Cy3glc) metabolism potentiating its conversion into methylated and glucuronidated derivatives (Me-Cy-Glucr and 3′-Me-Cy3glc). This effect was more pronounced in overweight and obese individuals. These results should prompt the attention of the scientific community to the fact that the kinetics of these compounds is influenced by ethanol and body mass index.

Recently, a crossover trial with healthy volunteers was performed using table and Port red wine in order to compare the pharmacokinetics of anthocyanins in these two red wines [49]. In this study, red wine anthocyanins were detected in their intact forms in both plasma and urine samples, but MvGlucr and PnGlucr were the two main derivatives detected after both red wines consumption. For the first time, and supported by the synthesis of Mv3Glucr, the main pathway followed by Mv3glc after absorption was described and involve the anthocyanidin conjugation with glucuronic acid after glucose removal.

According to these and other works performed with radiolabel anthocyanins [50], this class of pigments seem to be more bioavailable than previously reported. Nevertheless, the high impact of phase II conjugates detected in human plasma and urine, anthocyanin catabolites, seem also to be produced in huge amounts. The most important catabolites corresponded to products of anthocyanin degradation (i.e., benzoic, phenylacetic, and phenylpropanoic acids, phenolic aldehydes, and hippuric acid) and their corresponding phase II conjugates, which were found at 60- and 45-fold higher concentrations than their parent compounds in urine and plasma, respectively [50] (Fig. 5).

All in all, this data suggests that anthocyanins would be as bioavailable as other flavonoid subclasses, such as flavan-3-ols and flavones, which have relative bioavailabilities between 2.5% and 18.5% [51].

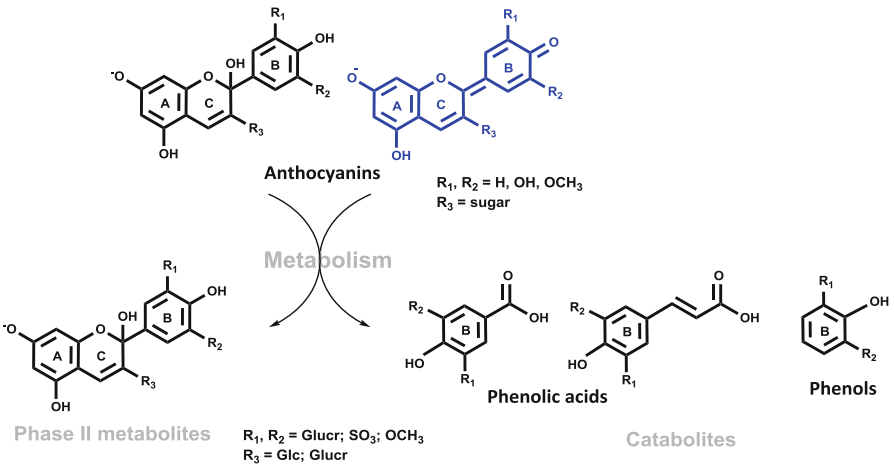


Fig. 5 Schematic representation of anthocyanins phase II metabolites and catabolites

3.3 Intestinal Tract

During the absorption process, anthocyanins can efficiently cross the intestinal wall [38]. To describe the intestinal transport mechanism, in vitro studies with Caco-2 cell line as an intestinal barrier model have been done. This cell line has an enterocyte phenotype after differentiation, and it expresses hexose transporters, ATP-binding cassette (ABC) gene family, and H^+-dependent monocarboxylic acid transporter in a similar way as small intestinal cells [52–55].

The pretreatment of caco-2 cells with red grape anthocyanin extract seems to increase (glucose transporter 2) GLUT2 expression, and this effect was positively correlated with anthocyanin and glucose transport [55]. In addition, cyanidin 3-O-glucoside absorption is dependent not only on the activity of GLUT2 but also on the activity of SGLT1 (sodium-dependent glucose transporter 1) although no implication of P-gp and MRP2 was observed [53]. Anthocyanins and anthocyanidins have moderate to high affinities for the human efflux transporter BCRP and moderate to low affinities for MDR1, so they may be actively transported out of intestinal tissues and endothelia, limiting their bioavailability [56]. Anthocyanidins may alter pharmacokinetics of drugs that act as BCRP and MDR1 substrates [56].

By using a phenolic berry extract containing 60% of anthocyanins, an opposite effect was observed as GLUT2 and SGLT1 mRNA expression was reduced by acute or longer pretreatments [52]. This contradictory results may once more highlight for the importance of matrix effect. Since the standards for metabolites detected in in vivo samples are not commercially available, no evidence for the transport mechanism has ever been described.

Besides the obvious implication of the small intestine in the absorption of anthocyanins, the huge impact of colon was also recently described. Using ileostomy subjects, a high proportion of anthocyanins from blueberry (up to 85%, depending on the attached sugar moiety) were found in the ileostomy bags unchanged; this amount would therefore reach the colon under physiological conditions and be subjected to microbial degradation [57]. Comparison of the bioavailability of anthocyanins in healthy subjects versus ileostomists revealed substantially higher amounts of anthocyanins and degradants in the plasma/urine of subjects with an intact gut [58]. The results suggested that the colon is an important site for absorption of bioactive components such as anthocyanins and their degradation products.

4 Obesity/Metabolic Syndrome

Epidemiological studies have established an association between unhealthy dietary behaviors and chronic diseases such as cardiovascular diseases (CVD) and type 2 diabetes, which are leading causes of death in high-income countries. In this context, the interest on functional food ingredients that might confer health benefits to the populations has increased.

Several reviews have been published concerning the effects of anthocyanins on obesity and metabolic syndrome, a set of interrelated risks factors for cardiovascular

disease and diabetes [59–62]. However, the number of robust and well-designed clinical trials about the effects of anthocyanins on obesity and metabolic syndrome is still limited to sustain health claims. Without solid scientific evidence, governmental authorities do not allow health claims on foods.

A search at the http://ClinicalTrials.gov database, a service of the US National Institutes of Health, for "anthocyanins" retrieved a total of 87 studies (last update February 9, 2018). From those addressing some of the metabolic syndrome features, three important randomized, double-blind placebo-controlled studies are highlighted.

One study was conducted on hypercholesterolemic participants, assigned either to the anthocyanin group ($n = 75$; 31 males and 44 females) or to the placebo group ($n = 75$; 32 males and 43 females), for 12 weeks. The anthocyanin capsules used (80 mg anthocyanins per capsule, 4 per day) provided a total daily intake of 320 mg anthocyanins. Compared with the control group, the authors observed significant increases in HDL cholesterol and a significant reduction in the LDL cholesterol concentrations [63].

In the second study conducted on a total of 58 diabetic patients, 160 mg of anthocyanins (80 mg anthocyanins per capsule) or placebo were provided twice daily ($n = 29$ in each group). Here, the authors found that anthocyanin supplementation significantly decreased serum LDL cholesterol (by 7.9%; $p < 0.05$) and triglycerides (by 23.0%; $p < 0.01$) and increased HDL cholesterol (by 19.4%; $p < 0.05$), compared with placebo, after the 24-week intervention period [64]. Anthocyanin supplementation exerted beneficial metabolic effects in subjects with type 2 diabetes by improving dyslipidemia, enhancing antioxidant capacity, and preventing insulin resistance [64].

Lastly, in the most recent study, a total of 160 eligible participants with either prediabetes or early untreated diabetes were recruited and randomly assigned to two intervention arms to receive either purified anthocyanins (320 mg/day) or placebo ($n = 80$ in each group). Compared with placebo, purified anthocyanins moderately reduced HbA1c (-0.14%; $p < 0.05$) and LDL cholesterol (-0.2 mmol/L; $p < 0.05$) after the 12-week intervention period [65].

These trials provided solid evidence on the effects of anthocyanins in the treatment of hypercholesteremia and diabetes by using commercialized capsules of purified anthocyanins extracted from bilberries and black currants (Medox®). The effects observed in these studies can be attributed exclusively to anthocyanins rather than other compounds (e.g., fibers) often present in anthocyanin-rich foods (e.g., berries).

Several mechanisms may explain the effects of anthocyanins on these metabolic syndrome features. Anthocyanins are antioxidant and anti-inflammatory, but only a few studies have considered their modulatory effects on the gut microbiota composition [66]. The effects of anthocyanins on gut microbiota are discussed in the next sections; nevertheless, it is important to bear in mind that the gut microbiota might compromise itself the effects of anthocyanins on obesity and metabolic syndrome.

The gut microbiota plays an important role in the bioavailability of anthocyanins. Anthocyanins are absorbed mainly in the intestine. However, those that are not

absorbed in the upper part of the gastrointestinal tract, in the colon might be metabolized by the existing bacteria, originating low molecular weight metabolites [67]. Given the enterohepatic recirculation, these compounds may prevail in the human body for several days [67]. This reveals the importance of the gut microbiota in anthocyanins metabolism. Since the gut microbiota differs between obese and lean individuals, this factor might be taken into account when evaluating the effects of anthocyanins in obese individuals [68]. In fact, two studies have shown that there are differences between normal weight and obese individuals regarding anthocyanins' metabolism [14, 69]. The disrupted gut microbiota as well as the generalized metabolic dysfunction unveiled by obese individuals may be behind the variability observed between these two groups. Obesity may comprise the metabolism of these compounds which deserves special consideration since obese individuals might be the ones who would benefit the most from anthocyanins intervention. It is important to note that anthocyanins are extensively metabolized in the organism (either by host or bacterial enzymes), and therefore, their metabolites are probably responsible for the health benefits of anthocyanins rather than the parent compounds that naturally exist in plants. Thus, future clinical trials should address this issue to achieve the optimal dose of anthocyanins according to the body mass index of the individuals.

5 Gut Microbiota Modulation

The human body harbors a collection of trillions of microorganisms. Bacteria, archaea, virus, fungi, and other eukaryotes live inside different organs establishing a symbiotic relationship with the host [70–72]. The microbes that collectively inhabit the gut – the gut microbiota – constitute the largest and most diverse community in the body [73]. Most of them are bacteria belonging to the *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, or *Verrucomicrobia* phylum [74].

Supported by the technological advances, the scientific community have been exploring the dynamics of such complex ecosystem. The role these microorganisms play in our health is still a mystery, but emerging evidence suggest that gut microbiota dysbiosis is implicated in a number of diseases ranging from localized gastrointestinal disorders to autoimmune, hepatic, respiratory, cardiovascular, oncologic, metabolic, neurologic, and psychiatric diseases [75].

The expansion of pathobionts (commensal microorganisms that can cause pathology under uncontrolled proliferation), the loss of beneficial bacteria, and the loss of diversity (microbial richness) are common characteristics of a dysbiotic state [76, 77]. These features have detrimental consequences for the host (may initiate obesity and metabolic diseases as well as neurologic disorders) and can be caused by environmental factors such as dietary modifications that encompass high contents of fat.

Diet is one of the most important factors that can modulate the gut microbiome and rapidly induce alterations in its composition [23]. High-fat and high-sucrose diets (HF diets) cause profound alterations in the gut microbial ecology, reducing bacterial diversity [24, 25]. In addition to HF diets, antibiotics, environmental

pollutants [28, 29], and food additives such as dietary emulsifiers [30–32] and noncaloric artificial sweeteners [33] have been shown to disrupt the gut microbiota (causing dysbiosis) in a similar way to what is observed in HF diet-induced obesity models. On the other hand, modulation of the gut microbiota composition by prebiotics might be a dietary strategy to prevent or counteract gut microbiota dysbiosis.

To fulfill the criteria of a previous prebiotic definition, the most studied prebiotics are nondigestible carbohydrates [78]. However, the definition of prebiotics has been modified to "a substrate that is selectively utilized by host microorganisms conferring a health benefit" [79]. Other substances, besides carbohydrates, might fit the updated definition if convincing evidence demonstrates their health benefits. Compounds such as polyphenols, namely, anthocyanins, may be considered prebiotics, according to this new definition.

Anthocyanins turn out to be particularly relevant since their bioavailability is considered to be low. Therefore, after consumption of anthocyanin-rich foods, it is expected that high amounts of anthocyanins reach the intestine to modulate bacterial growth at the same time they are metabolized by the existing bacteria.

Table 2 summarizes the studies evaluating the effects of anthocyanins on gut microbiota composition. There are substantial differences in the results since the type and the amount of anthocyanins used vary widely among the studies. Furthermore, the methodology used to characterize the gut microbiota composition is focused only on specific microbial groups which may not be sufficient to have a complete picture of the gut microbiota modifications that anthocyanins might bring about. For this reason, the gut microbiota composition of rats fed either a standard or a HF diet and supplemented with an anthocyanin-rich blackberry extract (BE) was recently evaluated, using next-generation sequencing (NGS) technology, the gold standard method. The dose chosen was 25 mg/kg body weight/day, easily achievable with 100 g of blackberries for a 60 kg man [14, 88]. Interestingly, BE was able to counteract some of the features of HF diet-induced dysbiosis by decreasing *Ruminococcus* and increasing *Sporobacter*, and, in the context of a standard diet, BE increased bacteria belonging to *Pseudoflavonifractor* genus [89].

6 Gut-Brain Axis

The gut-brain axis encompasses the strong bidirectional communication between the gut microbiota and the central nervous system (CNS) [90]. Multiple mechanisms may be involved in this bilateral communication: e.g., the gut microbiota has the capacity to produce many neurotransmitters and neuromodulators such as gamma-Aminobutyric acid (GABA), noradrenaline, serotonin, and acetylcholine [91, 92] and has the ability to control host tryptophan metabolism, regulating the fraction of tryptophan available for serotonin synthesis and, on the other hand, the production of neuroactive and neurotoxic metabolites along the kynurenine pathway [93–95]. As a result, modulation of the gut microbiota might be a tractable strategy to the development of novel therapeutics for complex CNS disorders.

Table 2 Studies reporting the effects of anthocyanins or anthocyanin-rich food sources on gut microbiota

Study design	Dose/duration	Methods	Changes in gut microbiota composition	Ref.
Fecal batch culture fermentation ($n = 3$)	Red wine extract (0.6 mg/ml) • 5, 10, 24, 30 and 48 h	FISH	= Bacteria (EUB mix) = Bifidobacterium spp. (Bif 164) = *Lactobacillus*/*Enterococcus* spp. (lab 158) = *Bacteroides* spp. (bac 303) = *C. histolyticum* (chis 150)	[80]
Fecal batch culture fermentation ($n = 3$)	Malvidin-3-glucoside (200 mg/l) • 5, 10, 24 h	FISH	↑ *Bifidobacterium* spp. (Bif 164) ↑ *Lactobacillus*/*Enterococcus* spp. (lab 158) = *Atopobium* spp. (Ato 291) = *Bacteroides* spp. (bac 303) ↓ *C. histolyticum* (chis 150) ↑ *Eubacterium rectale* – *Clostridium coccoides* (Erec 482) ↑ Total bacteria (EUB338)	[81]
Human crossover trial ($n = 10$)	Red wine (272 ml/day) and de-alcoholized red wine (272 ml/day) • 4 weeks	Real-time qPCR	↑ *Enterococcus* ↑ *Bifidobacterium* ↑ *Eggerthella lenta* ↑ *Blautia coccoides* – *Eubacterium rectale* groups	[82]
Male BALB/cJ mice. I/R-induced model ($n = 8$)	Bilberry powder (1.62 g/mouse) • 10 days	T-RFLP	= bacterial diversity (Shannon -wiener diversity index) Bacterial populations are influenced by dietary supplementation of bilberry	[83]
21-day-old offspring of Wistar rats ($n = 6$–9)	Jussara (*Euterpe edulis* Mart.) freeze-dried powder supplement (5 g/kg control diet) • Pregnancy and lactation	Real-time qPCR	↑ *Lactobacillus* spp.	[84]
Fecal suspension (pool of 14	Red cabbage and anthocyanin-rich extract digests • 48 h	Selective growth media	↑ *Lactobacillus* spp. ↑ *Bifidobacterium* spp. ↑ *Clostridium* spp. ↑ *Bacteroides* spp.	[85]

(*continued*)

Table 2 (continued)

Study design	Dose/duration	Methods	Changes in gut microbiota composition	Ref.
healthy volunteers)			↑ *Enterococcus* spp. ↑ *Enterobacteriaceae* ↑ Total anaerobic bacteria	
Anaerobic fermentation broth ($n = 8$)	Purple sweet potato anthocyanins • 6, 12, 24 h	FISH	↑ *Bifidobacterium* spp. (Bif 164) ↑ *Lactobacillus*/*Enterococcus* spp. (lab 158) ↓ *Bacteroides* spp. (bac 303) ↓ *C. histolyticum* (chis 150) = Total bacteria	[86]
Cross-sectional study ($n = 124$)	Strawberries (13.67 ± 29.47 mg anthocyanins/day)	Real-time qPCR	↓ *Lactobacillus*	[87]
Obese male C57BL/6 J Mice ($n = 8$)	Blueberry and black currant anthocyanins (400 μg/g HF diet) • 12 weeks	Gut low-density array (GULDA)	↑ *Bacteroidetes* ↑ Actinobacteria	[66]

FISH, fluorescence in situ hybridization; HF, high-fat; I/R, ischemic/reperfusion; qPCR, quantitative polymerase chain reaction

Anthocyanins have emerged as anti-inflammatory agents and are promising candidates for the prevention of neuroinflammation, a common hallmark of obesity-associated neuropsychiatry disorders and neurodegenerative diseases [60, 61, 96, 97]. The mechanisms underlying these effects might be related to the interaction of anthocyanins with neuron and microglia biology. Recent studies suggest that anthocyanins may protect neurons against neuroinflammatory injury by stimulating the production of proteins involved in synaptic plasticity [96, 98].

Anthocyanins and their metabolites are able to cross the blood-brain barrier [99]. Nonetheless, to be neuroprotective, anthocyanins do not necessarily need to reach the brain. By changing the gut microbiota and acting on the gut-brain axis, anthocyanins may exert a biological activity even without being absorbed. Alterations in gut microbial composition afforded by anthocyanins were shown to result in changes in the levels of tryptophan and kynurenic acid which has been implicated in CNS inflammation, excitation, and behavior (Fig. 6) [89]. Besides, the tryptophan/kynurenine ratio has been implicated in neuropsychiatry conditions such as depression and anxiety [95]. Therefore, by decreasing this ratio, anthocyanins might constitute a new promising strategy for the treatment of neuropshychiatry disorders.

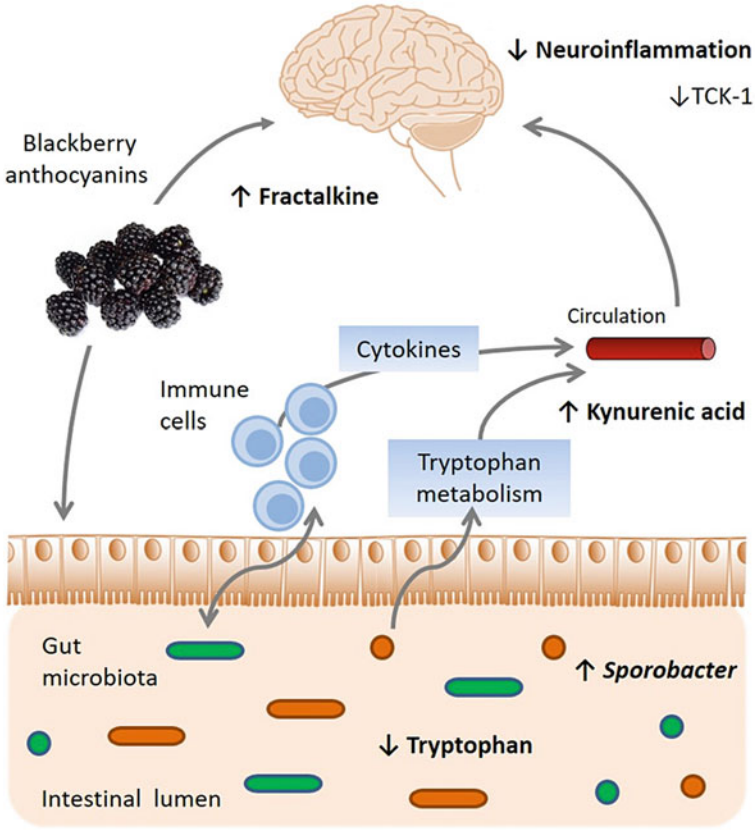


Fig. 6 Mechanisms behind the neuroprotective effects of anthocyanins, in the context of HF diet-induced obesity. Anthocyanins act directly in the brain increasing the expression of fractalkine, a chemokine extremely important in the crosstalk between neurons and microglia during synaptic plasticity [96]. On the other hand, anthocyanins modulate the gut microbiota composition, increasing the bacterial genus *Sporobacter* and alter host tryptophan metabolism. The amount of tryptophan available is decreased by anthocyanins to undergo the kynurenine pathway and originate kynurenic acid, a metabolite whose neuroprotective actions were recently identified [100, 101]. Through these routes, anthocyanins counteract the HF diet-induced neuroinflammation and may attenuate the neurological complications of obesity as well as neurodegenerative diseases. Anthocyanins might constitute, therefore, a new class of psychobiotics

7 Skin Health

Consumers have grown eager for natural solutions toward daily problems, paying more and more attention to natural sources of botanical products as bioactives in the products they consume. The cosmetic and skin health industry is no exception, and companies start to worry more about this issue, creating organic lines of products

and natural solutions to treat a variety of problems and diseases of the skin. At the same time, general concern from the population is rising toward the visible changes in skin structure and functionality associated with chronological aging that are aggravated by sun exposure. Consumers look up for innovation that englobes natural compounds when treating skin aging manifestations, as wrinkles and hyperpigmentation. On the other hand, while one out of three diagnosed cancers is a skin type cancer (nonmelanoma and melanoma), as revealed by the World Health Organization, WHO, there are not many products with natural bioactives to prevent these diseases.

Although cosmetic products with natural compounds and extracts usually claim a large variety of effects and benefits related to skin aging attenuation, truth is that there is a lack of scientific evidence to support those claims in terms of the role, stability, and absorption of such actives in cosmetic formulations. Despite this, fundamental knowledge regarding botanical compound bioactivity, particularly polyphenols and, most recently, anthocyanins, toward skin conditions is rising, and several works are now published about their role in skin aging, cancer, and other skin diseases. This section reviews the scientific literature existing on the potential of anthocyanins as skin actives to be used in preventing skin aging and skin diseases.

7.1 Skin Aging and UV Protection

The sun emits a wide spectrum of electromagnetic waves of which UV is the most cytotoxic, namely, UVB (295–315 nm) and UVA (325–400 nm), responsible for many skin diseases [102]. UV damage happens via two different mechanisms: the production of reactive oxygen species through the interaction of UVA with endogenous photosensitizers, which leads to the formation of oxidized products, such as lipid hydroperoxides and protein carbonyls, or the absorption of UVB by the heterocyclic bases of the DNA, causing damage to the genetic material. Hence, even if UV radiation possesses some health benefits, such as the stimulation of cholecalciferol (vitamin D) production, it is also responsible for some photosensitive diseases such as sunburn, immunosuppression, photocarcinogenesis, and premature skin aging (also called photoaging) [103].

Photoaging affects all layers of the skin, particularly epidermis and dermis [104]. The epidermis is the most external layer of the skin, and it is manly composed of keratinocytes. The dermis, located bellow the epidermis, is rich in connective tissue (collagen and elastic fibers), and it is the tissue responsible for the strength, extensibility, and elasticity of the skin. In physical and anatomical terms, skin aging is manifested by the appearance of skin wrinkles, dryness, thinning of the skin, loss of subcutaneous fat, and uneven pigmentation [105].

These visible changes of the skin depend on many molecular alterations which happen under UV irradiation. One of the key phenomena is the overexpression of metalloproteinases (MMPs), which are a family of zinc-dependent endopeptidases capable of degrading proteins of the ECM (the extracellular matrix), i.e., collagen

and elastin, which are important processes happening during skin regeneration and cell migration. When overexpressed, matrix maintenance is impaired, which leads to the loss of skin elasticity and resilience, leading to the appearance of wrinkles. At the same time, UV radiation triggers the increase in redox-sensitive transcription factors such as the nuclear factor-kappa B (NF-kB) and activator protein-1 (AP-1). On the other hand, as skin ages, cellular regeneration slows, making the recovery from mechanical injuries harder. This has led to an increasing research for compounds capable of blocking UV radiation or stopping its consequences, by a number of strategies, namely, by suppressing the oxidative damage, inhibiting MMP overexpression, modulating NF-kB/AP-1 pathways, and acting as wound-healing agents.

The harmful effects of UV radiation on the skin are usually fought using sunscreens. These are topically applied and can absorb or reflect UV radiation, preventing the damage to cellular material and diminishing the risk of skin diseases. There is a growing interest from the consumers' part toward the implementation of compounds from natural sources in general consumables, which is leading the markets toward natural alternatives. Botanical supplements with antioxidant activity have been proposed as sunscreen actives, usually in combination with well commonly used compounds [106]. Polyphenols [107–109] and particularly anthocyanins [110–112] have been described as potential agents in skin aging prevention, firstly because they absorb strongly in the visible and UV spectrums, with maximum absorbances in the ranges of 500–550 and 280–320 nm. This characteristic varies depending on their specific aglycone, sugar conjugation, and acylation patterns. An in vitro study with a cosmetic formulation containing anthocyanins from purple sweet potato (TNG73) investigated the potential of their absorbance patterns for dermatological purposes. Researchers found that, at a concentration of 0.61 mg/100 g of cream, anthocyanins could act as solar protectors, once they were shown to absorb 46% of the incident UV radiation [113], hence preventing damages to the dermal tissue.

The effects of anthocyanins in preventing skin damage have been studied using a different set of cellular and animal models, exploring which molecular pathways these compounds could be modulating (Fig. 7). Anthocyanins from blueberry could reduce UVA-stimulated ROS formation and lipid peroxidation in keratinocyte cells [114], and an extract from bog blueberry rich in cy3glc, petunidin-3-glucoside (Pt3glc), malvidin-3-glucoside (Mv3glc), and delphinidin-3-glucoside (Dp3glc) also prevented UVB-induced overexpression of MMPs and reduced the inflammatory response, modulating NF-kB- and MAPK-dependent pathways [115]. Another report showed that Cy3glc inhibited UV-induced translocation of the transcription factors NF-kB and AP-1, as well as the overexpression of IL-8, caspase-3 activation, and DNA fragmentation in human keratinocytes [116]. Blackberry anthocyanin-enriched fractions could reduce UV-mediated oxidative injury toward skin keratinocytes, by reducing reactive oxygen species and superoxide ion [117]. Skin photoprotection by anthocyanins has also been studied by Pornpimpa Phetpornpaisan et al., 2013, reporting that a black rice bran extract with high content of cyanidin-3-glucoside has a high antioxidative effect. This extract was able to inhibit MMP-2 and MMP-9 and possessed wound-healing effects by the

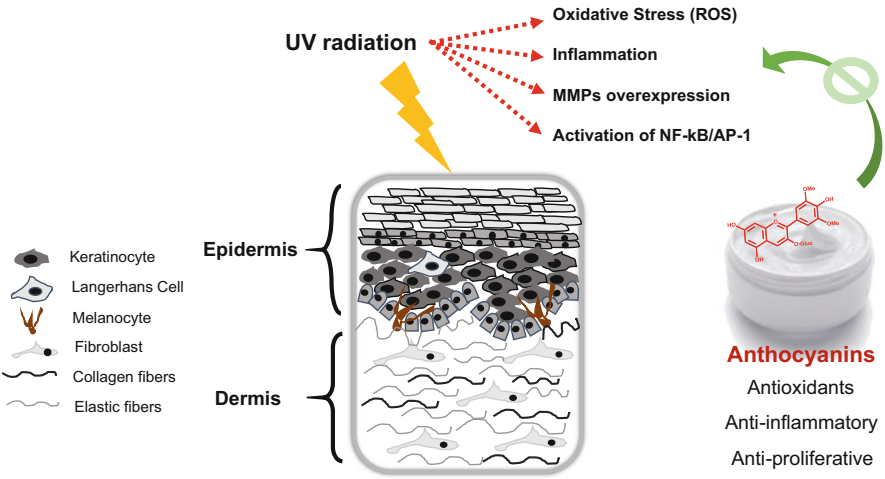


Fig. 7 Schematic representation of the skin layers and the main skin damages resulting from UV irradiation and potential actions of anthocyanins in pharmaceutical and cosmetic formulations

enhancement of collagen production and fibroblast proliferation [118]. A study using an anthocyanin-rich red orange extract showed that these compounds could reduce the translocation of NF-kB factor and AP-1 upon irradiation of cultured keratinocytes with UV light, as well as to reduce inflammation [119]. Green tea polyphenols were shown to be capable of preventing UV-induced immunosuppression [120], and cyanidin-3-glucopyranoside could prevent UVA-induced damage to keratinocytes [121]. At the same time, malvidin and cyanidin derivatives from açai fruit were able to counteract UVA-induced oxidative stress in fibroblasts, keeping the glutathione and lipid peroxidation levels close to the normal cellular values [122].

According to Tsoyi et al. (2008), anthocyanins from black soybean coats offer protection against UV radiation, not only to cultured keratinocytes but also in vivo to hairless mice skin, by reduction of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE(2)) through NF-kB-dependent pathways and by the prevention of the apoptotic cell death, inhibiting caspase-3 activation and reducing the levels of proapoptotic Bax protein [112]. In a different study, cyanidin-3-glucoside, the main anthocyanin in black soybean coats, was reported to inhibit the ROS/COX-2 pathway in HaCat cells (human normal keratinocytes cell line), scavenging reactive oxygen species by decreasing COX-2 expression [123].

Delphinidin, the most abundant anthocyanidin found in pomegranate fruit extract, has also shown a protective effect against oxidative stress and apoptosis in HaCat cells and in mouse skin in an in vivo experiment [124]. In another in vitro study, this anthocyanidin was capable of restoring the elastic modulus of irradiated keratinocytes, regenerating the mechanical properties of these cells [125], and of reducing the UV-induced MMP-1 expression in human dermal fibroblasts [126].

Using a reconstituted human skin – EpiD5erm™ FT-200, Afaq et al., 2009, explored the bioactives of pomegranate anthocyanin-enriched extracts. The extracts

were applied 1 h prior to a 12-h UVB irradiation period (60 mJ/cm^2), and it was noted that they could prevent UVB-induced damage to the dermal tissue [110]. These anthocyanins were able to significantly inhibit protein oxidation and to reduce the presence of cyclobutane pyrimidine dimers and 8-dihydro-2′-deoxyguanoside. On the other hand, anthocyanins showed some bioactive properties against the extracellular matrix of the skin, decreasing the UVB-induced overexpression of various MMPs, such as collagenase (MMP-1), gelatinase (MMP-2 and MMP-9), and elastase (MMP-12) [110].

Another group investigated the effect of encapsulated anthocyanins from *H. sabdariffa* on melanogenesis process using A375 melanocytes. It was found that these flavonoids, besides their potent antioxidant activity, could inhibit tyrosinase activity and suppress its expression alongside with the microphthalmia-associated transcription factor (MITF), involved in melanin production [127].

Another important feature of an aged skin is the slowing down of the cell cycle and the difficulty in regenerating injured skin. This leads to the investigation of compounds that, on one hand, may induce the production of collagen by fibroblasts on the dermis and, on the other hand, may accelerate recovery from injury. A few studies have tried to understand the role of anthocyanins in helping skin recover from wounding. Cell migration assays using HaCat and fibroblast cells were performed with black soybean anthocyanins (Cy3glc, Dp3glc and Pt3glc) in which the positive effect of anthocyanins in cellular migration was observed. At the same time, this study explored the production of VEGF (angiogenic factor) which was increased in both cell lines in the presence of anthocyanins. The authors also found that anthocyanins prevented TNF-α-mediated inflammatory responses, including ROS accumulation, NF-kB activation, and monocyte adhesion in endothelial cells [128].

Another model has been employed to study wound healing in a more reproducible way than with traditional assays such as the scratch assay, using a biophysical system – the electrical cell-substrate impedance sensing (ECIS). This system correlates impedance readings with biological behaviors of cells in culture. In one study, a model was developed to study the wound healing of skin cell monolayers such as keratinocytes (HaCat cell line) and fibroblasts [129]. Wound was electrically triggered with a higher voltage, and cells on top of the working electrode died. The healing was followed up by impedance readings, as the cells migrated and proliferated on top of the electrode, recovering from the damage. The time of recovery was analyzed in the absence or presence of red wine and blackberry anthocyanins, particularly mv3glc and cy3glc. It was shown that anthocyanins could reduce the time of recovery by more than half [130]. Although there are not many studies exploring the effects of anthocyanins in this phenomenon, the few existing suggest that anthocyanins may accelerate wound healing, promoting regeneration of the skin. This requires further studies with different anthocyanins and in a more reliable model of skin.

Even though these in vitro studies suggest the potential roles of anthocyanins in fighting photoaging, there is a huge gap in terms of dermatological preclinical and clinical studies performed with formulations containing anthocyanins. A study by

Leonel E. Rojo et al. (2013) showed that anthocyanins could be bound to protein-rich matrices and be stabilized without losing their pharmacological properties up to 50 weeks [131]. Anthocyanin-rich protein matrices were tested toward their inhibition ability of MMP-9, and it was observed that cranberry-rich protein matrix could abolish enzyme activity, followed by maqui berry and blueberry anthocyanins, inhibiting 85 and 80% of the enzyme activity, respectively. Another group recently performed an assay with a strawberry-based cosmetic formulation to test its efficacy against UV-induced damage to dermal fibroblasts [132]. They proposed the use of a strawberry/reduced CoQ10-based formulations as sunscreens, because it was observed that the conjugation of strawberry extract and CoQ10 could reduce ROS and pro-inflammatory markers, as well as to improve the mitochondrial function and the expression of antioxidant enzymes. Even though the class of polyphenols present in the extract was not identified, this kind of studies opens doors to the investigation of new ways to stabilize anthocyanins in formulations and to study their effect in vitro and in vivo in respect to aging conditions.

7.2 Anthocyanins and Skin Diseases

Anthocyanins have a variety of physiological functions, such as antioxidant activity and playing a protecting role in some chronic and age-related diseases that include some skin diseases and conditions.

A study performed using a three-dimensional reconstituted human skin model investigated the role of delphinidin in psoriasis. Psoriasis is an autoimmune, chronic, and inflammatory disease that affects 2–3% of the population [133]. The histologic marks reveal marked epidermal hyperproliferation and thickening, and the clinical features include skin inflammation with demarcated erythematous papules and scaly dermatological plaques [133]. Delphinidin has been reported to suppress proliferation and inflammation as well as to induce epidermal differentiation markers; thus this anthocyanidin is said to possess pro-differentiation effects [134]. In addition to this, delphinidin significantly decreased the thickness of the viable normal and hyperplastic epidermis, consolidating the differentiating and cornified layers of the skin equivalent [134]. Another study identified delphinidin as a novel potent dual PI3K/mTOR inhibitor, which possesses antipsoriatic activity in vitro in cell-free and cell culture, as well as in vivo in preclinical IMQ-induced psoriasis-like disease in Balb/c mice [135]. Another research group explored the effects of the topical application of delphinidin to flaky skin of mice and noted that it reduced the pathological markers of psoriasis lesions, by inducing the differentiation and reducing the proliferation and inflammation [136]. On the other hand, delphinidin also modulated the expression of tight junction proteins and increased the expression of protein JunB [136]. When using a three-dimensional reconstituted human skin model of psoriasis, researchers found that this anthocyanidin could significantly decrease the thickness of the viable normal and hyperplastic epidermis and consolidate the differentiating and cornified layers of the skin equivalent. This differentiation is correlated with an increase in expression of both caspase-14 and profilaggrin.

These set of studies indicate that delphinidin could be an important agent in ameliorating psoriasis lesions and suggest that preclinical and clinical studies should be performed to understand the efficacy of this anthocyanidin on the management of psoriasis. Heather R. Austin et al. (2014) investigated the potential role of cyanidin as an agent in psoriasis therapy by exploring its effect on the expression of late cornified envelope (LCE) genes as their deletion is a risk factor for psoriasis. In this study they show that this cyanidin upregulates the five LCE genes in cultures of differentiating primary human keratinocytes [137].

The effect of anthocyanins and other polyphenols from blueberry in atopic dermatitis has also been studied. This is a chronic and relapsing inflammatory skin disease with a prevalence of 10–20% and is more common among children [138]. Cyanidin-3-glucoside and quercetin fraction could inhibit inflammation of the lesion skin, correcting the Th1/Th2 balance and reducing IL-17. Anthocyanin-rich but not anthocyanidin-rich bilberry extract were shown to alleviate chronic pruritus of experimental dermatitis, probably by inhibition of mast cell degranulation [139].

Skin cancer is one of the most common of cancers in pale skin individuals, and it is directly related to sun exposure, namely, because of UV radiation. There are some studies regarding the use of anthocyanins as chemopreventive agents, particularly with pomegranate fruit extract (PFE), juice, and seed oil. Tests have been performed in cell culture, reconstituted human skin models and animal models of skin cancer and pomegranate was reported to exhibit immense potential for preventing UVB-induced skin cancer. PFE contains several polyphenols (such as catechins, gallic and ellagic acids) and anthocyanins. Pretreatment of human normal keratinocytes with PFE resulted in a dose- and time-dependent inhibition of UVB-induced phosphorylation of ERK1/2, JNK1/2, and p38 proteins and inhibition of the activation of nuclear factor-KB (NF-kB) pathway [140]. PFE treatment also resulted in a significant inhibition of UVA-induced expression of Ki-67 and PCNA and led to an enhanced expression of pro-apoptotic Bas and Bad with downregulation of anti-apoptotic Bcl-xL protein [141]. The chemopreventive properties of PFE were further evaluated in mice exposed to UVB radiation. Oral administration of PFE inhibited UVB-induced epidermal hyperplasia, leukocyte infiltration, and protein oxidation and also attenuate the activation of key inflammatory and cell proliferative pathways such as NF-kB and MAPK [142]. Oral administration of PFE in drinking water also reduced UVB-induced skin tumor incidence and tumor multiplicity in SKH-1 hairless mice [143]. In a study using delphinidin, it was also shown to inhibit UVB-induced COX-2 expression in JB6 P+ cells (a skin mouse cell line) by blocking the MAPKK4 and PI-3 K pathways and suppressing AP-1 and NF-kB activities, indicating that delphinidin may suppress UV-mediated skin carcinogenesis [144]. Diaconeasa Z. et al. (2017) performed a study on the effect of anthocyanins in melanoma inhibition. Different anthocyanin sources, namely, red grapes and chokeberries, were applied to melanoma cells and were able to reduce proliferation, to diminish mitochondrial membrane potential, and to induce oxidative damage, without showing these negative effects on normal skin cells [145].

A clinical study was performed to evaluate the effects of anthocyanins toward a skin condition. A formulation containing anthocyanins and glutathione was tested in

patients with erythema resulted from radiation therapy of breast cancer. This report does not present statistical significance or information on doses and types of anthocyanins used, but a trend was still observed toward improvement from the erythema in the group with the tested formulation. The theory behind this formulation is that while the breast is being irradiated, glutathione may clean up oxygen and carbon radicals formed, and anthocyanins may then help on the recycling of the glutathione so it can keep working.

Skin is the most superficial organ and the most exposed to environmental aggressions, UV radiation being one of the most important aggressors and the cause of skin photoaging and some skin diseases such as melanoma cancer. Oxidative damage, inflammation, apoptotic cell death, and overexpression of MMPs are some of the consequences of UV irradiation of the skin. In here it is suggested by accumulating scientific evidence that anthocyanins may play a role in delaying and fighting skin aging, as well as in protecting this organ against cancerous lesions. Even though the evidence obtained from different in vitro and in vivo studies shows the potential of these polyphenols, this might be insufficient to guarantee that the skin-protective effects are solely from anthocyanins, because in most studies, extracts are used and there is always the possibility that other compounds may be present. Furthermore, there is a gap in technology for the development of chemically stable and clinically effective anthocyanin-rich formulations for dermatological applications that must be addressed in the future.

8 Functional Foods and Cosmeceuticals

The concept of functional foods appeared first in Japan in the 1980s and mentioned foods that were developed specifically to promote health or to reduce the risk of disease. In Europe, the European Commission's Concerted Action on Functional Food Science, coordinated by the International Life Science Institute, defined functional foods as "a food that, together with the basic nutritional impact, has beneficial effects on one or more functions of the human organism thus either improving the general and physical conditions or/and decreasing the risk of the evolution of diseases" [146]. The amount of intake and form of the functional food should be as it is normally expected for dietary purposes. Consequently, it could not be in the form of pill or capsule just as normal food [147]. Therefore, they are usually considered to be those foods which are intended to be consumed as part of the normal diet and that contain bioactive components with potential health benefits such as vitamins, fatty acids, or dietary fiber or foods that include phytochemicals and other antioxidants or probiotics. These types of foods are being integrated into the corporate mainstream and being increasingly accepted by the public.

Anthocyanins are widely distributed in the human diet, and their chemical and biological functions have been extensively studied. These natural pigments are broadly used in the food industry as natural colorants, and their use is not only valuable for improving the overall appearance but also because anthocyanins have beneficial properties to our health. Anthocyanin extracts have been demonstrated to

play a role in several diseases and conditions, displaying antioxidative and radical-scavenging properties [148, 149], acting as chemopreventive agents [150], but also playing a role in diabetes [151–154]. Because of their coloring and biological properties, anthocyanins are now being considered as potential functional food ingredients and dietary supplements. This section will discuss the possibility of using anthocyanins in functional foods.

Anthocyanins are widely spread in fruits and vegetables, as well as in red wines which results in high ingestion and a daily intake ranging from several milligrams to hundreds of milligrams per person [155]. These flavonoids are now extensively studied and commonly used as food colorants especially in acidic foods that favor their chemistry, by stabilizing the flavylium cation form. Some vegetable sources like black carrot, red-fleshed and purple sweet potato, as well as red cabbage have been shown to provide high amount of acylated anthocyanins [156–158] that are more stable to pH than non-acylated [159]. An example of anthocyanins used as colorants is red rice, which was approved by the Chinese Ministry of Health as a modern food additive to increase the color and delicacy of meat, fish, and soybean products [160]. Besides this, red rice is also used as a functional food to treat abdominal pain and cardiovascular disease [161]. Even so and to this day, there are not many works performed on the manufacture of products that include anthocyanins as functional ingredients, but as the consumers interest on the incorporation of bioactives into popular foods is growing, innovative work is rising. An example of a product that can incorporate anthocyanins as functional ingredients is bread. This can be used by the production of anthocyanin-enriched flours as explored by Donatella Ficco et al. in 2017 [162]. They performed the micronization followed by air classification to select anthocyanin-rich fractions of durum and soft wheats and noted that the pigmented durum wheat showed the greatest acceptability in terms of aroma and taste of the bread obtained.

Anthocyanins have a variety of biological properties and may be used as potential functional foods against some metabolic syndromes such as obesity and type 2 diabetes [24]. Some anti-obesity strategies include the increase of physical activity and the consumption of non-starch polysaccharides and fiber, accompanied by the reduction of micronutrient-poor diets [163]. Another strategy that may be employed to fight obesity is related to the use of inhibitors of some digestive enzymes, such as α-glucosidase and α-amylase. Inhibitors may help retarding the increase of sugar in the bloodstream after a meal, and anthocyanins and other polyphenols, such as tannins, may be employed to this end [164]. On the other hand, cyanidin-3-glucoside (cy3glc) has been shown to reduce blood glucose and to enhance insulin sensitivity by downregulating retinol binding protein-4 expression in type 2 diabetic mice [165]. In two other studies, the effect of anthocyanins in diabetes was explored, and it was found that, even though black raspberry anthocyanins do not alter the development of obesity in mice that were fed an obesogenic high-fat diet [166], purified anthocyanins from blueberry as well as blueberry juice could prevent dyslipidemia and obesity in the same animal model [167]. Another study revealed that blueberry anthocyanins could reduce glucose production in hepatocytes and body weight gain [168]. Another source of anthocyanins, maqui berry rich in

delphinidin-3-sambubioside-4-glucoside, also decreased the glucose production and downregulated glucose-6-phosphatase in liver cells, which is a gluconeogenic enzyme [169]. Edirisinghe et al. (2011) also found that the strawberry anthocyanin pelargonidin-3-O-glucoside could reduce inflammation and increase insulin sensitivity in overweight adults [170]. These studies suggest the potential of anthocyanins to be used as functional foods to fight obesity and type 2 diabetes and to lead the way to the conception of new products enriched in these flavonoids to be consumed as supplement in patients with these conditions or as a fundamental tool to convert our diet in a functional one, preventing food-related diseases. Because anthocyanins are usually unstable outside their environment, work has been done toward the stabilization of anthocyanins for their use in the food industry which may be of importance when conceiving new functional foods [171–173]. Anthocyanin-loaded chitosan nanoparticles, for example, were found to slow the breakdown of anthocyanins in simulated gastrointestinal fluid and improved the stability of these compounds in a beverage [172].

Anthocyanins are now known as bioactives with potential health benefits extensively studied in several field such as at metabolic syndromes but also play a role in cardiovascular disease and possess anti-inflammatory, chemopreventive, and antioxidant activities, among others. The development of functional foods and supplements incorporating these compounds is now being promoted as the information on the biological properties of these flavonoids arises. Soon, it might be possible to purchase enriched beverages to aid in the treatment of diabetes or purple bread to incorporate in a more functional diet as well as other products usually consumed but improved not only in its nutritional value but also in health promoting and functional role in our diet.

References

1. Crozier A et al (2009) Dietary phenolics: chemistry, bioavailability and effects on health. Natural Product Reports
2. Charron CS et al (2009) Bioavailability of anthocyanins from purple carrot juice: effects of acylation and plant matrix. J Agric Food Chem 57:1226–1230
3. Delgado-Vargas F et al (2000) Natural pigments: carotenoids, anthocyanins, and betalains – characteristics, biosynthesis, processing, and stability. Crit Rev Food Sci Nutr 40:173–289
4. Oliveira J et al (2014) Previous and recent advances in pyranoanthocyanins equilibria in aqueous solution. Dyes Pigments 100:190–200
5. Fernandes I et al (2015) Multiple-approach studies to assess anthocyanin bioavailability. Phytochem Rev 14:899–919
6. Khanal RC et al (2010) Effect of heating on the stability of grape and blueberry pomace procyanidins and total anthocyanins. Food Res Int 43:1464–1469
7. Pina F et al (2015) Anthocyanins and derivatives are more than flavylium cations. Tetrahedron 71:3107–3114
8. Patras A et al (2010) Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends Food Sci Technol 21:3–11
9. Rodríguez R et al (2004) Mechanical properties of white and green asparagus: changes related to modifications of cell wall components. J Sci Food Agric 84:1478–1486
10. Chanliaud E et al (1999) In vitro synthesis and properties of pectin/Acetobacter xylinus cellulose composites. Plant J 20:25–35

11. Cheynier V (2005) Polyphenols in foods are more complex than often thought. Am J Clin Nutr 81:223s–229s
12. Fernandes A et al (2013) Effect of cyclodextrins on the thermodynamic and kinetic properties of cyanidin-3-O-glucoside. Food Res Int 51:748–755
13. Fernandes A et al (2014) Understanding the molecular mechanism of anthocyanin binding to pectin. Langmuir 30:8516–8527
14. Marques C et al (2016) Pharmacokinetics of blackberry anthocyanins consumed with or without ethanol: a randomized and crossover trial. Mol Nutr Food Res 60:2319–2330
15. Xiao D et al (2017) The effect of dietary factors on strawberry anthocyanins oral bioavailability. Food Funct 8:3970–3979
16. Walton MC et al (2009) Viscous food matrix influences absorption and excretion but not metabolism of blackcurrant anthocyanins in rats. J Food Sci 74:H22–HH9
17. Kamonpatana K et al (2014) Anthocyanin structure determines susceptibility to microbial degradation and bioavailability to the buccal mucosa. J Agric Food Chem 62:6903–6910
18. Ferrer-Gallego R et al (2015) New anthocyanin–human salivary protein complexes. Langmuir 31:8392–8401
19. Soares S et al (2013) Different phenolic compounds activate distinct human bitter taste receptors. J Agric Food Chem 61:1525–1533
20. Podsedek A et al (2017) Inhibitory potential of red cabbage against digestive enzymes linked to obesity and type 2 diabetes. J Agric Food Chem 65:7192–7199
21. McDougall GJ et al (2005) The inhibitory effects of berry polyphenols on digestive enzymes. Biofactors 23:189–195
22. Williamson G (2013) Possible effects of dietary polyphenols on sugar absorption and digestion. Mol Nutr Food Res 57:48–57
23. Jakobs S et al (2006) Natural flavonoids are potent inhibitors of glycogen phosphorylase. Mol Nutr Food Res 50:52–57
24. Castro-Acosta ML et al (2016) Berries and anthocyanins: promising functional food ingredients with postprandial glycaemia-lowering effects. Proc Nutr Soc 75:342–355
25. Castro-Acosta ML et al (2016) Drinks containing anthocyanin-rich blackcurrant extract decrease postprandial blood glucose, insulin and incretin concentrations. J Nutr Biochem 38:154–161
26. Wiese S et al (2009) Protein interactions with cyanidin-3-glucoside and its influence on α-amylase activity. J Sci Food Agric 89:33–40
27. Cahyana Y et al (2013) Interaction of anthocyanins with human serum albumin: influence of pH and chemical structure on binding. Food Chem 141:2278–2285
28. Ossman T et al (2016) Interaction of wine anthocyanin derivatives with lipid bilayer membranes. Comput Theor Chem 1077:80–86
29. Bonarska-Kujawa D et al (2012) Interaction of selected anthocyanins with erythrocytes and liposome membranes. Cell Mol Biol Lett 17:289–308
30. Tsuchiya H (2011) Effects of red wine flavonoid components on biomembranes and cell proliferation. Int J Wine Res 3:9
31. Sarma AD et al (1999) Anthocyanin-DNA copigmentation complex: mutual protection against oxidative damage. Phytochemistry 52:1313–1318
32. Mas T et al (2000) DNA triplex stabilization property of natural anthocyanins. Phytochemistry 53:679–687
33. Fernandes I et al (2014) Bioavailability of anthocyanins and derivatives. J Funct Foods 7:54–66
34. Lila MA et al (2016) Unraveling anthocyanin bioavailability for human health. Annu Rev Food Sci Technol 7:375–393
35. Kamonpatana K et al (2012) Susceptibility of anthocyanins to ex vivo degradation in human saliva. Food Chem 135:738. https://doi.org/10.1016/j.foodchem.2012.04.110
36. Mallery SR et al (2011) Effects of human oral mucosal tissue, saliva and oral microflora on intraoral metabolism and bioactivation of black raspberry anthocyanins. Cancer Prev Res (Phila) 4:1209–1221
37. Talavera S et al (2003) Anthocyanins are efficiently absorbed from the stomach in anesthetized rats. J Nutr 133:4178–4182

38. Fang J (2014) Bioavailability of anthocyanins. Drug Metab Rev 46:508–520
39. Oliveira H et al (2015) Experimental and theoretical data on the mechanism by which red wine anthocyanins are transported through human MKN-28 gastric cell model. J Agric Food Chem 63:7685
40. Atnip AA et al (2017) Time, concentration, and pH-dependent transport and uptake of anthocyanins in a human gastric epithelial (NCI-N87) cell line. Int J Mol Sci 18:446
41. Fernandes I et al (2012) A new approach on the gastric absorption of anthocyanins. Food Funct 3:508–516
42. He J et al (2010) Oxovitisins: a new class of neutral pyranone-anthocyanin derivatives in red wines. J Agric Food Chem 58:8814–8819
43. Fernandes I et al (2012) On the bioavailability of flavanols and anthocyanins: Flavanol–anthocyanin dimers. Food Chem 135:812–818
44. Faria A et al (2009) Bioavailability of anthocyanin-pyruvic acid adducts in rat. In: International Conference on Polyphenols and Health, Yorkshire, Leeds
45. Bub A et al (2001) Malvidin-3-glucoside bioavailability in humans after ingestion of red wine, dealcoholized red wine and red grape juice. Eur J Nutr 40:113–120
46. Bitsch R et al (2004) Bioavailability and biokinetics of anthocyanins from red grape juice and red wine. J Biomed Biotechnol 2004:293–298
47. Garcia-Alonso M et al (2009) Red wine anthocyanins are rapidly absorbed in humans and affect monocyte chemoattractant protein 1 levels and antioxidant capacity of plasma. J Nutr Biochem 20:521–529
48. Kuntz S et al (2015) Uptake and bioavailability of anthocyanins and phenolic acids from grape/blueberry juice and smoothie in vitro and in vivo. Br J Nutr 113:1044–1055
49. Fernandes I et al (2017) Pharmacokinetics of table and port red wine anthocyanins: a crossover trial in healthy men. Food Funct 8:2030–2037
50. Czank C et al (2013) Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a 13C-tracer study. Am J Clin Nutr 97:995–1003
51. Manach C et al (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S–242S
52. Alzaid F et al (2013) Regulation of glucose transporter expression in human intestinal Caco-2 cells following exposure to an anthocyanin-rich berry extract. PLoS One 8:e78932
53. Zou TB et al (2014) The role of sodium-dependent glucose transporter 1 and glucose transporter 2 in the absorption of cyanidin-3-o-beta-glucoside in Caco-2 cells. Nutrients 6:4165–4177
54. Hribar U et al (2014) The metabolism of anthocyanins. Curr Drug Metab 15:3–13
55. Faria A et al (2009) Absorption of anthocyanins through intestinal epithelial cells – putative involvement of GLUT2. Mol Nutr Food Res 53:1430–1437
56. Dreiseitel A et al (2009) Berry anthocyanins and anthocyanidins exhibit distinct affinities for the efflux transporters BCRP and MDR1. Br J Pharmacol 158:1942–1950
57. Williamson G et al (2010) Colonic metabolites of berry polyphenols: the missing link to biological activity? Br J Nutr 104:S48–S66
58. Mueller D et al (2017) Human intervention study to investigate the intestinal accessibility and bioavailability of anthocyanins from bilberries. Food Chem 231:275–286
59. Azzini E et al (2017) Antiobesity effects of anthocyanins in preclinical and clinical studies. Oxidative Med Cell Longev 2017:2740364
60. Lee YM et al (2017) Dietary anthocyanins against obesity and inflammation. Nutrients 9:1089
61. Li D et al (2017) Health benefits of anthocyanins and molecular mechanisms: update from recent decade. Crit Rev Food Sci Nutr 57:1729–1741
62. Guo H et al (2015) The update of anthocyanins on obesity and type 2 diabetes: experimental evidence and clinical perspectives. Rev Endocr Metab Disord 16:1–13
63. Zhu Y et al (2011) Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. Clin Chem 57:1524–1533

64. Li D et al (2015) Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients. J Nutr 145:742–748
65. Yang L et al (2017) Role of purified anthocyanins in improving Cardiometabolic risk factors in Chinese men and women with prediabetes or early untreated diabetes-a randomized controlled trial. Nutrients 9:pii E1104
66. Overall J et al (2017) Metabolic effects of berries with structurally diverse anthocyanins. Int J Mol Sci 18:pii: E422
67. Kay CD et al (2017) Anthocyanins and flavanones are more bioavailable than previously perceived: a review of recent evidence. Annu Rev Food Sci Technol 8:155–180
68. Ley RE et al (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444:1022–1023
69. Novotny JA et al (2017) The effect of obesity and repeated exposure on pharmacokinetic response to grape polyphenols in humans. Mol Nutr Food Res 61:1700043
70. Grice EA et al (2011) The skin microbiome. Nat Rev Microbiol 9:244–253
71. Mark Welch JL et al (2016) Biogeography of a human oral microbiome at the micron scale. Proc Natl Acad Sci U S A 113:E791–E800
72. Man WH et al (2017) The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol 15:259–270
73. Sender R et al (2016) Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 14:e1002533
74. Arumugam M et al (2011) Enterotypes of the human gut microbiome. Nature 473:174–180
75. Lynch SV et al (2016) The human intestinal microbiome in health and disease. N Engl J Med 375:2369–2379
76. Levy M et al (2017) Dysbiosis and the immune system. Nat Rev Immunol 17:219–232
77. Petersen C et al (2014) Defining dysbiosis and its influence on host immunity and disease. Cell Microbiol 16:1024–1033
78. Gibson GR et al (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17:259–275
79. Hill C et al (2014) Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506–514
80. Sanchez-Patan F et al (2012) In vitro fermentation of a red wine extract by human gut microbiota: changes in microbial groups and formation of phenolic metabolites. J Agric Food Chem 60:2136–2147
81. Hidalgo M et al (2012) Metabolism of anthocyanins by human gut microflora and their influence on gut bacterial growth. J Agric Food Chem 60:3882–3890
82. Queipo-Ortuno MI et al (2012) Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. Am J Clin Nutr 95:1323–1334
83. Jakesevic M et al (2013) Effects of bilberry (*Vaccinium myrtillus*) in combination with lactic acid bacteria on intestinal oxidative stress induced by ischemia-reperfusion in mouse. J Agric Food Chem 61:3468–3478
84. Almeida Morais C et al (2014) Jussara (*Euterpe edulis* Mart.) supplementation during pregnancy and lactation modulates the gene and protein expression of inflammation biomarkers induced by trans-fatty acids in the colon of offspring. Mediat Inflamm 2014:987927
85. Podsedek A et al (2014) Matrix effects on the stability and antioxidant activity of red cabbage anthocyanins under simulated gastrointestinal digestion. Biomed Res Int 2014:365738
86. Zhang X et al (2016) The modulatory effect of anthocyanins from purple sweet potato on human intestinal microbiota in vitro. J Agric Food Chem 64:2582–2590
87. Fernandez-Navarro T et al (2016) Bioactive compounds from regular diet and faecal microbial metabolites. Eur J Nutr 57:487–497
88. Nair AB et al (2016) A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 7:27–31

89. Marques C (2018) Unraveling the effects of anthocyanins in metabolic health and disease: insights on bioavailability and gut microbiota modulation. University of Porto, Porto
90. Mayer EA et al (2015) Gut/brain axis and the microbiota. J Clin Invest 125:926–938
91. Barrett E et al (2012) Gamma-Aminobutyric acid production by culturable bacteria from the human intestine. J Appl Microbiol 113:411–417
92. Roshchina V (2010) Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells. In: Lyte M et al (eds) Microbial endocrinology – Interkingdom signaling in infectious disease and health. Springer New York
93. O'Mahony SM et al (2015) Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behav Brain Res 277:32–48
94. Lovelace MD et al (2017) Recent evidence for an expanded role of the kynurenine pathway of tryptophan metabolism in neurological diseases. Neuropharmacology 112:373–388
95. Kennedy PJ et al (2017) Kynurenine pathway metabolism and the microbiota-gut-brain axis. Neuropharmacology 112:399–412
96. Meireles M et al (2015) The impact of chronic blackberry intake on the neuroinflammatory status of rats fed a standard or high-fat diet. J Nutr Biochem 26:1166–1173
97. Carvalho FB et al (2017) Anthocyanins control neuroinflammation and consequent memory dysfunction in mice exposed to lipopolysaccharide. Mol Neurobiol 54:3350–3367
98. Meireles M et al (2016) Anthocyanin effects on microglia M1/M2 phenotype: consequence on neuronal fractalkine expression. Behav Brain Res 305:223–228
99. Faria A et al (2014) Flavonoid metabolites transport across a human BBB model. Food Chem 149:190–196
100. Wu HQ et al (2010) The astrocyte-derived alpha7 nicotinic receptor antagonist kynurenic acid controls extracellular glutamate levels in the prefrontal cortex. J Mol Neurosci 40:204–210
101. Salimi Elizei S et al (2017) Kynurenic acid downregulates IL-17/1L-23 axis in vitro. Mol Cell Biochem 431:55–65
102. Svobodova A et al (2006) Ultraviolet light induced alteration to the skin. Biomed Pap 150:25–38
103. Taylor MDCR et al (1996) Sun exposure and skin disease. Annu Rev Med 47:181–191
104. Giangreco A et al (2008) Epidermal stem cells are retained in vivo throughout skin aging. Aging Cell 7:250–259
105. Giacomoni PU (2008) Advancement in skin aging: the future cosmeceuticals. Clin Dermatol 26:364–366
106. Baliga MS et al (2006) Chemoprevention of photocarcinogenesis by selected dietary botanicals. Photochem Photobiol Sci 5:243–253
107. Afaq F et al (2011) Polyphenols: skin Photoprotection and inhibition of Photocarcinogenesis. Mini Rev Med Chem 11:1200–1215
108. Kao E-S et al (2007) Effects of polyphenols derived from fruit of *Crataegus pinnatifida* on cell transformation, dermal edema and skin tumor formation by phorbol ester application. Food Chem Toxicol 45:1795–1804
109. Kim J et al (1998) Protective effects of green tea polyphenols on the ultraviolet-induced dermal extracellular damage. J Dermatol Sci 16:S127
110. Afaq F et al (2009) Protective effect of pomegranate derived products on UVB-mediated damage in human reconstituted skin. Exp Dermatol 18:553–561
111. Lila MA (2004) Anthocyanins and human health: an in vitro investigative approach. J Biomed Biotechnol 2004:306–313
112. Tsoyi K et al (2008) Protective effect of anthocyanins from black soybean seed coats on UVB-induced apoptotic cell death in vitro and in vivo. J Agric Food Chem 56:10600–10605
113. Chan C-F et al (2010) Influence of purple sweet potato extracts on the UV absorption properties of a cosmetic cream. J Cosmet Sci 61:333–341
114. Calò R et al (2014) Protective effect of *Vaccinium myrtillus* extract against UVA- and UVB-induced damage in a human keratinocyte cell line (HaCaT cells). J Photochem Photobiol B Biol 132:27–35

115. Bae J-Y et al (2009) Bog blueberry anthocyanins alleviate photoaging in ultraviolet-B irradiation-induced human dermal fibroblasts. Mol Nutr Food Res 53:726–738
116. Cimino F et al (2006) Effect of Cyanidin-3-O-glucoside on UVB-induced response in human keratinocytes. J Agric Food Chem 54:4041–4047
117. Murapa P et al (2012) Anthocyanin-rich fractions of blackberry extracts reduce UV-induced free radicals and oxidative damage in keratinocytes. Phytother Res 26:106–112
118. Phetpornpaisan P et al (2014) A local Thai cultivar glutinous black rice bran: a source of functional compounds in immunomodulation, cell viability and collagen synthesis, and matrix metalloproteinase-2 and -9 inhibition. J Funct Foods 7:650–661
119. Cimino F et al (2007) Protective effects of a red orange extract on UVB-induced damage in human keratinocytes. Biofactors 30:129–138
120. Roh E et al (2017) Molecular mechanisms of green tea polyphenols with protective effects against skin photoaging. Crit Rev Food Sci Nutr 57:1631–1637
121. Tarozzi A et al (2007) Protective Effects of Cyanidin-3-O-β-glucopyranoside Against UVA-induced Oxidative Stress in Human Keratinocytes. Photochem Photobiol 81:623–629
122. Petruk G et al (2017) Malvidin and cyanidin derivatives from açai fruit (*Euterpe oleracea* Mart.) counteract UV-A-induced oxidative stress in immortalized fibroblasts. J Photochem Photobiol B Biol 172:42–51
123. He Y et al (2017) Cyanidin-3-O-glucoside inhibits the UVB-induced ROS/COX-2 pathway in HaCaT cells. J Photochem Photobiol B Biol 177:24–31
124. Afaq F et al (2007) Delphinidin, an Anthocyanidin in pigmented fruits and vegetables, protects human HaCaT keratinocytes and mouse skin against UVB-mediated oxidative stress and apoptosis. J Investig Dermatol 127:222–232
125. Sobiepanek A et al (2016) The effect of delphinidin on the mechanical properties of keratinocytes exposed to UVB radiation. J Photochem Photobiol B Biol 164:264–270
126. Lim T-G et al (2013) NADPH oxidase is a novel target of delphinidin for the inhibition of UVB-induced MMP-1 expression in human dermal fibroblasts. Exp Dermatol 22:428–430
127. Hwang J-M et al (2013) Inhibitory effect of liposome-encapsulated anthocyanin on melanogenesis in human melanocytes. Pharm Biol 51:941–947
128. Nizamutdinova IT et al (2009) Anthocyanins from black soybean seed coats stimulate wound healing in fibroblasts and keratinocytes and prevent inflammation in endothelial cells. Food Chem Toxicol 47:2806–2812
129. Yang JM et al (2016) A quantitative cell modeling and wound-healing analysis based on the electric cell-substrate impedance sensing (ECIS) method. Comput Biol Med 69:134–143
130. Evora A et al (2017) The effect of anthocyanins from red wine and blackberry on the integrity of a keratinocyte model using ECIS. Food Funct 8:3989–3998
131. Roopchand DE et al (2013) Food-compatible method for the efficient extraction and stabilization of cranberry pomace polyphenols. Food Chem 141:3664. https://doi.org/10.1016/j.foodchem.2013.06.050
132. Gasparrini M et al (2017) Strawberry-based cosmetic formulations protect human dermal fibroblasts against UVA-induced damage. Nutrients 9:605
133. Nestle FO et al (2009) Psoriasis. N Engl J Med 361:496–509
134. Chamcheu JC et al (2013) Delphinidin, a dietary antioxidant, induces human epidermal keratinocyte differentiation but not apoptosis: studies in submerged and three-dimensional epidermal equivalent models. Exp Dermatol 22:342–348. https://doi.org/10.1111/exd.12140
135. Chamcheu Jean Christopher AVM, Stephane E, Mario S, Siddiqui IA, Satyshur KA, Syed DN, Dodwad S-JM, Maria-Ines C-R, Jack LB, Wood GS, Hasan M (2017) Dual inhibition of PI3K/Akt and mTOR by the dietary antioxidant, delphinidin, ameliorates psoriatic features in vitro and in an imiquimod-induced psoriasis-like disease in mice. Antioxid Redox Signal 26:49–69
136. Pal HC et al (2015) Topical application of delphinidin reduces psoriasiform lesions in the flaky skin mouse model by inducing epidermal differentiation and inhibiting inflammation. Br J Dermatol 172:354–364

137. Austin HR et al (2014) Regulation of late cornified envelope genes relevant to psoriasis risk by plant-derived cyanidin. Biochem Biophys Res Commun 443:1275–1279
138. Leung DYM et al (2001) Cellular and immunologic mechanisms in atopic dermatitis. J Am Acad Dermatol 44:S1–S12
139. Yamaura K et al (2012) Anthocyanins, but not anthocyanidins, from bilberry (*Vaccinium myrtillus* L.) alleviate pruritus via inhibition of mast cell degranulation. J Food Sci 77: H262–H2H7
140. Afaq F et al (2004) Pomegranate fruit extract modulates UVB-induced activation of nuclear factor kappa B and phosphorylation of mitogen activated protein kinases in normal human epidermal keratinocytes. Cancer Res 45:932
141. Syed DN et al (2006) Photochemopreventive effect of pomegranate fruit extract on UVA-mediated activation of cellular pathways in normal human epidermal keratinocytes. Photochem Photobiol 82:398–405
142. Khan N et al (2012) Pomegranate fruit extract inhibits UVB-induced inflammation and proliferation by modulating NF-κB and MAPK signaling pathways in mouse skin†. Photochem Photobiol 88:1126–1134
143. Afaq F et al (2008) Inhibitory effect of oral feeding of pomegranate fruit extract on UVB-induced skin carcinogenesis in SKH-1 hairless mice. Cancer Res 68:1246
144. Kwon JY et al (2009) Delphinidin suppresses ultraviolet B-induced cyclooxygenases-2 expression through inhibition of MAPKK4 and PI-3 kinase. Carcinogenesis 30:1932–1940
145. Diaconeasa Z et al (2017) Melanoma inhibition by anthocyanins is associated with the reduction of oxidative stress biomarkers and changes in mitochondrial membrane potential. Plant Foods Hum Nutr 72:404
146. Roberfroid MB (2000) Concepts and strategy of functional food science: the European perspective. Am J Clin Nutr 71:1660s–1664s
147. No authors listed (2007) Scientific concepts of functional foods in Europe Consensus document. Br J Nutr 81:S1–S27
148. Astadi IR et al (2009) In vitro antioxidant activity of anthocyanins of black soybean seed coat in human low density lipoprotein (LDL). Food Chem 112:659–663
149. Kähkönen MP et al (2003) Antioxidant activity of anthocyanins and their Aglycons. J Agric Food Chem 51:628–633
150. Fimognari C et al (2004) Induction of apoptosis in two human leukemia cell lines as well as differentiation in human promyelocytic cells by cyanidin-3-O-β-glucopyranoside. Biochem Pharmacol 67:2047–2056
151. Zhang Y et al (2004) Insulin secretion and cyclooxygenase enzyme inhibition by cabernet sauvignon grape skin compounds. J Agric Food Chem 52:228–233
152. Jayaprakasam B et al (2005) Insulin secretion by bioactive anthocyanins and Anthocyanidins present in fruits. J Agric Food Chem 53:28–31
153. Andriambeloson E et al (1997) Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. Br J Pharmacol 120:1053–1058
154. Liu Z et al (2005) Black raspberry extract and fractions contain angiogenesis inhibitors. J Agric Food Chem 53:3909–3915
155. Horbowicz M et al (2008) Anthocyanins of fruits and vegetables – their occurrence, analysis and role in human. Veg Crop Res Bull 68:5–22
156. Xu J et al (2015) Characterisation and stability of anthocyanins in purple-fleshed sweet potato P40. Food Chem 186:90–96
157. Xiong S et al (2006) Stability and antioxidant activity of black currant anthocyanins in solution and encapsulated in glucan gel. J Agric Food Chem 54:6201–6208
158. Ahmadiani N et al (2014) Anthocyanins contents, profiles, and color characteristics of red cabbage extracts from different cultivars and maturity stages. J Agric Food Chem 62:7524–7531
159. Stintzing FC et al (2004) Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends Food Sci Technol 15:19–38

160. Ma J et al (2000) Constituents of red yeast Rice, a traditional Chinese food and medicine. J Agric Food Chem 48:5220–5225
161. Deng G-F et al (2013) Phenolic compounds and bioactivities of pigmented Rice. Crit Rev Food Sci Nutr 53:296–306
162. Ficco DBM et al (2018) Production of anthocyanin-enriched flours of durum and soft pigmented wheats by air-classification, as a potential ingredient for functional bread. J Cereal Sci 79:118–126
163. Ba S et al (2007) Diet, nutrition and the prevention of excess weight gain and obesity. Public Health Nutr 7:123–146
164. Boath AS et al (2012) Berry polyphenols inhibit digestive enzymes: a source of potential health benefits? Food Digestion 3:1–7
165. Sasaki R et al (2007) Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to downregulation of retinol binding protein 4 expression in diabetic mice. Biochem Pharmacol 74:1619–1627
166. Prior RL et al (2010) Dietary black raspberry anthocyanins do not Alter development of obesity in mice fed an obesogenic high-fat diet. J Agric Food Chem 58:3977–3983
167. Prior RL et al (2010) Purified blueberry anthocyanins and blueberry juice Alter development of obesity in mice fed an obesogenic high-fat diet. J Agric Food Chem 58:3970–3976
168. Roopchand DE et al (2013) Blueberry polyphenol-enriched soybean flour reduces hyperglycemia, body weight gain and serum cholesterol in mice. Pharmacol Res: Off J Ital Pharmacol Soc 68:59. https://doi.org/10.1016/j.phrs.2012.11.008
169. Rojo LE et al (2012) In vitro and in vivo anti-diabetic effects of anthocyanins from Maqui Berry (*Aristotelia chilensis*). Food Chem 131:387–396
170. Edirisinghe I et al (2011) Strawberry anthocyanin and its association with postprandial inflammation and insulin. Br J Nutr 106:913–922
171. Cortez R et al (2017) Natural pigments: stabilization methods of anthocyanins for food applications. Compr Rev Food Sci Food Saf 16:180–198
172. He B et al (2017) Loading of anthocyanins on chitosan nanoparticles influences anthocyanin degradation in gastrointestinal fluids and stability in a beverage. Food Chem 221:1671–1677
173. Stebbins NB et al (2017) Stabilization of anthocyanins in blackberry juice by glutathione fortification. Food Funct 8:3459–3468

Wine Polyphenols and Health

39

Giovanna Giovinazzo, Maria A. Carluccio, and Francesco Grieco

Contents

Abstract

The various polyphenol families present in wine are important for a number of technological properties of wine such as clarity, hue, and palatal taste. Dietary polyphenols are associated with a wide range of health benefits, protecting against chronic diseases and promoting healthy aging. However, basic and clinical science is showing that the reality is much more complex than this and that several issues, notably daily intake, bioavailability, or in vivo antioxidant activity, are yet to be resolved. The concentration of phenolic compounds in wine is determined by viticulture and vinification practices, peculiar of different countries. Interesting are the effects of different yeast strains on the final concentration of polyphenols in red wine. We here summarize the recent findings concerning

G. Giovinazzo (✉) · F. Grieco
Institute of Sciences of Food Production (ISPA), National Research Council, Lecce, Italy
e-mail: giovanna.giovinazzo@ispa.cnr.it; francesco.grieco@ispa.cnr.it

M. A. Carluccio
Institute of Clinical Physiology (IFC), National Research Council, Lecce, Italy
e-mail: maria@ifc.cnt.it

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_81

the effects of specific classes of polyphenol (soluble acids, flavonols, and stilbenes) on human health and propose future directions for research to increase the amount of these healthy compounds in wine.

Keywords
Wine · Soluble acids · Flavonols · Stilbenes · Yeast · Health

1 Introduction

Wine contains a large and diverse class of phenolic compounds known variously as "polyphenols" or "biophenols." These compounds contribute to the characteristic colors and flavors of wine and act as natural wine preservatives that allows a long aging process [1]. Polyphenols are extracted during crushing and fermentation when the juice is in contact with the grape skins and seeds. The amount of phenolic compounds in red wine is about six times higher than that in white wine because red juice has longer contact time with the grape skins and seeds. The concentration of polyphenolic compounds in red wine is approximately 1800–3000 mg/L [2].

There is emerging evidence that a functional diet can help in modulating the immune system responses to the inflammation processes through a variety of mechanisms based on the absorption and utilization by the human metabolism of specific compounds.

Polyphenols are the principal compounds related to the wine consumption benefits due to their antioxidant and free radical scavenging properties. This class of compounds has been proven to exert important health effects, acting against cancer pathologies [3] as well as reactive oxygen species (ROS) which are considered the main cause of different cardiovascular and neurodegenerative diseases.

Grapevine (*Vitis* spp) is the most cultivated fruit crop in the world, with an area dedicated to viticulture. The berries are harvested primarily for winemaking but also to provide fresh table grapes, raisins, and other minor products. Phenolic composition of grape is genetically driven and greatly affected by environmental factors. A major challenge for breeding of grapevine cultivars adapted to climate change and with high potential for winemaking is to dissect the complex plant metabolic response involved in adaptation mechanisms. Among plant products are the polyphenols, a large family of secondary metabolites, which are involved in plant responses to biotic and abiotic stresses. The most represented polyphenols in plants are the flavonoids, the cinnamic and benzoic acids, and the stilbenes. They derive from the phenylpropanoid metabolism, but flavonoids are ubiquitous in plants, whereas stilbenes are specific to certain plant families.

Other polyphenols, as the phytoalexins, are antimicrobial compounds synthesized in response to pathogen or herbivore attack. However, other roles have been described for stress-induced polyphenols, including the defense signaling of responses and protection against ultraviolet (UV) light damage [4, 5].

Phenolic compounds are secondary metabolites synthesized [4] during normal development of the berry grape in response to stress conditions.

A large-scale experiment involving cultivation of an association panel of a large number (more than 200) *V. vinifera* cultivars designed to represent the genetic and phenotypic variation encountered in cultivated grapevine and metabolomics analysis targeted to a large number of polyphenolic compounds (polyphenomics) was performed. Chemometrics analysis of the data showed large differences in polyphenol composition related to genetic factors, environmental factors (i.e., water stress), and genetic-environment interactions. Correlation networks shed light on the relationships between the different polyphenol metabolites and related biosynthetic pathways. In addition, detailed polyphenomics analysis confirmed that polyphenol reactions described in wine take place in the berries.

Finally, was reported a large-scale study demonstrating an influence of environmental influence (water stress) on the different classes of polyphenols but also cultivar differences in the types and extents of drought responses, with different molecules affected either positively or negatively and different impacts on grape and wine quality [6].

Grape phenolic compounds comprise several families, divided between non-flavonoids (hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes) and flavonoids, based on the same C6-C3-C6 skeleton (flavonols, dihydroflavonols, flavan-3-ols, and anthocyanins). Each family is represented by several compounds differing by their hydroxylation level and by substitution of the hydroxy groups (methylation, glycosylation, acylation). For example, anthocyanins, the red grape pigments, are based on six aglycones, which can be mono- or di-glucosylated and further acylated with acetic, p-coumaric, and caffeic acid, giving rise to a large number of compounds [6]. Grape flavan-3-ols also show high diversity. They include several monomers (catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin 3-gallate) that are the constitutive units of oligomers and polymers (proanthocyanidins or condensed tannins), with degrees of polymerization ranging from 2 to over 100 in grape skin [6].

The flavonoid family includes the flavonols, such as myricetin, quercetin, and kaempferol, which exist both as aglycones and sugar conjugates. The non-flavonoids encompass hydroxybenzoic acids such as gallic acid, hydroxycinnamic acids, including p-coumaric, caffeic, and caftaric acids, and the stilbenes, such as *trans*-resveratrol and *cis*-resveratrol [5]. The synthesis of stilbenes in grape berry tissues is activated in response to fungal attack, to berry injury, and to ultraviolet irradiation [5].

The healthy physiological effects are especially associated to flavonoids and stilbenes [7], namely, quercetin, (+)-catechin, gallic acid, and trans-resveratrol [8]. The stilbene trans-resveratrol has gained great attention, and a number of scientific papers have appeared related to the moderate consumption of red wine for its ability to inhibit platelet aggregation and LDL oxidation and its beneficial effects in health. Since *trans*-resveratrol is postulated to be involved in the health benefits associated with a moderate consumption of red wine, it is one of the most extensively studied natural products.

The various polyphenol families present in wine [7, 9] are important for a number of technological properties of wine [7]. The knowledge about their qualitative and

quantitative profile in grapes is very important to predict wine aging attitude and can help to solve problems related to color stability, especially in the case of red wines that are destined to long aging periods [10]. The wine aging also changes the phenolic composition, as these compounds can suffer diverse transformations, like oxidation processes, condensation and polymerization reactions, and extraction from wood, usually associated to the changes in color and colloidal stability [11], flavor, bitterness, and astringency [12, 13]. The polyphenolic fingerprint can be useful for the classification of wines, since it can give us information about the variety, the geographic and winery origin, and even the applied winemaking technology [14].

During winemaking, only a fraction of the grape flavonoids is selectively transferred to the wine and a final yield strongly depending on the management of the contact of the liquid must, containing berry skin and seeds, with the solid parts of the grape bunches and on the grape variety [10].

The data concerning the extractable phenolics of the grape cannot be simply generalized to predict the wine composition, since a high variability in the extraction yield from grape to wine is introduced by the technological factors governing the winemaking process (such as temperature, duration and intensity of the liquid-solid contact, final ethanol concentration).

Many conditions (i.e., genetic, agronomic, technological, storage, etc.) linked to each other by complex and multifactorial phenomena affect both profiles and concentrations of bioactive compounds, either in grape or in wine [15].

2 Soluble Acids, Flavonols, and Stilbenes: Bioactive Compounds in Wine

Wine is a complex mixture of hundreds of molecules, some of them showing important biological properties, while others are mainly associated with its organoleptic characteristics. In particular, we describe specific classes of polyphenols such as phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonols, and stilbenes (Fig. 1).

2.1 Phenolic Acids

In wine, there are two groups of phenolic acids: hydroxybenzoic acids and hydroxycinnamic acids [16]. Hydroxybenzoic acids, including gallic acid, protocatechuic acid, gentisic acid, p-hydroxybenzoic acid, vanillic acid, and syringic acid, derive from benzoic acid. Chlorogenic acid, as the main constituent of the hydroxycinnamic acid derivative group, increased with harvest time delay, and the same occurred with sinapic acid. The converse was true of caffeic acid and ferulic acid, which were also esterified with tartaric acid as the known compounds caftaric acid and fertaric acid, respectively [16, 17]. Hydroxycinnamic acids have gained an increasing interest in health because they are known to be potent antioxidants.

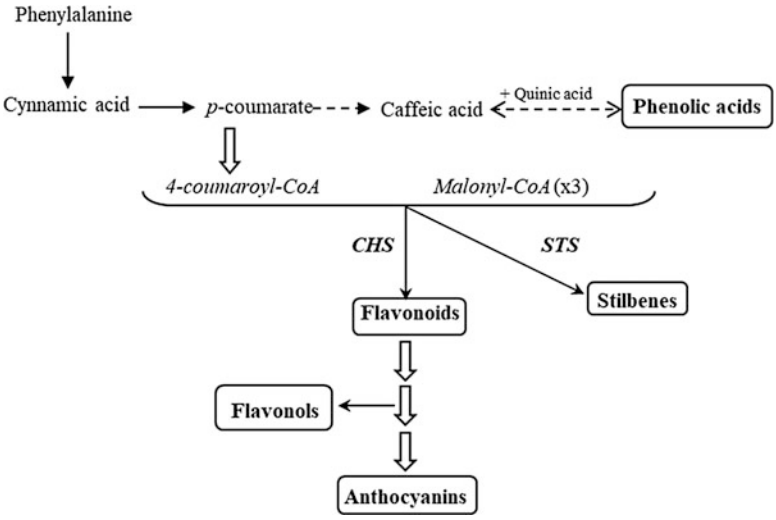


Fig. 1 Polyphenol biosynthesis pathway in grape tissues. *CHS* chalcone synthase, *STS* stilbene synthase

These compounds have been described as chain-breaking antioxidants acting through radical scavenging activity that is related to their hydrogen or electron donating capacity and to the ability to delocalize/stabilize the resulting phenoxyl radical within their structure. The free radical scavenger ability of antioxidants can be predicted from standard one-electron potentials.

Phenolic acids represent important fraction of wine phenolics, but their biological effects have been scarcely investigated. The interrelationship between antioxidative capacity and vasodilatory activity, two potentially beneficial biological effects, of phenolic acids from wine was examined. Antioxidative and vasodilatory effects of phenolic acids relate to the number of hydroxyl groups in the phenyl ring, degree of compactness and branching of molecules, and three-dimensional distributions of atomic polarizability of the tested molecules [18]. Caffeic acid has been shown to have neuroprotective effects against injury induced by 5-S-cysteinyl-dopamine, against Aβ-induced neurotoxicity and by inhibiting peroxynitrite-induced neuronal injury [19, 20]. Ferulic acid has been cited as an antidiabetic effect by lowering blood glucose and by increasing plasma insulin [21].

2.2 Flavonols

Four aglycones belong to the flavonols class: myricetin, quercetin, kaempferol, and isorhamnetin. The diversity in the flavonols structure is due to changes in the basic skeleton introduced by enzymes such as glycosyl transferase, methyl transferase, and rhamnosyl-transferase. In one plant species, dozens of different flavonoids may be present, and many of these are conjugated to various types of sugars. Both the basic

structure and the level of glycosylation determine the biological function and bioavailability of polyphenols in the human and animal intestines. Depending on their structure, these molecules have diversified activities.

Flavonols are found throughout the plant foods. The best-known flavonols are quercetin and kaempferol. Quercetin, kaempferol, myricetin, and isorhamnetin are flavonols presents in grape skins and stems as several different glycosides. Quercetin accumulates in grape skins to protect against damage from ultraviolet (UV) light. There are high concentrations of quercetin in wine made from sun-exposed grapes. Quercetin is readily extracted from grape skins during fermentation. Stems may contribute additional flavonols in whole cluster fermentations.

Quercetin glycosides may be hydrolyzed in wine to form quercetin aglycone. This process is similar to the hydrolysis that can occur with anthocyanins. Unlike anthocyanins, flavonol aglycones are stable in wine and can be used to monitor hydrolysis reactions. Quercetin may cause problems as a precipitate in bottled wines. Flavonols can interact with anthocyanins, enhancing their red color in a process known as co-pigmentation. This process may also help anthocyanin color stability.

Dietary flavonols inhibit LDL oxidation and so reduce the primary risk factor for atherosclerosis and related diseases. The animal studies are supported by human epidemiological studies, which show inverse correlations between the occurrence of CVD, certain cancers, and age-related degenerative diseases and the consumption of flavonol-rich diets [22–25]. Flavonols have been linked to protective effects against several specific cancers, including leukemia and pancreatic, breast, cervical, prostate, uterine, and urinary tract cancers. Subjects with regular flavonol intake have a 10–60% lower incidence of these types of cancer compared with subjects with low flavonol intake.

This protective activity results from both the action of flavonols as stimulators of antioxidant defenses and their direct inhibitory effects on cellular proliferation. Quercetin consumption has been reported to be inversely associated with breast cancer incidence [26].

2.3 Stilbenes

Stilbenes are a class of compounds with multiple pharmaceutically relevant properties. The stilbenes production in grape berry tissues is considered to be a part of the general defense mechanism since they display strong antifungal and antimicrobial activities [27].They are a group of plant phytoalexin polyphenols found in high concentrations in grapes, berries, nuts, and teas. In plants, their main function is to protect the plants against pathogens and fungi; therefore, their content is highly variable and increases with stress exposure. UV radiation, heavy metal exposure, and fungal infection may thus be utilized to enrich grapes with stilbenoids [28, 29].

Resveratrol (3,4_,5-trihydroxystilbene) may be found especially in red wine, grapes, and berries, at a concentration ranging from 0.1 to 15 mg/L. Nowadays, the primary source of resveratrol is the roots of perennial plant *Polygonum cuspidatum* (Japanese knotweed) [29]. Due to the positive health effects attributed

to resveratrol through so-called French paradox, its biological activity has been extensively studied. Most biological effects are assigned to trans-resveratrol, the more stable of the two isoforms. However, also cis-resveratrol, which is formed from trans-resveratrol upon UV light exposure, exhibits certain anti-inflammatory activity. Unless otherwise stated, the activities mentioned here apply to trans-resveratrol. Stilbenes are non-nitrogen polyphenols with acidic and amphiphilic character which causes their enrichment in biomembranes, where many of their targets occur (COX, 5-LOX, protein kinase B) [30]. Structurally, stilbenoids possess two aromatic rings connected by an ethylene or ethene bridge with a variety of substituents. Even though the presence of double bond suggests that stilbenoids exist in cis- as well as trans-form, the trans-form is more stable and the biologically relevant form. In nature, stilbenoids are synthesized from phenylalanine through multiple enzymatic reactions [31]. Stilbenoids are heterogeneously spread throughout the plant kingdom. They are especially abundant in *Gnetaceae*, *Pinaceae*, *Cyperaceae*, *Fabaceae*, *Dipterocarpaceae*, and *Vitaceae* families [32]. Resveratrol has a function of phytoalexin produced by specific plant species in response to biotic and abiotic challenges. It is thought to be one of the principal agents in the health-promoting effects of red wine [33]. Results of clinical studies show that the most important source of resveratrol and piceid are wines (98.4%) and grape and grape juices (1.6%), whereas peanuts, pistachios, and berries contribute to less than 0.01% [34]. Wine is the major source of resveratrol and piceid to the diet, ranging from 95% and 98.7% for *trans*-resveratrol and *trans*-piceid to 99.9% and 99.7% for *cis*-resveratrol and *cis*-piceid, respectively. Other food items such as grapes contribute by amount of 3.8% of total trans-resveratrol, whereas other contributors such as pistachios or berries provide less than 1% of the dietary total amount of trans-resveratrol and trans-piceid.

Resveratrol possesses numerous important bioactivities, including anti-inflammatory, antioxidant, anti-aggregatory functions, and modulation of lipoprotein metabolism [35–37]. It has also been shown to detain chemo preventive properties against certain forms of cancer and cardiovascular disorders and to have positive effects on longevity [38–42].

Anticancer activity of this compound is mainly due to induction of apoptosis via several pathways, as well as alteration of gene expressions, leading to a decrease in tumor initiation, promotion, and progression [43]. Resveratrol blocks the growth of lymphoma cells and increases their rate of cell death [44]. Resveratrol sensitizes chemotherapy-resistant lymphoma cells to treatment with paclitaxel-based chemotherapy [45], also reduces the production of growth factors, such as vascular endothelial growth factor and interleukin [33], and may reduce the ability of lymphoma cells to spread to other organs [46]. Finally, it was demonstrated that in vitro administration of resveratrol favorably altered gene expression in the androgen axis and in cell cycle regulators, providing potential anticancer benefit for prostate cancer [47]. Moreover, *trans*-resveratrol appears to protect against diabetes [48] and neurodegenerative disorders [49], due to induction of sirtuin 1 genes [50]. *Trans*-resveratrol might also contribute to increasing the life span of metazoans and mice by miming the effect of caloric restriction and thus decreasing age-related signs [51, 52]. Experimental studies have shown that resveratrol exhibits

both an anti-inflammatory and cardioprotective potential by inhibiting the expression of inflammatory mediators and the monocyte adhesion to vascular endothelial cells [53, 54]. Although resveratrol exhibits potent anticancer activities against transformed cells, its effectiveness is limited by its poor bioavailability, and as a dietary phytonutrients, it is most effective against tumors with which it comes in direct contact, such as skin cancers and tumors of the gastrointestinal tract. Furthermore, inhibition of sirtuin 1 by both pharmacological and genetic means abolished protein de-acetylation and autophagy as stimulated by resveratrol, but not by piceatannol, indicating that these compounds act through distinct molecular pathways. In support of this notion, resveratrol and piceatannol synergized in inducing autophagy as well as in promoting cytoplasmic protein de-acetylation [55].

3 Technological Approaches to Enhance Polyphenol Content and Antioxidant Activity in Wine

The winemaking steps determine the phenolic content of red wines that able the extraction of phenolic compounds from the grape berries. Numerous winemaking procedures have been developed to enhance the extraction of phenolic molecules, by preventing the several motives that affect the release of polyphenols from the berry tissues [56, 57]. Several investigations have studied the effect of the fermentation temperature during the winemaking process on the extraction of polyphenols, thus demonstrating that their concentration increased when wines were produced at higher fermentation temperatures [58, 59]. Moreover, it has recently shown the efficacy of thermo-vinification to improve a number of factors, such as the antioxidant potential and the polyphenolic and resveratrol content, in Pinot noir, Prokupac, Merlot, and Cabernet Sauvignon wines [60].

The impact of maceration time and of the utilization of enological additives (enzymes, sulfur dioxide) on the polyphenol content has been studied [57]. Gambuti and coworkers [61] have examined the effects of those factors during vinification of Aglianico grape must. The authors indicated that the simultaneous addition, during the pre-fermentation stage, of pectolytic enzymes and SO_2 increased the release of these molecules from grape tissues, thus resulting in a higher concentration of polyphenols in the produced wine. Comparable evidences were recently obtained by investigating experimental vinification of grape must from the Vranec cultivar [62, 63]. Also in this case, both increased maceration time and SO_2 addition augmented the final concentration of total polyphenols, anthocyanins, flavonoids, and flavan-3-ols in the final product, this effect action not dependent by the wine aging.

The action of diverse winemaking approaches in determining polyphenolic profile of red wines obtained from the Italian red cultivar “Negroamaro” has been recently studied. Were compared three different pre-fermentative steps: the traditional (7 days of maceration at 25 °C), the cryomaceration, (24 h at 0 °C using dry ice), and ultrasound (37 kHz, 150 W, 15 min at 30 °C) [64]. The authors demonstrated that the ultrasound action enhanced the release of all polyphenol classes,

whereas the cryomaceration only improved the anthocyanin content in the produced wine.

This evidence has been recently supported by a recent study of Ferraretto and Celotti [65]. They evaluated the effect of high-power ultrasound (20 kHz) on the phenolic structure of red wines and demonstrated that this physical treatment promote the polymerization of the phenolic compounds as the wine matures and consequently speed up the aging course of wines.

Another investigation [66] has evaluated the consequence of different technological approaches on the polyphenolic profile and the antioxidant activity of wine produced from grape of the Italian cultivar Primitivo. The addition of tannins was more efficient in enhancing the concentration of phenolic molecule when compared in musts to the other considered technologies and the aging to get better wine antioxidant activity. Furthermore, a recent report indicated that protracted post-fermentation maceration up to 50 days increased the polyphenol concentration and, consequently, the potential healthy effect of the obtained wine [67]. However, a number of reports do not agree with this hypothesis.

However in contrast with the above findings, Mulero and coworkers [68] did not detect variation in the concentrations of the different types of phenolic compounds in three wines from Monastrell grapes, produced by separately adopting the protracted maceration, must fermentation by adding enzymes, or the traditional procedure above three technological approaches.

The pulsed electric field (PEF) technology represents a very promising approach because of its ability to enhance the extraction of polyphenolic compounds throughout the vinification process. The PEF pretreatments on Cabernet Sauvignon must gave substantial differences in the produced wines, since the same PEF-treated must showed an increase of 97% in the content of total flavonols of 32% in the content of total phenolics and of 62%, in the color, when compared to the untreated control [59]. The above findings were confirmed by similar investigations that analyzed the vinification of Cabernet [69], Merlot [70], and Syrah [71] grape musts after the application of the PEF step. Moreover, PEF treatment allowed the acceleration and enhancement in the extraction of phenolic compounds throughout the maceration step of winemaking process [72].

An interesting research have recently assessed the effects of a novel fermentation technologies based on the "Ganimede" that is able to trap the carbon dioxide (CO2) generated during the alcoholic fermentation on the phenolic contents of Cabernet Sauvignon wines [73] indicating that this device was able to increase the concentration of anthocyanins superior in the wines produced.

The mechanisms through which yeast influences the color and content of polyphenolic compounds of wine are currently being researched, but three modes of interaction between the yeasts and the polyphenolic component have been already identified. Some strains of yeast adsorb polyphenols on the cell wall. However, although it has been shown that yeast is one of the factors able of inducing the reduction of the polyphenol concentration in wines, it is unknown whether their adsorption on the cell walls is the only mechanism responsible of this behavior. The amount of biomass produced during the alcoholic fermentation is capable to adsorb

on the cell walls a significant amount of polyphenols. This capacity is likely to be strain-specific, since different yeast strains have a different composition of the cell wall. In fact, it could be hypothesized that specific yeast strains could perform a "differentiated" adsorption of the diverse polyphenol classes. The second type of interaction between yeast strains and the wine polyphenol is related to the microbial β-glucosidase enzymatic activity. In fact, the greatest part of anthocyanins is found in the wine as glucosidase derivatives (linked to a sugar); thus, in this state they are much less sensitive to chemical or enzymatic oxidation; therefore the β-glucosidase activity decreases color and stability, since it produces free anthocyanins in wine. The third mechanism regards the strain-specific secretion throughout the alcoholic fermentation, by some wine yeast, of polysaccharides capable to combine with the polyphenols and to form with them stabile complexes.

It has been shown that different yeast metabolites, including pyruvic acid, can react with the anthocyanins of the grapes giving rise to stable pigments during the maturation and aging of red wines [74]. Taken together, the above considerations highlight the pivotal role played by yeasts in modifying the polyphenolic profile of wines.

Indeed, several investigations have highlighted the ability of different wine yeast strains and of enological additives of microbial origin to improve the phenolic profile of red wines. The addition of β-glucanases or other yeast-derived enzyme preparations as enological additives increased the antioxidant potential of sparkling wines [75]. On the opposite, yeast lees have been demonstrated to lower the amounts of polyphenolic compounds [76] and anthocyanins [74] in wines, because they formed stabile complexes with the mannoproteins released by yeasts after their yeast.

Even though previous studies did not show any effect of yeast starters on the polyphenolic profile of Pinot noir [77], Cabernet Franc, and Merlot [78] wines, several recent reports indicated a correlation between the utilized yeast strain and the antioxidant capacity of the produced wine.

Brandolini and coworkers [79] carried out an investigation by evaluating the properties of wines produced by the separate inoculation of 19 strains of *Saccharomyces cerevisiae* in the same grape must. The antioxidant capacity and the polyphenol profile detected in the different wines were extremely different, thus showing the strain-specific yeast feature to differentially adsorb polyphenols during the vinification process. Kostadinović and coworkers [62] showed analogous evidence on Vranec and Merlot wines in Macedonia. The authors used different starter cultures to carry out vinification tests, where the strains demonstrated that they were capable to affect specifically the *trans*-resveratrol concentration and antioxidant activities in the final wines.

The employment of different yeast starter strains allowed the production of Pinot noir wines with a dissimilar polyphenolic content [80]. This study has analyzed how yeast selection can modify phenolic content in Pinot noir wine. In fact, five different yeast starters were tested in multiple vinifications, where the *Saccharomyces cerevisiae* strain RC212 was able to raise conspicuously the concentrations of total pigment, anthocyanin, and tannins. Recently, Carrascosa and collaborators [81] have recently shown that different yeast strains were able to produce Albariño wines

denoted with a specific polyphenol composition. The above findings were further confirmed by a recent report [82], where an unambiguous correlation between the yeast starter utilized to promote the fermentation process and chemical profile of wine was recognized, thus underlining the strain-specific yeast property to modify the color and the polyphenolic composition of the final product.

Moreover, a recent study has produced the identification of yeast starter cultures able to enhance the quality of the wine produced from the Italian red cultivar "Gaglioppo" a cultivar with reduced synthesis of anthocyanins [83]. The obtained evidences further evidenced the strain-specific capacity of wine yeast to modify the final amounts of total anthocyanins, total polyphenols, and total tannins.

Recently, Giovinazzo and coworkers (manuscript in preparation) highlighted a positive role of autochthonous yeast starter cultures for the enhancement of polyphenol content throughout the industrial production of Negroamaro wine. The statistical assessment of the experiment showed that the use of autochthonous strains increased the concentrations of several classes of polyphenols in the produced wines when compared to wines produced from the Sama grape must with commercial starter strain.

Taken together, the above-described scientific outcomes emphasize the relevance of the development and the industrial application of innovative biotechnological approaches in order to exalt the presence of healthy molecules in wine, thus improving "functional parameters" with the consequential enhancement of the final wine quality.

4 Wine Polyphenol Mechanism of Action Against Cardiovascular Diseases

Wine polyphenols have garnered much attention, especially with regard to their potential role in the protection against cardiovascular diseases. Indeed, red wine is thought to be responsible for the "French paradox" [84], a term used to describe the low incidence of cardiovascular disease in the French population despite their high intake of saturated fats.

Many preclinical and some clinical studies have identified a number of mechanisms and targets by which specific wine polyphenols could exert benefits against cardiovascular diseases. Wine polyphenols, including flavonols and resveratrol, have been shown to modulate the expression of inflammation-related genes involved in the atherosclerotic process as well as in chronic degenerative diseases.

Flavonols (quercetin, kaempferol, and myricetin) significantly downregulate the coordinated expression of the endothelial adhesion molecules, E-selectin, vascular cell adhesion molecule (VCAM)-1, and intercellular adhesion molecule (ICAM)-1, in human cultured endothelial cells activated by inflammatory triggers [85], thus decreasing the adhesion and subsequent trans-endothelial migration of monocytes into the intima of the vascular wall, i.e., processes that constitute the initial steps in the development of atherosclerosis. Flavonols have also been reported to significantly downregulate the expression of monocyte chemoattractant protein (MCP)-1

and macrophage colony-stimulating factor (M-CSF); both pro-inflammatory endothelial proteins guide monocytes into the subendothelial space, during inflammatory state [85]. The anti-inflammatory flavonol effects were mediated by the reduced activation of NF-κB and AP-1, whose binding sites are present in the promoter region of the pro-inflammatory genes including VCAM-1, ICAM-1, E-selectin, as well as MCP-1 and M-CSF [86]. Furthermore, flavonols also affected the inflammatory response by downregulating the expression of inflammation-related genes, like interleukin (IL)-6, IL-4, and tumor necrosis factor (TNF)-α. In human vascular endothelial cells, our research group reported that quercetin reduced inflammatory angiogenesis, a key pathogenic process contributing to atherosclerotic lesion formation, progression, and vulnerability, through inhibition of the pro-inflammatory enzyme cyclooxygenase (COX)-2 and gelatinases, the matrix metalloproteinase (MMP)-9 [87].

A limitation of the anti-inflammatory effect by flavonols in these in vitro studies is the use of flavonols at supraphysiological concentrations and as aglycone forms. It has been proven that after oral absorption, flavonols are rapidly converted to circulating conjugates through glucuronidation, sulfidation, or methylation. This accounts for the very low aglycone concentrations in human plasma (in the nanomolar range).

Few studies have investigated the effects of flavonol metabolites on vascular cell function. It has been shown that human quercetin plasma metabolites at physiologically significant concentrations were able to inhibit COX-2 expression in human lymphocytes. Furthermore, Tribolo et al. showed that quercetin and its phase II metabolites affected the expression of VCAM-1, ICAM-1, and MCP-1 in inflamed endothelial cells [88]. However, at 10 μM, quercetin metabolites showed a reduced ability to decrease the stimulated expression of these genes when compared with quercetin. This suggested that the chemical transformation of quercetin during phase II metabolism resulted in a reduction of bioactivity, at least with respect to regulation of inflammatory gene expression. However, at a vascular level, quercetin glucuronides can be freed of their sugar moiety by a deconjugation process performed by β-glucuronidases, and the free aglycone is delivered to tissues, particularly under inflammatory conditions [89]. In a vascular co-culture model represented by human arterial smooth muscle cells and endothelial cells, quercetin and its phase II metabolites at physiologically relevant concentration significantly decreased the stimulated expression of the vasoconstrictor endothelin-1 [90], involved in the endothelial regulation of vascular tone.

Many results obtained in cell culture studies have been replicated in animal model but not in human trials. However, several human studies have shown that quercetin can reduce blood pressure in hypertensive patients [91], although the precise mechanisms have not been elucidated.

In addition to flavonols, the anti-inflammatory action of wine polyphenols is exerted by resveratrol.

Resveratrol, like quercetin, has been reported to modulate the expression of inflammation-related genes involved in the cellular processes that control adhesion and migration of monocytes to vascular endothelium [92, 93]. We reported that

a

GROUPS	POLYPHENOLS	PWPE		NWPE	
		µg/mL	µmol/L	µg/mL	µmol/L
HYDROXYCINNAMIC ACIDS	*p*-Coumaric acid	0.04 ± 0.03	0.24	0.04 ± 0.02	0.27
	Caffeic acid	1.26 ± 0.54	6.98	1.52 ± 0.81	8.41
	Caftaric acid	8.23 ± 1.12	26.37	8.01 ± 0.98	25.64
Total Hydroxycinnamic Acids		*9.53*		*9.57*	
FLAVONOLS	Kaempferol	0.05 ± 0.02	0.18	0.08 ± 0.04	0.30
	Quercetin	0.13 ± 0.05	0.42	0.14 ± 0.03	0.47
	Myricetin	0.04 ± 0.01	0.12	0.09 ± 0.02	0.29
Total Flavonols		*0.22*		*0.31*	
STILBENES	*trans*-Resveratrol	0.06 ± 0.02	0.24	0.02 ± 0.01	0.10
	trans-Piceid	0.20 ± 0.05	0.51	0.09 ± 0.03	0.24
Total Stilbenes		*0.26*		*0.11*	
TOTAL POLYPHENOLS		10.00		10.00	

b

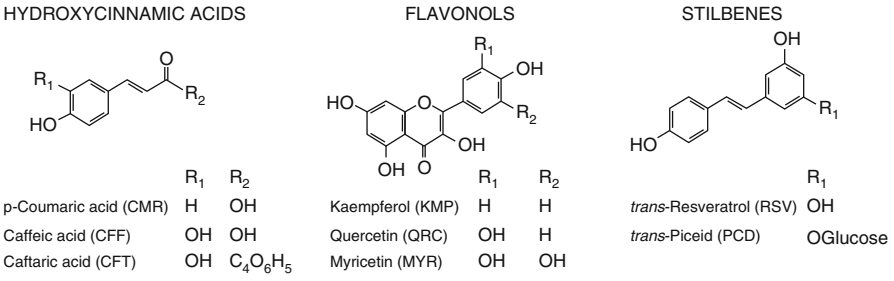


Fig. 2 Characterization of Primitivo wine polyphenol extract (PWPE) and Negramaro wine polyphenol extract (NWPE) polyphenol content and chemical structure of polyphenols. (**a**) Polyphenol content PWPE and NWPE (10 µg/mL). (**b**) Chemical structures of polyphenol groups identified in red wine extracts: hydroxycinnamic acids (*CFF* caffeic acid, *CMR* p-coumaric acid, *CFT* caftaric acid), flavonols (*KMP* kaempferol, *QRC* quercetin, *MYR* myricetin), and stilbenes (*RSV* trans-resveratrol, *PCD* trans-piceid)

resveratrol decreased monocytoid cell adhesion to human endothelial cells via reduction of VCAM-1 gene expression and by suppressing VCAM-1 promoter activity (see Figs. 2 and 3) [15]. In addition to VCAM-1, resveratrol also inhibited ICAM-1 and E-selectin, as well as MCP-1 and M-CSF [85, 93]. In human endothelial cells, monocytes, and smooth muscle cells, resveratrol strongly inhibited the expression of MMPs [85, 93, 94], responsible for the degradation of extracellular matrix, an essential event in atherosclerotic process, thus preventing plaque development, progression, and vulnerability. Furthermore, in endothelial and mononuclear cells, resveratrol inhibited, in a dose-dependent manner, the stimulated expression of tissue factor [96], a key regulator in the extrinsic pathway of blood coagulation. These anti-inflammatory and anti-thrombotic effects of resveratrol were at least in part mediated by lowered levels of intracellular ROS and the reduced activation of redox-sensitive transcription factors, NF-κB and AP-1 [85, 93, 96]. Part

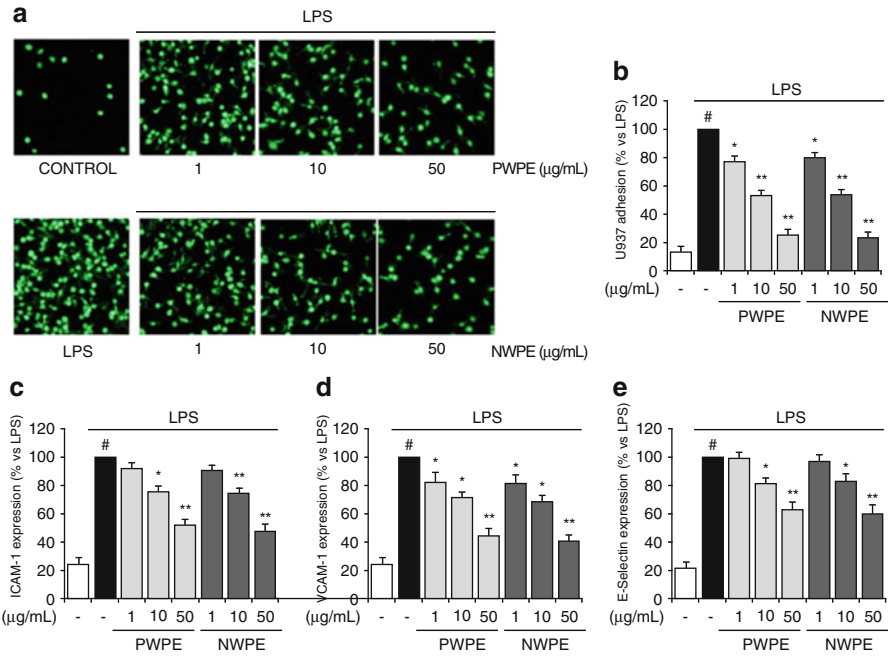


Fig. 3 Inhibitory effects of PWPE and NWPE on the monocyte adhesion to endothelial monolayer and on the expression of endothelial adhesion molecules. (**a**, **b**) HUVEC were pretreated with Primitivo wine polyphenol extract (PWPE) and Negramaro wine polyphenol extract (NWPE) (1–50 μg/mL) or vehicle (control) for 1 h and then stimulated with LPS 0.5 μg/mL for 16 h. HUVEC were co-cultured with calcein AM-labeled U937 monocytes for 1 h. The number of adherent U937 cells was monitored by fluorescence microscope (**a**) or measured by the fluorescence plate reader (**b**). (**c**–**e**) Cell surface expression of ICAM-1 (**c**), VCAM-1 (**d**), and E-Selectin (**e**) was analyzed by cell surface EIA. Each experiment was performed in triplicate. Data are expressed as the percentage of LPS-induced expression (mean ± SD). #p <0.01 versus control; *p <0.05; **p <0.01 versus lipopolysaccharide (LPS) alone

of the beneficial effects of resveratrol was also mediated by the upregulation of endothelial nitric oxide synthase (eNOS), involved in the regulation of vascular homeostasis. Most of the studies about the cardiovascular beneficial effect of resveratrol were performed by using its aglycone form; however it has been shown that piceid, a glycosidic form of resveratrol, also preserved the vascular anti-inflammatory properties, although at a lesser extent [85, 94].

As a critical point of in vitro studies, the cardio-vasculo-protective effects of resveratrol have been shown to occur at supraphysiological (10 μM) concentrations, which cannot be achievable through dietary intake. However, some beneficial properties of resveratrol have been also observed at dietary doses. In human endothelial cells, resveratrol at physiological concentrations decreased the stimulated expression of VCAM-1, ICAM-1, and MCP-1 [54], as well as the cytokines, IL-6 and CCXL2. A significant increase in eNOS expression in HUVEC has been reported also at lower concentration of resveratrol (1 μM), following repeated

stimulation for 6 days [97]. Physiological concentrations (0.1–1 μM) of resveratrol have also been reported to modulate the expression of genes involved in cell proliferation, blood pressure regulation, oxidative stress response, and autophagy in endothelial cells [98].

Another critical point for resveratrol efficacy is its low bioavailability. It is rapidly absorbed, metabolized, and excreted; however, in spite of its low bioavailability, evidence that beneficial activities occur in humans is beginning to emerge, and this phenomenon has been described as the "resveratrol paradox." This paradox could thus be related to a possible action of resveratrol metabolites [99] and/or to a synergistic effect of resveratrol with other polyphenols or micronutrients. Accordingly, resveratrol as a blend of polyphenols from grape extracts exhibited a greater inhibitory effects on the expression of inflammatory markers in vascular cell than resveratrol alone [85, 95], suggesting the occurrence of a synergism among resveratrol and other polyphenols.

Though resveratrol's potential utility in preventive medicine has been demonstrated using in vitro models, few clinical trials have also evaluated the effects of resveratrol on clinically relevant biomarkers. In healthy individuals, Agarwal and collaborators evaluated the effects of 1-month resveratrol treatment on endothelial response and plasma biomarkers [100]. Exposing cultured human coronary artery endothelial cells to plasma drawn post-resveratrol supplement resulted in significantly lower mRNA expression of VCAM-1, ICAM-1, and IL-8 than plasma drawn from the same subjects at baseline. This clinical trial highlighted for improved gene expression in vascular endothelium by resveratrol. A triple-blind, randomized, placebo-controlled, 1-year treatment with a resveratrol-containing grape supplement on stable patients with coronary artery disease [101] showed dose-dependently an increase of the anti-inflammatory serum adiponectin and a decrease of plasminogen activator inhibitor-1. Moreover, the transcriptional profiling showed a down-regulation of pro-inflammatory genes and a modulation of inflammatory transcription factors, confirming previous in vitro findings [102].

Moreover, in peripheral blood mononuclear cells of type 2 diabetes and hypertensive patients with coronary artery disease [102], the supplementation with resveratrol-containing grape supplement significantly reduced the expression of the pro-inflammatory cytokines CCL3, IL-1, and TNF-α and modulated inflammatory-related microRNAs. These clinical studies support the conclusion of beneficial anti-inflammatory and immunomodulatory effects of grape extract enriched in resveratrol for secondary prevention of patients with coronary artery diseases.

5 Conclusion

The precise nature of the role played by polyphenols in human health has been largely highlighted in these last years. A better knowledge concerning the composition and dynamics of polyphenol profile in red grape will help vinedresser and winemakers in producing grape-derived products and wines with high content of

phenolic antioxidants and considerable antioxidant activity, maintaining optimal organoleptic properties and a significant link with the original terroir.

Even though, a better understanding is still requested about the several different cellular mechanisms and complex pathways involved in wine polyphenol metabolism, the present findings suggest that the contribution of antioxidant phenols through a reasonable daily drinking of red wines may offer additional protection against in vivo oxidation and other damages of human cell components.

Acknowledgments This research was partially supported by the Apulia Region in the framework of the Projects NEWINE (Bando "Ricerca e sperimentazione in Agricoltura"; Project code PRS_042), SOLBIOGRAPE (Bando "Ricerca e sperimentazione in Agricoltura"; Project code PRS_053), and DOMINA APULIAE (POR Puglia FESR – FSE 2014-2020-Azione 1.6. – InnoNetwork; Project code AGBGUK2).

References

1. Yung JY, Saliba AJ, Prenzler PD (2010) Should red wine be considered a functional food? Compr Rev Food Sci Food Saf 9:530–551
2. Goldberg DM, Tsang E, Karumanchiri A, Diamandis EP, Soleas G, Ng E (1996) Method to assay the concentrations of phenolic constituents of biological interest in wines. Anal Chem 68:1688–1694
3. Giovinazzo G, Grieco F (2015) Functional properties of grape and wine polyphenols. Plant Foods Hum Nutr 70:454–462
4. Chong MF, Macdonald R, Lovegrove JA (2010) Fruit polyphenols and CVD risk: a review of human intervention studies. Br J Nutr 104(S3):S28–S39
5. Jandet P, Douillet-Breuil AC, Bessis R, Debord S, Sbaghi M, Adrian M (2002) Phytoalexins from the *vitaceae*, biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. J Agric Food Chem 50:2731–2741
6. Pinasseau L, Vallverdú-Queralt A, Verbaere A, Roques M, Meudec E, Le Cunff L, Péros J-P, Ageorges A, Sommerer N, Boulet J-C, Terrier N, Cheynier V (2017) Cultivar diversity of grape skin polyphenol composition and changes in response to drought investigated by LC-MS based metabolomics. Front Plant Sci 8:1826
7. Paixao N, Pereira V, Marques JC, Camara JS (2008) Quantification of polyphenols with potential antioxidant properties in wines using reverse phase HPLC. J Sep Sci 31: 2189–2198
8. Soleas GJ, Diamandis EP, Goldberg DM (2001) The world of resveratrol. In: American Institute for Cancer Research (eds) Nutrition and Cancer Prevention. Advances in Experimental Medicine and Biology, vol 492. Springer, Boston, MA, pp 159–182
9. Arnous A, Makris DP, Kefalas P (2001) Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. J Agric Food Chem 49:5736–5742
10. Mattivi F, Zulian C, Nicolini G, Valenti L (2002) Wine, biodiversity, technology, and antioxidants. Ann N Y Acad Sci 957:37–56
11. Saucier C, Little D, Glories Y (1997) First evidence of acetaldehyde-flavanol condensation products in red wine. Am J Enol Vitic 48:370–373
12. Sánchez-Iglesias M, González-Sanjosé ML, Pérez-Magariño S, Ortega-Heras M, González-Huerta C (2009) Effect of micro-oxygenation and wood type on the phenolic composition and colour of an aged red wine. J Agric Food Chem 57:11498–11509
13. Berta Baca-Bocanegra J, Nogales-Bueno J, Hernández-Hierro M, Heredia FJ (2018) Evaluation of extractable polyphenols released to wine from cooperage byproduct by near infrared hyperspectral imaging. Food Chem 20:206–212

14. Spranger MI, Climaco MC, Sun BS, Eiriz N, Fortunato C, Nunes A, Leandro MC, Avelar ML, Belchior AP (2004) Differentiation of red winemaking technologies by phenolic and volatile composition. Anal Chim Acta 513:151–161
15. Restuccia D, Sicari V, Pellicanò TM, Spizzirri UG, Loizzo MR (2017) The impact of cultivar on polyphenol and biogenic amine profiles in Calabrian red grapes during winemaking. Food Res Int 102:303–312
16. Cabrita MJ, Torres M, Palma V, Alves E, Patão R, Costa Feritas AM (2008) Impact of malolactic fermentation on low molecular weight phenolic compounds. Talanta 74:1281–1286
17. Gil-Muñoz R, Gómez-Plaza E, Martínez A, López-Roca JM (1999) Evolution of phenolic compounds during wine fermentation and post-fermentation: influence of grape temperature. J Food Compos Anal 12:259–272
18. Teixeira J, Gaspar A, Garrido EM, Garrido J, Borges F (2013) Hydroxycinnamic acid antioxidants: an electrochemical overview. Bio Med Res Int 2013:Article ID 251754. 11 pages
19. Sul D, Kim HS, Lee D, Joo SS, Hwang KW, Park SY (2009) Protective effect of caffeic acid against beta-amyloid-induced neurotoxicity by the inhibition of calcium influx and tau phosphorylation. Life Sci 84:257–262
20. Vauzour D, Houseman EJ, George TW, Corona G, Garnotel R, Jackson KG, Sellier C, Gillery P, Kennedy OB, Lovegrove JA, Spencer JP (2010) Moderate champagne consumption promotes an acute improvement in acute endothelial-independent vascular function in healthy human volunteers. Br J Nutr 103:1168–1178
21. Jung EH, Ran Kim S, Hwang IK, Youl HT (2007) Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db mice. J Agric Food Chem 55:9800–9804
22. Vina J, Gomez-Cabrera MC, Borras C (2007) Fostering antioxidant defences, up-regulation of antioxidant genes or antioxidant supplementation. Br J Nutr 98:S36–S40
23. Mink PJ, Scrafford CG, Barraj LM (2007) Flavonoid intake and cardiovascular disease mortality, a prospective study in post-menopausal women. Am J Clin Nutr 85:895–909
24. Seeram NP, Adams LS, Hardy ML, Heber D (2004) Total cranberry extract versus its phytochemical constituents, anti-proliferative and synergistic effects against human tumour cell lines. J Agric Food Chem 52:2512–2517
25. Soobratte MA, Bahorun T, Aruoma OI (2006) Chemopreventive actions of polyphenolic compounds in cancer. Biofactors 27:19–35
26. Fink BN, Steck SE, Wolff MS, Britton JA, Kabat GC, Schroeder JC, Teitelbaum SL, Neugut AI, Gammon MD (2007) Dietary flavonoid intake and breast cancer risk among women on Long Island. Am J Epidemiol 165:514–523
27. Zhu L, Zhang Y, Lu J (2012) Phenolic contents and compositions in skins of red wine grape cultivars among various genetic backgrounds and originations. Int J Mol Sci 13:3492–3510
28. Dvorakova M, Landa P (2017) Anti-inflammatory activity of natural stilbenoids: a review. Pharm Res 124:126–145
29. Piotrowska H, Kucinska M, Murias M (2012) Biological activity of piceatannol: leaving the shadow of resveratrol. Mutat Res 750:60–82
30. Neves AR, Lúcio M, Lima JLC, Reis S (2012) Resveratrol in medicinal chemistry: a critical review of its pharmacokinetics drug-delivery, and membrane interactions. Curr Med Chem 19:1663–1681
31. Poulose SM, Thangthaeng N, Miller MG, Shukitt-Hale B (2015) Effects of pterostilbene and resveratrol on brain and behaviour. Neurochem Int 89:227–233
32. Rivière C, Pawlus AD, Mérillon JM (2012) Natural stilbenoids: distribution in the plant kingdom and chemotaxonomic interest in *Vitaceae*. Nat Prod Rep 29:1317–1333
33. Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 5:493–506
34. Zamora-Ros R, Andres-Lacueva C, Lamuela-Raventos RM, Berenguer T, Jakszyn P, Martinez C, Sanchez M, Navarro C, Chirlaque M, Tormo M-J, Quiros J, Amiano P, Dorronsoro M, Larranaga N, Barricarte A, Ardanaz E, Gonzalez C (2008) Concentrations of resveratrol and derivatives in foods and estimation of dietary intake in a Spanish population,

European Prospective Investigation into Cancer and Nutrition (EPIC)-Spain cohort. Br J Nutr 100:188–196
35. Gerard E, Mullin MD (2011) Red wine, grapes, and better health-resveratrol. Nutr Clin Pract 26:722–723
36. D'Introno A, Paradiso A, Scoditti E, D'Amico L, De Paolis A, Carluccio MA, Nicoletti I, DeGara L, Santino A, Giovinazzo G (2009) Anti-oxidant and anti-inflammatory properties of tomato fruit synthesising different amount of stilbenes. Plant Biotechnol J 7:422–429
37. Frei B (2004) Efficacy of dietary antioxidants to prevent oxidative damage and inhibit chronic disease. J Nutr 134:3196–3198
38. Kundu JK, Surth YJ (2008) Cancer chemopreventive and therapeutic potential of resveratrol, mechanistic perspectives. Cancer Lett 269:243–261
39. Nguyen A, Martinez M, Stamos MJ, Moyer MP, Planutis K, Hope C, Holcombe RF (2009) Results of a phase I pilot clinical trial examining the effect of plant-derived resveratrol and grape powder on Wnt pathway target gene expression in colonic mucosa and colon cancer. Cancer Manag Res 1:25–37
40. Fonar Y, Frank D (2011) FAK and WNT signaling: the meeting of two pathways in cancer and development. Anti Cancer Agents Med Chem 11:600–606
41. Kaminski BM, Steinhilber D, Stein JM, Ulrich S (2011) Phytochemicals resveratrol and sulforaphane as potential agents for enhancing the anti-tumor activities of conventional cancer therapies. Curr Pharm Biotechnol 67:1167–1178
42. Vergara D, Simeone P, Toraldo D, Del Boccio P, Vergaro V, Leporatti S, Pieragostino D, Tinelli A, De Domenico S, Alberti S, Urbani A, Salzet M, Santino A, Maffia M (2012) Resveratrol down regulates Akt/GSK and ERK signalling pathways in OVCAR-3 ovarian cancer cells. Mol Biosyst 8:1078–1087
43. Udenigwe CC, Ramprasath VR, Aluko RE, Jones PJ (2008) Potential of resveratrol in anticancer and anti-inflammatory therapy. Nutr Rev 66:445–454
44. Bruno R, Ghisolfi L, Priulla M, Nicolin A, Bertelli A (2003) Wine and tumours: study of resveratrol. Drugs Exp Clin Res 29:257–261
45. Jazirehi AR, Bonavida B (2004) Resveratrol modifies the expression of apoptotic regulatory proteins and sensitizes non-Hodgkin's lymphoma and multiple myeloma cell lines to paclitaxel-induced apoptosis. Mol Cancer Ther 3:71–84
46. Dulak J (2005) Nutraceuticals as anti-angiogenic agents: hopes and reality. J Physiol Pharmacol 56:51–67
47. Jones SB, DePrimo SE, Whitfield ML, Brooks JD (2005) Resveratrol induced gene expression profiles in human prostate cancer cells. Cancer Epidemiol Biomark Prev 14:596–604
48. Sharma S, Chopra K, Kulkarni SK (2007) Effect of insulin and its combination with resveratrol or curcumin in attenuation of diabetic neuropathic pain, participation of nitric oxide F TNF-alpha. Phytother Res 21:278–283
49. Anekonda TS (2006) Resveratrol a boon for treating Alzheimer's disease? Brain Res Rev 52:316–326
50. Wodd JG, Rogina B, Lavu S, Howitz K, Hefland SL, Tatar M, Sinclair D (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 430:686–689
51. Park SJ, Ahmad F, Philp A, Baar K, Williams T, Luo H, Ke H, Rehmann H, Taussig R, Brown AL, Kim MK, Beaven MA, Burgin AB, Manganiello V, Chung JH (2012) Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. Cell 14:421–433
52. Pearson KJ1, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, Swindell WR, Kamara D, Minor RK, Perez E, Jamieson HA, Zhang Y, Dunn SR, Sharma K, Pleshko N, Woollett LA, Csiszar A, Ikeno Y, Le Couteur D, Elliott PJ, Becker KG, Navas P, Ingram DK, Wolf NS, Ungvari Z, Sinclair DA, de Cabo R (2008) Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab 8:157–168

53. Carluccio MA, Ancora MA, Massaro M, Carluccio M, Scoditti E, Distante A, Storelli C, De Caterina R (2007) Homocysteine induces VCAM-1 gene expression through NF-kappa B and NAD(P)H oxidase activation, protective role of Mediterranean diet polyphenolic antioxidants. Am J Physiol Heart Circ Physiol 293:2344–2354
54. Csiszar A, Smith K, Labinskyy N, Orosz Z, Rivera A, Ungvari Z (2006) Resveratrol attenuates TNF-α-induced activation of coronary arterial endothelial cells: role of NF-κB inhibition. Am J Physiol-Heart Circ Physiol 291:H1694–H1699
55. Pietrocola F, Mariño G, Lissa D, Vacchell IE, Malik SA, Niso-Santano M, Zamzami N, Galluzzi L, Maiuri MC, Kroemer G (2012) Pro-autophagic polyphenols reduce the acetylation of cytoplasmic proteins. Cell Cycle 11:3851–3860
56. Amri A, Chaumeil JC, Sfar S, Charrueau C (2012) Administration of resveratrol, what formulation solutions to bioavailability limitations ? J Control Rel 158:182–193
57. González-Neves G, Gil G, Favre G, Baldi C, Hernández N, Traverso S (2013) Influence of winemaking procedure and grape variety on the color and composition of young red wines. S Afr J Enol Vitic 34:138–146
58. Gambacorta G, Antonacci D, Pati S, la Gatta M, Faccia M, Coletta A, La Notte E (2011) Influence of winemaking technologies on phenolic composition of Italian red wines. Eur Food Res Technol 233:1057–1066
59. El Darra N, Turk MF, Ducasse MA, Grimi N, Maroun RG, Louka N, Vorobiev E (2016) Changes in polyphenol profiles and color composition of freshly fermented model wine due to pulsed electric field, enzymes and thermos vinification pretreatments. Food Chem 194:944–950
60. Atanacković M, Petrović A, Jović S, Gojković-Bukarica L, Bursać M, Cvejić J (2012) Influence of winemaking techniques on the resveratrol content, total phenolic content and antioxidant potential of red wines. Food Chem 131:513–518
61. Gambuti A, Strollo D, Erbaggio A, Lecce L, Moio L (2007) Effect of winemaking practices on color indexes and selected bioactive phenolics of Aglianico wine. J Food Sci 72: S623–S628
62. Kostadinović S, Wilkens A, Stefova M, Ivanova V, Vojnoski B, Mirhosseini H, Winterhalter P (2012) Stilbene levels and antioxidant activity of Vranec and Merlot wines from Macedonia: effect of variety and enological practices. Food Chem 135:3003–3009
63. Ivanova V, Vojnoski B, Stefova M (2012) Effect of winemaking treatment and wine aging on phenolic content in Vranec wines. J Food Sci Technol 492:161–172
64. Coletta A, Trani A, Faccia M, Punzi R, Dipalmo T, Crupi P, Antonacci D, Gambacorta G (2013) Influence of viticultural practices and winemaking technologies on phenolic composition and sensory characteristics of Negroamaro red wines. Int J Food Sci Technol 4811: 2215–2227
65. Ferraretto P, Celotti E (2016) Preliminary study of the effects of ultrasound on red wine polyphenols. CyTA-J Food 14:529–535
66. Baiano A, Terracone C, Gambacorta G, La Notte E (2009) Phenolic content and antioxidant activity of Primitivo wine: comparison among winemaking technologies. J Food Sci 74: 258–267
67. Francesca N, Romano R, Sannino C, Le Grottaglie L, Settanni L, Moschetti G (2014) Evolution of microbiological and chemical parameters during red wine making with extended post-fermentation maceration. Int J Food Microbiol 17:84–93
68. Mulero J, Zafrilla P, Cayuela JM, Martínez-Cachá A, Pardo F (2011) Antioxidant activity and phenolic compounds in organic red wine using different winemaking techniques. J Food Sci 76:C436–C440
69. Cholet CL, Delsart C, Petrel M, Gontier E, Grimi N, L'Hyvernay A, Ghidossi R, Vorobiev E, Mietton-Peuchot M, Geny L (2014) Structural and biochemical changes induced by pulsed electric field treatments on Cabernet Sauvignon grape berry skins: impact on cell wall total tannins and polysaccharides. J Agric Food Chem 62:2925–2934

70. Delsart C, Ghidossi R, Poupot C, Cholet C, Grimi N, Vorobiev E, Milisic V, Peuchot MM (2012) Enhanced extraction of phenolic compounds from merlot grapes by pulsed electric field treatment. Am J Enol Vitic 63:205–211
71. Puértolas E, Saldaña G, Alvarez I, Raso J (2010) Effect of pulsed electric field processing of red grapes on wine chromatic and phenolic characteristics during aging in oak barrels. J Agric Food Chem 58:2351–2357
72. Luengo E, Alvarez I, Raso J (2015) Phenolic extraction: pulsed electric fields: a technology for improving phenolic extraction in red wines. Wine Vitic J 301:17–21
73. Bai B, He F, Yang L, Chen F, Reeves MJ, Li J (2013) Comparative study of phenolic compounds in Cabernet Sauvignon wines made in traditional and Ganimede fermenters. Food Chem 1414:3984–3992
74. Morata A, Gomez-Cordoves C, Subervolia J, Bartolome B, Colomo B, Suarez JA (2003) Adsorption of anthocyanins by yeast cell walls during fermentation of red wines. J Agric Food Chem 51:4084–4088
75. Rodriguez-Nogales JM, Fernández-Fernández E, Gómez M, Vila-Crespo J (2012) Antioxidant properties of sparkling wines produced with β-glucanases and commercial yeast preparations. J Food Sci 77:1005–1010
76. Mazauric JP, Salmon JM (2005) Interactions between yeast lees and wine polyphenols during simulation of wine aging: I analysis of remnant polyphenolic compounds in the resulting wines. J Agric Food Chem 53:5647–5653
77. Girard B, Yuksel D, Cliff MA, Delaquis P, Reynolds AG (2001) Vinification effects on the sensory, colour, and GC profiles of Pinot noir wines from British Colombia. Food Res Int 34:483–499
78. Mazza G, Fukumoto L, Delaquis P, Girard B, Ewert B (1999) Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Colombia. J Agric Food Chem 47:4009–4017
79. Brandolini V, Fiore C, Maietti A, Tedeschi P, Romano P (2007) Influence of *Saccharomyces cerevisiae* strains on wine total antioxidant capacity evaluated by photo-chemiluminescence. World J Microbiol Biotechnol 23:581–586
80. Carew AL, Smith P, Close DC, Curtin C, Dambergs RG (2013) Yeast effects on Pinot noir wine phenolics, color, and tannin composition. J Agric Food Chem 6141:9892–9898
81. Carrascosa AV, Bartolome B, Robredo S, Leon A, Cebollero E, Juega M, Nunez YP, Martinez MC, Martinez-Rodriguez AJ (2012) Influence of locally-selected yeast on the chemical and sensorial properties of Albariño white wines. LWT-Food Sci Technol 46: 319–325
82. Tufariello M, Chiriatti MA, Grieco F, Perrotta C, Capone S, Rampino P, Tristezza M, Mita G, Grieco F (2014) Influence of autochthonous *Saccharomyces cerevisiae* strains on volatile profile of Negroamaro wines. LWT Food Sci Technol 58:35–48
83. Caridi A, De Bruno A, De Salvo E, Piscopo A, Poiana M, Sidari R (2017) Selected yeasts to enhance phenolic content and quality in red wine from low-pigmented grapes. Eur Food Res Technol 2433:367–378
84. Renaud S, de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339:1523–1526
85. Calabriso N, Scoditti E, Massaro M, Pellegrino M, Storelli C, Ingrosso I, Giovinazzo G, Carluccio MA (2016) Multiple anti-inflammatory and anti-atherosclerotic properties of red wine polyphenolic extracts: differential role of hydroxycinnamic acids, flavonols and stilbenes on endothelial inflammatory gene expression. Eur J Nutr 55:477–489
86. Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T (1995) Transcriptional regulation of endothelial cell adhesion molecule: NF-kB and cytokine-inducible enhancer. FASEB J 9:899–909
87. Scoditti E, Calabriso N, Massaro M, Pellegrino M, Storelli C, Martines G, De Caterina R, Carluccio MA (2012) Mediterranean diet polyphenols reduce inflammatory angiogenesis through MMP-9 and COX-2 inhibition in human vascular endothelial cells: a potentially protective mechanism in atherosclerotic vascular disease and cancer. Arch Biochem Biophys 527:81–89

88. Tribolo S, Lodi F, Connor C, Suri S, Wilson VG, Taylor MA, Needs PW, Kroon PA, Hughes DA (2008) Comparative effects of quercetin and its predominant human metabolites on adhesion molecule expression in activated human vascular endothelial cells. Atherosclerosis 197:50–56
89. Shimoi K, Saka N, Nozawa R, Sato M, Amano I, Nakayama T, Kinae N (2001) Deglucuronidation of a flavonoid, luteolin monoglucuronide, during inflammation. Drug Metab Dispos 29:1521–1524
90. Lodi F, Winterbone MS, Tribolo S, Needs PW, Hughes DA, Kroon PA (2012) Human quercetin conjugated metabolites attenuate TNF-alpha-induced changes in Vasomodulatory molecules in a HUASMCs/HUVECs co-culture model. Planta Med 78:1571–1573
91. Boomgaarden I, Egert S, Rimbach G, Wolffram S, Muller MJ, Doring F (2010) Quercetin supplementation and its effect on human monocyte gene expression profiles in vivo. Br J Nutr 104:336–345
92. Tome-Carneiro J, Larrosa M, Gonzalez-Sarrias A, Tomas-Barberan FA, Garcia-Conesa MT, Espin JC (2013) Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. Curr Pharm Des 19:6064–6093
93. Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, Visioli F, Distante A, De Caterina R (2003) Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. Arterioscler Thromb Vasc Biol 23:622–629
94. Lee B, Moon SK (2005) Resveratrol inhibits TNF-alpha-induced proliferation and matrix metalloproteinase expression in human vascular smooth muscle cells. J Nutr 135:2767–2773
95. Calabriso N, Massaro M, Scoditti E, Pellegrino M, Ingrosso I, Giovinazzo G, Carluccio MA (2016) Red grape skin polyphenols blunt matrix metalloproteinase-2 and-9 activity and expression in cell models of vascular inflammation: protective role in degenerative and inflammatory diseases. Molecules 21(9):1147
96. Pendurthi UR, Williams JT, Rao LVM (1999) Resveratrol, a polyphenolic compound found in wine, inhibits tissue factor expression in vascular cells – a possible mechanism for the cardiovascular benefits associated with moderate consumption of wine. Arterioscler Thromb Vasc Biol 19:419–426
97. Takizawa Y, Kosuge Y, Awaji H, Tamura E, Takai A, Yanai T, Yamamoto R, Kokame K, Miyata T, Nakata R, Inoue H (2013) Up-regulation of endothelial nitric oxide synthase (eNOS), silent mating type information regulation 2 homologue 1 (SIRT1) and autophagy-related genes by repeated treatments with resveratrol in human umbilical vein endothelial cells. Br J Nutr 110:2150–2155
98. Ungvari Z, Bagi Z, Feher A, Recchia FA, Sonntag WE, Pearson K, de Cabo R, Csiszar A (2010) Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. Am J Phys Heart Circ Phys 299:H18–H24
99. Bresciani L, Calani L, Bocchi L, Delucchi F, Savi M, Ray S, Brighenti F, Stilli D, Del Rio D (2014) Bioaccumulation of resveratrol metabolites in myocardial tissue is dose-time dependent and related to cardiac hemodynamics in diabetic rats. Nutr Metab Cardiovasc Dis 24:408–415
100. Agarwal B, Campen MJ, Channell MM, Wherry SJ, Varamini B, Davis JG, Baur JA, Smoliga JM (2013) Resveratrol for primary prevention of atherosclerosis: clinical trial evidence for improved gene expression in vascular endothelium. Int J Cardiol 166:246–248
101. Tome-Carneiro J, Gonzálvez M, Larrosa M, Yáñez-Gascón MJ, García-Almagro FJ, Ruiz-Ros JA, Tomás-Barberán FA, García-Conesa MT, Espín JC et al (2013) Grape resveratrol increases serum adiponectin and downregulates inflammatory genes in peripheral blood mononuclear cells: a triple-blind, placebo-controlled, one-year clinical trial in patients with stable coronary artery disease. Cardiovasc Drug Ther 27:37–48
102. Tome-Carneiro J, Larrosa M, Yáñez-Gascón MJ, Dávalos A, Gil-Zamorano J, Gonzálvez M, García-Almagro FJ, Ruiz Ros JA, Tomás-Barberán FA, Espín JC, García-Conesa MT (2013) One-year supplementation with a grape extract containing resveratrol modulates inflammatory-related microRNAs and cytokines expression in peripheral blood mononuclear cells of type 2 diabetes and hypertensive patients with coronary artery disease. Pharmacol Res 72:69–82

Natural Estrogenic Substances, Origins, and Effects

40

Catherine Bennetau-Pelissero

Contents

Abstract

Some natural substances have been scientifically identified as estrogenic since the late 1930s when they were found to be deleterious at high doses for cattle

C. Bennetau-Pelissero (✉)
Department of Life Science and Health, University of Bordeaux, Bordeaux, France

Department Feed and Food, Bordeaux Sciences Agro, Gradignan, France
e-mail: catherine.bennetau@u-bordeaux.fr; catherine.bennetau@agro-bordeaux.fr

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_10

reproduction. Several compounds belonging to different chemical families are considered here: isoflavonoids, coumestans, lignans, and resorcylic acid lactones. This list is not exhaustive. The vegetable sources of these compounds are probably not all identified yet, but all the compounds presented here were shown to act as endocrine disruptors, i.e., modifying the hormonal natural balance, at dietary doses either in human, in cattle, or in other vertebrates.

Estrogenic compounds mimic estradiol activities and can interact nearly with all biological functions in lower and higher vertebrates. Some mollusks are also sensitive to estrogens. The effective dose is crucial to consider since as other endocrine disruptors the natural substances may have opposite effects at low, dietary, and pharmacological concentrations. Different cell pathways are triggered by the natural estrogenic substances, including some that are not influenced by estradiol itself, and this explains why their effect is not a monotonic dose–response line. This questions the classical toxicological approach which considers acute exposure (short and high concentrations) as the key of the toxicity evaluation. The history of human exposure to isoflavones was recently casted on doubt, reinforcing the need for careful study of these compounds' occurrence and effects on humans. It is clear now that the traditional soy food makings were able to remove isoflavones from foodstuffs. This is no longer the case in modern processing, and this means that the exposure to this estrogenic substances has increased markedly in recent times. Estrogens in mammals can have both beneficial and harmful effects which are evoked here.

Keywords

Natural estrogens · Food sources · Modern exposure · Bioavailability · Mechanism of actions · Breast cancer · Bone health · Thyroid · Reproductive disruption

1 Introduction

Nowadays, as the concern on endocrine disruptors is back to front stage, scientists realize that natural estrogenic compounds which were ignored for ages have to be included in the panel of active and potentially deleterious compounds [1]. This is mainly due to the recent discovery that humans were not traditionally exposed to natural estrogenic compounds [1] and that this recent exposure is synchronized with that to other anthropoid endocrine disruptors such as pesticides or wrapping agents [2]. Humans were not traditionally exposed to natural estrogenic compounds because they used to prepare all grain legumes, which are the major sources of estrogenic substances in human diet, by prolonged soaking, cooking, or simmering in water. The water was then discarded, and because natural estrogenic compounds are mainly under a glycosidic form in plants, they leaked into the water during the cooking steps and were then eliminated with water [3]. Reproductive problems were recently reported in multigenerational toxicological studies in animal models [4] and

in humans [5]. They occur at actual dietary exposure levels, although the acute toxicity studies performed classically on short-term durations and with pharmacological concentrations do not show major adverse effects [6]. Since endocrine disruptors were discovered, the toxicology studies dealing with reproductive issues were moved to multigenerational exposure and to low doses. Following this guidance, studies can show hormonal disruption of reproduction including epigenetic effects visible even in generations not exposed per se but issued from exposed genitors [7]. For the compounds studied here, the toxicity studies all converge in claiming an estrogenic activity. The present chapter is therefore focusing on the so-called phytoestrogens taken in their large definition since it included the mycotoxins zearalenone (ZEN) and zearalenol (ZOL) produced on grains by fungi. They also focus on natural estrogenic substances produced by plants or by the human gut after plant-substance absorption, i.e., coumestans, isoflavones, isoflavans, and enterolignans. Flavonoids can also have estrogenic effects, but at nutritional exposure, their effects remain low enough to be neglected here. However, if tomorrow a synergy with other anthropological endocrine disruptors taken at environmental doses is demonstrated, they will also have to be considered. Sources and concentrations, roles in plants, and effects in plant consumers are exposed together with the questions remaining on their beneficial and deleterious effects in human and domestic animal consumers.

2 The Natural Estrogenic Substances and Their Sources

2.1 Estrogenic Effects

The estrogenicity of a substance being anthropogenic or natural relies on two major pillars: its efficiency on estrogen-dependent gene transcription which is approached by its efficient dosage in in vivo and in vitro systems and its bioavailability at the target cell. The estrogenic effects of natural substances are based on their chemical structures which share some similarities with that of the 17β-estradiol (17β-E_2) molecule, which is the most potent estrogen in vertebrate animals. 17β-E_2 is an aromatized C18 steroid with hydroxyl groups at 3- and 17-β-position (Fig. 1a). In humans, it is produced primarily by the cyclic ovaries and the placenta. It is also produced by the brain, the adrenal cortex, and the adipose tissue of men and postmenopausal women. It is also crucial for male tract development [8] and adequate sperm production [9]. As seen in Fig. 1, 17β-E_2 is a three-dimensional molecule with its two hydroxyl groups at a distance of 10 angstroms (Å). 17β-E_2 bears a phenolic ring called cycle A.

The hydroxyl groups form an angle allowing the best interaction with the specific estradiol receptors (ERs) [10]. These receptors are proteins with specific quaternary structures allowing estradiol binding via a ligand-binding domain (LBD) and a subsequent activation of gene transcription via phosphorylation processes and activation of transcription factors. This transcription phenomenon can either follow the canonical route or be induced via cell phosphorylation pathways. The canonical

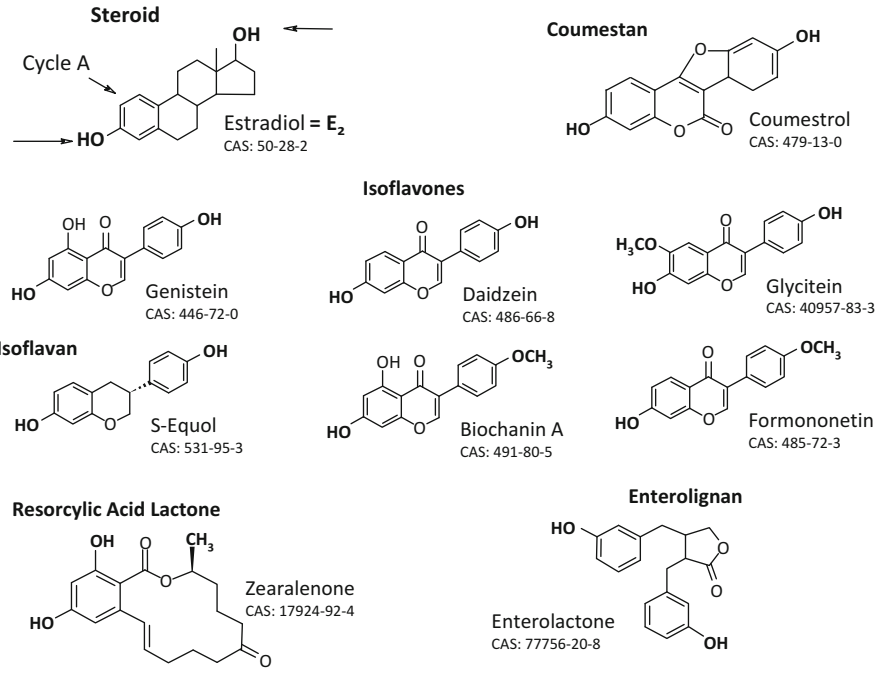


Fig. 1 17β-Estradiol structure together with the structures of the main phytoestrogen families: isoflavones, coumestans lignans, and resorcylic acid lactones

route involves a direct DNA receptor interaction, and it is known to rely on both ERα and ERβ estradiol receptor subtypes. These two estradiol receptors derive from two different genes: ESR1 located in humans on chromosome 6 and ESR2 located on chromosome 14, respectively [11]. The two receptors derive from a common ancestor but evolved differently, and nowadays they do not exhibit the same primary structure, and their activation by various ligands can be rather different.

These receptors present a LBD with a hydrophobic pocket constituted by the E/F domain (C-terminal domain) of the protein and a part of the A/B domain (N-terminal domain) folded in its vicinity [11]. The E/F domain is constituted of several helix structures, and the spatial position of the H12 helix depends on the chemical structure of the ligand. Estradiol taken as reference is a full agonist of its ERs. Other estrogens can exhibit selective modulating effects depending on the way they fit into the ligand-binding pocket of the ERs. If their structure induces a structural conformation of the H12 helix which does not allow the recruitment of all transcription factors, then the estrogenic effect can be partial. Because the transcription factors are not the same in all cell types, referring to their differentiating history, then some compounds can induce the transcription of estrogen-dependent genes in certain cell types only. This situation refers to the SERM (selective estrogen receptor modulators) concept [12]. The 17β-E_2 spatial structure

is so relevant for the ligand–receptor interaction that the natural 17-α-isomer of estradiol binds only weakly to the ERs and exhibits little estrogenic–genomic activity, considering its natural concentrations, in estrogen-responsive tissues [13]. 17β-E_2 physiological plasma concentrations in women usually vary from 10 to 20 pg/mL (i.e., from 36 to 72 pM) in the metestrous phase of the female cycle to 500–600 pg/mL (i.e., 2 to 4 nM) in the estrous phase of the female cycle. During pregnancy these 17β-E_2 levels can reach values over 30 ng/mL (i.e., over 110 nM). In the meantime, 17β-E_2 levels in men are below 50 pg/mL and below 40 pg/mL in postmenopausal women (i.e., below 200 pM in both cases). Estradiol receptors coded by ESR1 and ESR2 are also present in cell membranes. This is due to the palmitoylation of the ERs at the AA corresponding to the 451 DNA base [14] since a mutation at this point prevents membrane location. The physiological roles of the membrane forms of the ER subtypes are currently under investigation [15]. A role of the membrane ERs as transmembrane channels for steroid hormones is evoked by some authors [16].

Other estradiol receptors were found in cell endoplasmic reticulum and mitochondria membranes, the so-called GPER or GPR-30. This is a completely different molecule belonging to the seven transmembrane G-protein-coupled receptors of the rhodopsin receptor family. These receptors were shown to react with estradiol and with several estrogens, including phytoestrogens [17, 18], and to induce cell signaling pathways and growth factor receptor cross talk [19]. Finally and although these last receptors do not transmit estrogenic effects, the orphan estrogen-related receptors, i.e., ERRα, ERRβ, and ERRγ, were found to bind with different affinities several categories of phytoestrogens [20]. In some cases, the ERRs are involved in physiological processes known to be influenced by estrogens. The ERRs' function is currently under investigation, but they seem to be involved in the cell energy balance and considering an integrative view in metabolic syndrome in humans.

Because of a partial similarity (Fig. 1), the affinities of isoflavones for the ERs are lower than that of 17β-E_2. They range from 1/1000 to 1/10,000 for ERα when genistein (GEN) and kievitone are considered (with the following order of magnitude decrease: GEN > daidzein (DAID) > glycitein > biochanin A > formononetin > phaseolin > kievitone) [11, 21]. For ERβ the affinity of isoflavones is known to be higher, 1/60 and 1/150 for GEN and DAID, respectively. Other compounds like glyceolin were also found to exhibit estrogenic activity. However, these activities are not fully characterized yet [22] even though they are expressed at nutritional doses. Although the affinities of the natural estrogens for the ERs can be low, natural estrogenic substances can be present at mg level in edible plants, and some of them can reach plasma concentrations 10,000 to 100,000 times higher than those of 17β-E_2. Namely, isoflavones which present the highest bioavailability can reach micromolar concentrations in plasma, whereas 17β-E_2 is currently found at picomolar to nanomolar (pregnancy) concentrations in those same plasmas. Cell bioavailability specifically controlled by phase III enzymes (ABC transporters) acting after phase I (CYP) and II (Glut and Sult) enzymes can also play a role in the tissue-specific activities of the natural substances. See [11] for more details.

2.2 Isoflavones

The estrogenic isoflavonoids studied here include GEN, DAID, glycitein, and the DAID metabolite *S*-equol produced by the gut flora and which is an isoflavan (Fig. 1). Two other isoflavones are methylated precursors of estrogenic compounds, the so-called formononetin and biochanin A (Fig. 1). All these compounds are secondary metabolites from legumes and mainly present in Fabaceae. Some of them contain high amounts of isoflavones with estrogenic properties (i.e., up to several hundred mg/100 g of dry matter [23]). The most concentrated Leguminosae are, namely, black beans (a variety of soybean) [21], soy (*Glycine max*) [24], kudzu (*Pueraria lobata* or *tuberosa*) [25], alfalfa (*Medicago* sp.) [23], and clover (*Trifolium* sp.) [26]. Estrogenic isoflavones are also found in a smaller quantity in pulses traditionally edible in Western countries like beans, lentils, broad beans, and chick-peas, but in these vegetable sources, the concentrations are about 500 times lower than in the plants previously cited [27, 28] (Table 1). These compounds were also found in more than 300 different plants, including many species of Leguminosae like *Baptisia*, *Cytisus*, *Dalbergia*, *Genista*, *Lupinus*, *Medicago*, *Phaseolus*, *Teline*, *Trifolium*, and *Ulex* and in several species of Rosaceae like *Prunus* [29, 30]. In Western countries, these plants were traditionally used as sources of antifertility agents [29, 30].

2.3 Coumestans

The main estrogenic substance in the coumestan family is coumestrol (COUM) although it also exists as methylated substances (4′-*O*-methyl and 7-*O*-methyl derivatives) which were found in alfalfa (*Medicago sativa*) [39]. When they reach the liver, the methylated forms can be demethylated in COUM by phase I enzymes. Coumestrol estrogenic potency is by far the highest in vitro especially through ERα [40]. However, its bioavailability appears to be lower than that of isoflavones in rat [41]. No data were found in humans since this compound is considered as toxic, and therefore, pharmacokinetic studies have not been published yet in humans. In addition, this compound is present in significant amounts in alfalfa especially after fungi's attacks (*Pseudopeziza medicaginis*) [42] or in clover sprouts [43]. However, if these pulses can be used for animal feeding, they are only anecdotally used in humans, and in that case, it is essentially as dietary supplements. According to [44], clover sprouts, raw, contain 14.079 mg COUM/100 g; kala chana, mature seeds, raw, contain 6.130 mg COUM/100 g; and pinto beans, mature seeds, raw, contain 1.805 mg COUM/100 g. In addition, alfalfa seeds, sprouted, raw, contain 1.596 mg COUM/100 g; mung beans, mature seeds, sprouted, raw, contain 0.932 mg COUM/100 g; and split peas, mature seeds, raw, contain 0.812 mg/100 g. Still according to [44], soymilk, original and with vanilla, unfortified, can contain 0.807 mg COUM/100 g, and soybeans, mature seeds, sprouted, raw, can contain 0.341 mg COUM/100 g. However, soybean does not seem to be a constant source of COUM. It is likely that COUM synthesis depends on the plant culture conditions and its contamination by fungi.

Table 1 Isoflavones and coumestrol in several edible plants

	Isoflavones					Coumestans		
Fruits and vegetables	Biochanin A (CAS 491-80-5)	Daidzein (CAS 486-66-8)	Formononetin (CAS 485-72-3)	Genistein (CAS 446-72-0)	Glycitein (CAS 40957-83-3)	Coumestrol (CAS 479-13-0)	Total	Authors
	µg/100 g of fresh weight						µg/100 g	
Alfalfa sprout	67	152	3,899	118	na	105	4,341	[31]
Alfalfa sprout	nd	nd	340	nd	na	4,680	5,020	[32]
Alfalfa sprout (freeze-dried)	nd	nd	5,170	nd	na	72,010	77,180	[32]
Almond	25	<1	<1	1	<1	na	26	[33]
Apricot (dry)	tr	nd	nd	tr	na	nd	0	[31]
Asparagus (white)	na	nd	na	nd	na	na	0	[34]
Asparagus (white)	nd	58	nd	tr	na	tr	58	[31]
Asparagus (green)	na	nd	na	nd	na	na	0	[34]
Basilic	na	nd	na	nd	na	na	0	[34]
Beet	na	nd	na	nd	na	na	0	[34]
Black bean freeze-dried	nd	77,440	nd	79,640	na	nd	157,080	[32]
Black bean seeds 1, dry	nd	69,850	nd	61,220	na	nd	131,070	[32]
Black bean seeds 2, boiled	nd	26,950	nd	27,710	na	nd	54,660	[32]
Black trumpet mushrooms	na	nd	na	nd	na	na	0	[34]
Black-eyed bean seeds, dry	173	nd	nd	nd	na	nd	173	[32]
Brazil nut	13	6	<1	85	<1	na	104	[33]

(*continued*)

Table 1 (continued)

	Isoflavones					Coumestans		
Fruits and vegetables	Biochanin A (CAS 491-80-5)	Daidzein (CAS 486-66-8)	Formononetin (CAS 485-72-3)	Genistein (CAS 446-72-0)	Glycitein (CAS 40957-83-3)	Coumestrol (CAS 479-13-0)	Total	Authors
	µg/100 g of fresh weight						µg/100 g	
Broad beans (grilled)	nd	nd	210	129	na	nd	339	[32]
Broccoli	nd	44	nd	nd	na	nd	44	[31]
Broccoli	nd	5	nd	7	na	na	12	[35]
Brocoli sprouts	nd	44	nd	nd	na	na	44	[27]
Brocoli sprouts	nd	44	nd	nd	na	nd	44	[31]
Brussel sprouts	nd	tr	nd	nd	na	nd	0	[31]
Brussel sprouts	na	nd	na	nd	na	na	0	[34]
Capers	na	nd	na	nd	na	na	0	[34]
Carrotts	nd	2	nd	2	na	nd	4	[31]
Carrotts	5	nd	nd	nd	na	na	5	[31]
Cashew nuts	7	nd	2	2	1	na	12	[33]
Celery	na	nd	na	nd	na	na	0	[34]
Ceps	na	nd	na	nd	na	na	0	[34]
Chanterelle	na	nd	na	nd	na	na	0	[34]
Chick pea	126	nd	nd	640	na	6,130	6,896	[32]
Chick pea	2	40	140	60	na	na	242	[35]
Chinese peas, boiled	101	nd	nd	nd	na	nd	101	[32]
Chinese peas, freese-dry	126	nd	nd	nd	na	nd	126	[32]
Clover sprout	440	nd	2,280	350	na	28,060	31,130	[32]
Clover sprout	751	71	4,020	71	na	98	5,011	[31]

Clover sprout freese-dried	8,810	nd	561,140	6,940	na	561,140	1,138,030	[32]
Coconuts	6	nd	nd	nd	4	na	10	[33]
Coconuts powder	na	nd	na	nd	na	na	0	[34]
Codea	nd	50	nd	tr	na	na	50	[31]
Dog Rose Berry	na	nd	na	nd	na	na	0	[34]
Garbanzo bean seeds, dry	152	nd	nd	nd	na	nd	152	[32]
Garbanzo bean seeds, dry	1.52	nd	nd	nd	na	na	1.52	[36]
Garbanzo bean seeds, dry	1,394	nd	52	67	na	na	1,513	[31]
Garbanzo bean seeds, dry	2,822	33	258	82	na	na	3,195	[35]
Garlic	tr	nd	nd	tr	na	nd	0	[31]
Garlic	nd	nd	23	1	na	na	24	[35]
Ginger	na	nd	na	nd	na	na	0	[34]
Grape	na	nd	na	nd	na	na	0	[34]
Grape	nd	tr	nd	tr	na	nd	0	[31]
Grapefruit	tr	36	tr	27	na	50	113	[31]
Grapefruit	na	nd	na	nd	na	na	0	[34]
Great northern bean seeds, dry	60	nd	nd	nd	na	nd	60	[32]
Green bean freese-dried	nd	nd	211	nd	na	nd	211	[32]
Green bean fresh boiled	tr	nd	tr	nd	na	nd	0	[32]
Green bean fresh raw	nd	nd	15	nd	na	nd	15	[32]

(*continued*)

Table 1 (continued)

	Isoflavones					Coumestans		
Fruits and vegetables	Biochanin A (CAS 491-80-5)	Daidzein (CAS 486-66-8)	Formononetin (CAS 485-72-3)	Genistein (CAS 446-72-0)	Glycitein (CAS 40957-83-3)	Coumestrol (CAS 479-13-0)	Total	Authors
	µg/100 g of fresh weight						µg/100 g	
Haricots en grains	838	11	215	73	na	na	1,137	[35]
Hazelnut	12	<1	<1	9	<1	na	21	[33]
Horse radish	na	nd	na	nd	na	na	0	[34]
Kiwi	na	nd	na	nd	na	na	0	[34]
Large lima beans seeds, boiled	nd	nd	10	nd	na	nd	10	[32]
Leek	na	nd	na	nd	na	na	0	[34]
Letuce	na	nd	na	nd	na	na	0	[34]
Licorice	nd	293	1,493	599	na	nd	2,385	[31]
Mungo bean sprout	tr	387	26	424	na	2,000	2,837	[31]
Mungo bean sprout	nd	745	nd	1,902	na	na	2,647	[37]
Mungo bean sprout	nd	nd	tr	nd	na	na	0	[35]
Mungo bean sprout	nd	nd	tr	nd	nd	na	0	[32]
Mungo bean sprout freese-dry	nd	tr	nd	nd	na	na	0	[32]
Mungo Beans	nd	nd	61	nd	na	na	61	[32]
Olive	na	nd	na	nd	na	na	0	[34]

Orange (juices)	tr	tr	tr	tr	na	53	53	[31]
Origan	na	nd	na	nd	na	na	0	[34]
Peach	nd	nd	nd	nd	na	nd	0	[31]
Peanuts (fresh)	8	<1	4	48	10	na	70	[33]
Pear	na	nd	na	nd	na	na	0	[34]
Pecan nut	30	nd	2	2	nd	na	34	[33]
Pine nuts	27	<1	<1	4	<1	na	31	[33]
Pink Bean	nd	nd	105	nd	na	nd	105	[32]
Pinto bean	56	nd	nd	nd	na	361	417	[32]
Plums	nd	nd	nd	nd	na	nd	0	[31]
Red Bean	196	nd	nd	9	na	na	205	[31]
Red Bean	nd	10	nd	5	na	na	15	[35]
Red Bean boiled	41	nd	nd	nd	na	nd	41	[32]
Red Bean dry	560	nd	nd	nd	na	na	560	[36]
Red Bean dry	nd	20	nd	520	na	na	540	[31]
Red Bean dry	4	42	nd	98	na	na	144	[38]
Red Bean dry	nd	nd	nd	310	na	na	310	[32]
Red Bean freese-dried	132	nd	nd	nd	na	nd	132	[32]
round split peas, dry	nd	nd	nd	nd	na	811	811	[32]
Rutabaga	na	nd	na	nd	na	na	0	[34]
Shitake	na	nd	na	nd	na	na	0	[34]
Small lima bean seeds (dry)	37	nd	55	nd	na	nd	92	[32]
Small white bean seeds, dry	nd	nd	82	74	na	nd	156	[32]
Soft potatoes	nd	nd	nd	tr	na	nd	0	[31]

(*continued*)

Table 1 (continued)

	Isoflavones					Coumestans		
Fruits and vegetables	Biochanin A (CAS 491-80-5)	Daidzein (CAS 486-66-8)	Formononetin (CAS 485-72-3)	Genistein (CAS 446-72-0)	Glycitein (CAS 40957-83-3)	Coumestrol (CAS 479-13-0)	Total	Authors
	µg/100 g of fresh weight						µg/100 g	
Sunfolower seeds	nd	nd	nd	nd	na	nd	0	[31]
Turnip	na	nd	na	nd	na	na	0	[34]
Wallnuts	17	1	<1	11	<1	na	29	[33]
Yellow split peas, dry	86	nd	nd	nd	na	nd	86	[32]
Yellow split peas, dry	nd	726	nd	nd	na	nd	726	[32]

2.4 Lignans

Lignans are not estrogenic substances per se, but some compounds in the lignan family (Fig. 2) can be transformed into enterolignans exhibiting low or partial estrogenic effects [45]. As far as it is known, the classical precursors of the estrogenic metabolites enterolactone (ENL) and enterodiol (END) are lariciresinol, matairesinol, medioresinol, pinoresinol, secoisolariciresinol, sesamin, sesamolin, and syringaresinol [46, 47]. Figure 2 gives their chemical formula.

However, in rats it was shown that lignin could be metabolized into enterolignans via its hydrolysis into lignans [48]. The current sources of enterolignan precursors are given in Table 2. However, this list is most likely not exhaustive.

Therefore, the exposure to enterolignan precursors is far to be precise, and in some populations, data are missing. As a consequence, the exposure is more reliably evaluated based on blood or urine measurements of enterolignans. This is particularly true since the ability to form enterolignans varies largely between human subjects [11].

2.5 Resorcylic Acid Lactones

The estrogenic resorcylic acid lactones are the mycotoxins ZEN and ZOL types α and β. All are produced by fungi of the *Fusarium* family developing on maturing corn, wheat, barley, rye oats, soybeans, sorghum, peanuts, and other food and feed

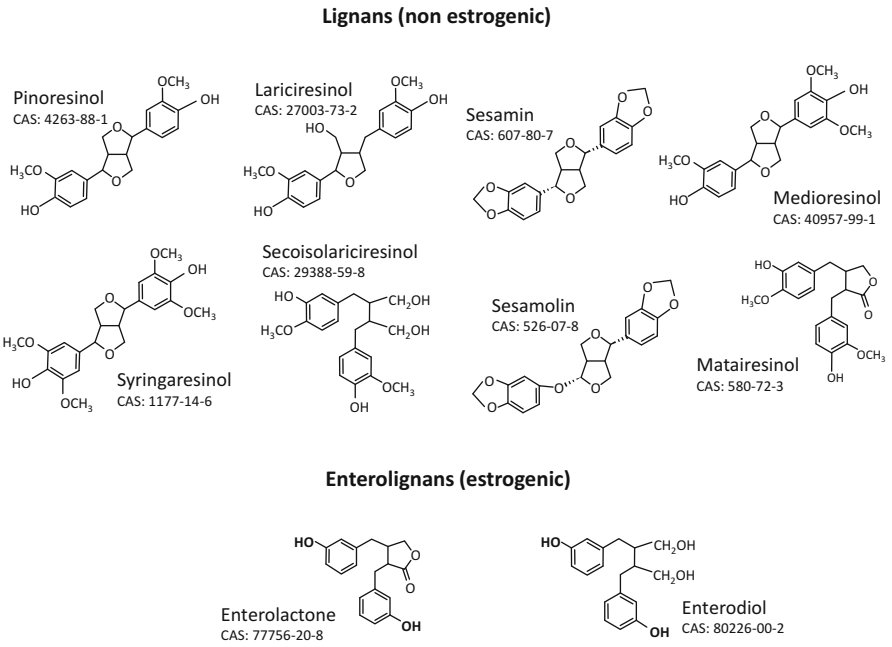


Fig. 2 Chemical structure of enterolignans and of some of their known precursors

Table 2 Lignan content in several edible fruits and vegetables

Fruits and vegetables	Secoisolariciresinol (CAS 148244-82-0)	Matairesinol (CAS 580-72-3)	Lariciresinol (CAS 27003-73-2)	Pinoresinol (CAS 487-36-5)	Syringaresinol (CAS 21453-69-0)	Medioresinol (CAS 40957-99-1)	Total	Author
	µg/100 g of wet weight						µg/100 g	
Alfalfa sprouts	tr	nd	na	na	na	na	0	[31]
Ananas	7	18	24	3	na	na	52	[49]
Apple	na	3	55	na	na	na	58	[49]
Apricot dry	328.3	tr	na	na	na	na	328.3	[31]
Artichoke	171.45	0	153.76	3,479.71	21.89	56.65	3,883.47	[46]
Asparagus	743	14	92	na	na	na	849	[49]
Asparagus	68	tr	na	na	na	na	68	[31]
Avocado	47	6	31	272	na	na	356	[49]
Baby corn	na	na	25	16	na	na	41	[49]
Baby corn	7	na	14	na	na	na	21	[49]
Bambou sprouts	38	na	na	na	na	na	38	[49]
Banana	nd	17	na	19	na	na	36	[49]
Basilic	546	1.9	na	na	na	na	547.9	[34]
Black trumpet mushrooms	1.3	0.1	na	na	na	na	1.4	[34]
Broad Bean	240.39	34.89	319.23	24.67	57.34	4	681.29	[46]
Broccoli	tr	nd	na	na	na	na	0	[31]
Brussel Sprouts	30	nd	na	na	na	na	30	[31]
Brussel Sprouts	21	0.6	na	na	na	na	21.6	[34]
Capers	44.5	15.1	na	na	na	na	59.6	[34]

Carob bean	1,266.51	108.41	1,770.74	294.99	12,965.7	336.08	16,742	[46]
Carrots	38	tr	na	na	na	na	38	[31]
Cauliflower	91	na	36	85	na	na	212	[49]
Celery	12	na	16	43	na	na	71	[49]
Celery	9.9	0	na	na	na	na	9.9	[34]
Ceps	0.6	0.3	na	na	na	na	1	[34]
Chanterelles	3.9	7.3	na	na	na	na	11.2	[34]
Cherry tomato	17	na	43	11	na	na	71	[49]
Cherry tomato	16	na	38	19	na	na	73	[49]
Clover sprouts	nd	nd	na	na	na	na	0	[31]
Coconut (powder)	34	0	na	na	na	na	34	[34]
Cucumber	41	na	65	na	na	na	106	[49]
Dog Rose berry	78.8	2	na	na	na	na	80.8	[34]
Eggplant	8	na	40	51	na	na	99	[49]
Garlic	55	na	84	45	na	na	184	[49]
Garlic	27	37	na	na	na	na	64	[31]
Ginger	na	16	na	15	na	na	31	[49]
Ginger	21.3	0	na	na	na	na	21.3	[34]
Grape	tr	52	na	na	na	na	52	[31]
	µg/100 g de poids frais						µg/100 g	
Grape	2	1.3	na	na	na	na	3.3	[34]
Grapefruit	nd	tr	na	na	na	na	0	[31]
Grapefruit	26.3	0	na	na	na	na	26.3	[34]
Green Asparagus	78.3	3.4	na	na	na	na	81.7	[34]

(*continued*)

Table 2 (continued)

Fruits and vegetables	Secoisolariciresinol (CAS 148244-82-0)	Matairesinol (CAS 580-72-3)	Lariciresinol (CAS 27003-73-2)	Pinoresinol (CAS 487-36-5)	Syringaresinol (CAS 21453-69-0)	Medioresinol (CAS 40957-99-1)	Total	Author
Green Pepper	5	na	73	6	na	na	84	[49]
Grilled corn	14.46	0	15.33	0	331.55	3.01	364.35	[46]
Horse radish	14	0	na	na	na	na	14	[34]
Horse radish	37	49	219	16	na	na	321	[49]
Kiwi fruit	174.6	1.2	na	na	na	na	175.8	[34]
Kiwi fruit	106	na	20	13	na	na	139	[49]
Leak	11.8	0	na	na	na	na	11.8	[34]
Lentils	1.38	245.17	233.24	86.93	196.63	17	781.1	[46]
Mung bean sprouts	82	1	32	33	na	na	148	[49]
Mung bean sprouts	tr	tr	na	na	na	na	0	[31]
Nashi	7	na	21	na	na	na	28	[49]
Olive	55.9	2.7	na	na	na	na	58.6	[34]
Orange Navelle	14	na	128	24	na	na	166	[49]
Orange Valencia	56	na	193	51	na	na	300	[49]
Oranges (Juice)	tr	tr	na	na	na	na	0	[31]
Oregano (dry)	44.4	1	na	na	na	na	45.4	[34]
Oriental Pears	0.28	0	6.9	179.99	23.43	9	220.21	[46]
Paprika	8	79	2	na	na	na	89	[49]
Pea	129	na	9	6	na	na	144	[49]
Pea	na	na	59	50	na	na	109	[49]

Pea	2.73	6.79	150.59	79.81	175.36	24.2	439.48	[46]
Pea	1	98.82	83.73	7.11	1.82	0	192.63	[46]
Pea	2.61	87.75	133.75	8	1.81	0	234.14	[46]
Peach	11	na	38	83	na	na	132	[49]
Peach	28	nd	na	na	na	na	28	[31]
Pear	2	na	34	2	na	na	38	[49]
Pear	9.9	0.7	na	na	na	na	10.6	[34]
Plums	16	na	20	42	na	na	78	[49]
Plums	76	tr	na	na	na	na	76	[31]
Pumpkin	20	na	29	na	na	na	49	[49]
Pumpkin	29	3	58	8	na	na	98	[49]
Radish	na	na	29	43	na	na	72	[49]
Radish	1	15	na	40	na	na	56	[49]
Red bean	nd	nd	na	na	na	na	0	[31]
Red Pepper	9	na	73	1	na	na	83	[49]
Rice Bread	33	4	47	44	na	na	128	[49]
Rice Bread	7	1	18	16	na	na	42	[49]
Rutabaga	3.7	0.6	na	na	na	na	4.3	[34]
Salade (Letuce)	105.6	0.6	na	na	na	na	106.2	[34]
Shitake	0.2	0	na	na	na	na	0.2	[34]
Spanish melon	23	na	69	12	na	na	104	[49]
Spinach	8	na	110	31	na	na	149	[49]
Spinach	13	1	79	15	na	na	108	[49]
Strawberry	51	na	33	21	na	na	105	[49]
Strawberry	61.37	18.3	134	5.21	60.83	3.89	284.49	[46]
Sunflower seed	128	nd	na	na	na	na	128	[31]

(*continued*)

Table 2 (continued)

Fruits and vegetables	Secoisolariciresinol (CAS 148244-82-0)	Matairesinol (CAS 580-72-3)	Lariciresinol (CAS 27003-73-2)	Pinoresinol (CAS 487-36-5)	Syringaresinol (CAS 21453-69-0)	Medioresinol (CAS 40957-99-1)	Total	Author
Sweet potato	65	2	79	16	na	na	162	[49]
Sweet potato	nd	41	na	na	na	na	41	[31]
Tomato	3	na	11	12	na	na	26	[49]
Turnip	6.3	0	na	na	na	na	6.3	[34]
Watermelon	5	na	67	5	na	na	77	[49]
White Asparagus	28.7	0.6	na	na	na	na	29.3	[34]

crops in the field and in grain during transportation or storage. Zearalenone and ZOL (Fig. 1) are mainly produced by *F. graminearum* and *F. semitectum* [50]. Due to its structural similarity to the naturally occurring estrogens, ZEN is an estrogenic mycotoxin that induces obvious estrogenic effects in animals [51]. Zearalenone and ZOL productions are favored by high-humidity and low-temperature conditions. They can occur simultaneously with other mycotoxins such as deoxynivalenol (DON) and less frequently with aflatoxins [52]. Zearalenone is stable in food under regular cooking temperatures but can be partially eliminated under deep heating [53]. In the human diet, the main sources are grain milling products, for human consumption, e.g., breakfast cereals, breads, and rolls. Cooked pastas only contain small amounts of ZEN, and meat and meat products are always below the detection limits (Table 3).

3 Human Exposure

3.1 Isoflavones

Isoflavones nowadays are present in almost all domestic animal food except when an alternative breeding practice is adopted, namely, if grass or other human residues are used instead of the conventional diet. In most animal breeds, the protein input is brought by pulses. The most nutritionally interesting are soy, alfalfa, or clover. All three can contain natural estrogenic compounds at high concentrations as previously mentioned. However, estrogenic isoflavones, although having a good bioavailability, have a low distribution volume and are usually eliminated for the most part during the 24 h coming after intake. Because animals are currently fasting for at least 12 h before slaughtering, the amount of isoflavones remaining in their flesh is generally low [55]. Meat or fish, even though they have been fed pulses during their life, are not good isoflavone vectors in human diet.

In human, the exposure is mainly due to soy intake, although clover, alfalfa, and kudzu extracts can be used in food supplements. Until recently, this exposure was thought to be safe assuming that isoflavones had always been part and parcel of the human diet in Asia. However, it was recently shown that all traditional Asian recipes, which were elaborated sometimes over several centuries, included prolonged cooking, simmering, or soaking in water. These steps empirically removed the glycosylated isoflavones which are soluble in water but heat resistant. As an example, for traditional tofu, a rough soy meal is simmered and soaked in water for a total of 2–3 h before curdling. Then, water is squeezed out by pressing to make the soy cheese. In the same way, kudzu, which contains high levels of isoflavones with inverse proportions of GEN and DAID compared to soy, is directly powdered to prepare medicines. It is also boiled in water to be eaten as we could do with potatoes. The boiling step can last over 2 h and removes isoflavones. In 2004, looking at isoflavone intakes in rural Chinese women who are more likely to have kept their traditional tofu making, Liu and co-workers [56] found that 65% of them had an isoflavone intake below 15 mg/day, the majority being below 5 mg/day. Nowadays,

Table 3 Occurrence of ZEN in unprocessed grains from EFSA [54]

Food group	Nb	>LOD	Concentrations (μg/kg)					
		%	LB/UB	Mean	P50	P75	P95	Max
Unprocessed grains	9,877	41	LB	33	0.0	15	160	2,969
			UB	40	7.0	27	161	2,969
Wheat	5,318	38	LB	22	0.0	7.0	89	2,969
			UB	27	5.0	20	90	2,969
Barley	1,071	37	LB	10	0.0	5.0	49	775
			UB	13	5.0	10	50	775
Corn	2,460	56	LB	76	16	76	319	2,700
			UB	87	40	78	319	2,700
Oats	596	23	LB	21	0.0	0.0	76	1,590
			UB	23	1.5	5.0	98	1,590
Rice	43	7.0	LB	0.8	0.0	0.0	10	15
			UB	5.5	5.0	5.0	10	15
Sorghum	55	53	LB	96	50	147	450	700
			UB	116	50	147	450	700

N, number of samples; > LOD, indicates the percentage of results above the LOD or LOQ; *LB*, lower-bound; *UB*, upper-bound; (lower-bound, values below the detection limit are extrapolated to 0; upper-bound, values below the detection limit are extrapolated to detection limit)
P50, 50th percentile; P75, 75th percentile; P95, 95th percentile
[a]If $N < 60$, then the calculated P95 should be considered only as an indicative value [54]

in Western countries as in the developed countries of Asia, soy is essentially produced using industrial techniques. In all cases, cooking steps were reduced in time to reduce energy costs, and water is used either in small proportion (steaming) or not used at all. As a consequence, the amounts of isoflavones can be very high. Therefore, the amount of isoflavones in modern products can be high when a portion is considered (Table 4). Soy is also incorporated in processed food as soy flakes (Table 4) which can contain from 90 to 182 mg of isoflavones for 100 g [1]. Therefore, isoflavones can be present in significant amounts in processed food containing soy flakes (Table 4).

Why is there such amount of isoflavones in modern soy preparation? This is because when the industrial processes were set up, in the 1930s, hardly anything was known about isoflavones, and the processes were designed essentially to prevent protein degradation. As an example, defatted soy flakes are treated with hexane which removes fat and concentrates isoflavones in the protein matrix. The matrix is then extruded. Extrusion destroys most of the anti-nutritional factors but not isoflavones. Generally speaking, in the industry, the cooking steps were shortened to save energy and costs. When it was possible to assay isoflavones accurately, in the early 1980s, the assays were performed in several popular areas essentially eating industrial soy-based products produced in mass for city populations. The study by Liu [56] is one of the very few dealing with exposure in remote Chinese areas still consuming soy food traditionally cooked. It showed that the largest proportion of rural women was exposed to low doses of isoflavones. Therefore, nowadays and

Table 4 Quantities of isoflavones measured in modern dishes based on soy or containing soy as an ingredient

	Genistein (μg/g)	Daidzein (μg/g)	Total (μg/g)	Reasonable portion size	Intake for 1 portion (μg)
Foodstuffs based on soy juice					
Tonyu 1	91.37 (± 6.52)	49.57 (± 3.47)	140.94	1 bowl (350 mL)	49,330
Tonyu 2	51.32 (± 8.16)	39.83 (± 4.39)	91.14	1 bowl (350 mL)	31,899
Yogurts	44.70 (± 3.17)	37.40 (± 3.61)	82.20	1 yogurts	8,220
Soft yogurt	125.00 (± 8.75)	129.19 (± 9.12)	254.31	1 yogurt	30,510
Vanilla soy cream	29.82 (± 2.16)	19.39 (± 1.36)	49.21	1 cup (100 g)	4,921
Caramel soy cream	40.00 (± 2.86)	17.90 (± 0.7)	57.89	1 cup (100 g)	5,789
Soy juice chocolate taste	107.00 (± 7.49)	70.00 (± 4.92)	178.75	1 mug (330 mL)	58,987
Vanilla dessert	159.50 (± 11.16)	63.20 (± 4.42)	224.10	1 cup (100 g)	22,410
Powdered soy “milk”	1,310.00 (± 81.62)	1,070.00 (± 64.96)	2,390.00	1 mug	49,790
Herb cheese made of soy	368.39 (± 25.86)	357.23 (± 22.99)	725.62	50 g	36,280
Cream substitute made of soy	70.08 (± 5.11)	63.16 (± 4.42)	134.95	20 mL	2,700
Foodstuffs based on soy protein					
Sausages made of soy (1)	82.21 (± 5.76)	40.64 (± 2.87)	122.85	2 sausages (90 g)	11,060
Sausages made of soy (2)	134.15 (± 10.55)	66.95 (± 5.53)	201.10	3 sausages (80 g)	16,090
Sausages made of soy (3)	259.50 (± 10.42)	231.00 (± 20.70)	490.50	2 sausages (90 g)	44,145
Soy biscuits with figs	95.38 (± 6.36)	87.74 (± 6.16)	183.12	4 biscuits (80 g)	14,650
Buckwheat pancakes with tofu	228.50 (± 15.86)	154.00 (± 10.35)	382.50	1 pancake (100 g)	38,250
Soy pancakes with tomatoes	202.30 (± 14.16)	116.92 (± 8.56)	319.22	1 pancake (100 g)	31,920
Soy pancakes “provençale”	227.15 (± 15.57)	129.48 (± 11.02)	356.63	1 pancake (100 g)	35,663
Smoked tempeh	165.33 (± 11.56)	112.00 (± 7.84)	277.33	3 slices (50 g)	13,870

(*continued*)

Table 4 (continued)

	Genistein (μg/g)	Daidzein (μg/g)	Total (μg/g)	Reasonable portion size	Intake for 1 portion (μg)
Instant powder for drinks	99.69 (± 6.96)	106.11 (± 7.35)	205.80	3 spoons	8,026
Japanese soft tofu	117.87 (± 10.29)	70.92 (± 2.88)	188.79	100 g	18,879
Natural tofu	225.27 (± 75.14)	117.22 (± 7.43)	342.49	125 g	42,810
Japanese soft tofu	117.87 (± 10.29)	70.92 (± 2.88)	188.79	100 g	18,879
Traditional tofu[a]	224.43 (± 11.79)	100.92 (± 12.48)	325.35 (± 24.26)	100 g	32,535
Whey from traditional tofu[a]	744.36 (± 39.89)	459.93 (± 60.17)	1,204.30 (± 100.06)	60 mL	72,258
Tofu	48.00 (± 3.26)	46.16 (± 3.16)	95.27	1 portion (100 g)	9,530
Breaded tofu (1)	150.33 (± 10.52)	71.22 (± 5.57)	221.55	1 portion (100 g)	22,150
Breaded tofu (2)	289.29 (± 6.04)	188.49 (± 2.00)	477.78	1 portion (100 g)	47,778
Tofu with garlic	216.74 (± 9.60)	138.25 (± 4.09)	354.99	1 portion (80 g)	28,400
Smoked tofu	273.54 (± 15.33)	178.54 (± 5.50)	452.08	1 portion (100 g)	45,210
Legumes mixed with tonyu sauce	98.81 (± 1.90)	62.90 (± 6.35)	161.71	1 dish (300 g)	48,510
Vegan steak tomato and onions	332.44 (± 11.39)	222.65 (± 3.29)	555.09	1 steak (90 g)	49,960
Vegan "Bolognese for pasta"	244.73 (± 81.73)	155.40 (± 8.25)	400.13	120 g	48,020
Conventional soy grain	490.98 (± 6.62)	347.68 (± 78.26)	838.67	100 g	85,421
Toasted soy grain (appetizers)	1,360.00 (± 51.32)	1117.42 (± 119.03)	2,477.42	20 g	49,550
Slimming dish (soup)	223.59 (± 15.46)	135.44 (± 9.24)	359.03	1 pack (50 g)	16,510
Slimming dish (breakfast)	185.03 (± 11.95)	98.36 (± 7.56)	283.39	1 pack (50 g)	13,030
Slimming dish (meal)	287.17 (± 18.53)	193.64 (± 13.22)	480.81	1 pack (50 g)	22,110
Soy lecithin extract (1)	0.17 (± 0.01)	0.68 (± 0.04)	0.97	10 g	9.7
Soy lecithin extract (2)	0.86 (± 0.06)	2.36 (± 0.16)	3.52	10 g	35.2

(*continued*)

Table 4 (continued)

	Genistein (μg/g)	Daidzein (μg/g)	Total (μg/g)	Reasonable portion size	Intake for 1 portion (μg)
Foodstuffs with hidden soy					
Minced beef pie (Parmentier)	4.66 (± 0.32)	1.53 (± 0.11)	6.20	1 portion (300 g)	1,860
Minced beef portions	73.92 (± 5.11)	49.34 (± 3.43)	12.22	1 steak	12,220
Stuffed tomatoes	33.02 (± 2.82)	26.94 (± 1.96)	59.99	2 tomatoes	8,960
Stuffed cabbages	33.04 (± 2.55)	25.48 (± 1.53)	58.48	2 cabbages	9,040
Meatballs (1)	78.14 (± 6.16)	54.23 (± 3.56)	132.37	4 balls (125 g)	16,546
Meatballs (2)	82.55 (± 6.68)	59.60 (± 2.59)	142.15	4 balls (125 g)	17,768
Minced veal (breaded)	55.96 (± 3.86)	35.32 (± 2.45)	91.28	1 steak (100 g)	9,128
Brownies	65.24 (± 4.56)	43.92 (± 3.15)	109.16	3 Pieces (90 g)	9,824

Figures are mean ± SD of three measures performed on three different microtitration plates
[a]The traditional tofu is an industrial product

especially in developed Asian countries where soy is traditionally eaten but where the processing has been industrialized, the isoflavone intakes have risen dramatically, sometimes over the 45 mg/day which shown to have an effect on premenopausal menstrual cycle [57]. According to recent studies, the mean human exposure in Japan lies between 45 and 60 mg/day [58], in Korea the highest value recorded was 33.6 mg/day for adults [59], and in China it tends to increase nowadays [60] with 16.2 mg/day for adults and 27 mg/day for adolescents and a large interindividual variation. In Western countries, the exposure to isoflavones also rose recently although it is most likely underestimated. Usually, in Western countries, the isoflavone intake is based on the evaluation of pulse consumption and essentially based on soy which is the major provider [61]. Therefore, many countries consider the intake below 2 mg/day especially because they tend to calculate a mean intake over the total population and taking only soy food intake into account. In the USA, the exposure was estimated to be less than 2.7 mg/day in 2001 [31] and confirmed later by subsequent studies [62, 63]. However, the amount of isoflavones in modern soy-based food is higher than that found in the past in traditional foodstuffs (Table 4). Hence, soy juices which are very popular in the West, together with their side products (yogurts and creams), can be good vectors of isoflavones, since in this case, isoflavones are concentrated at the juice filtration step [1]. Soy is also prepared as flakes containing large amount of isoflavones. As already

Table 5 Isoflavone intakes by infants fed with soy-based infant formula in different countries

Countries	Isoflavones mg/liter	Daily isoflavone intake (mg/kg bw)	Reference
USA	20.9–47	2.3 to 9.3	[68]
UK	18–46.7	1.7 to 4.4	[68, 69]
Australia	17.2–21.9		[68]
New Zealand	17.1–33	2.9 to 3.8	[68, 70]
Brazil	10–47.4	0.8–1.6 to 6.6	[68, 71, 72]
France	19.4–42.3	2.3 to 6.04	[73]

mentioned, flakes are incorporated into many recipes marketed as traditional but made by the food industry. Soy is then incorporated as an ingredient for its nutritional and technological properties or for economic reasons. Most studies have underestimated this exposure, but it is so significant that in recent studies no urine samples were found to be devoid of isoflavones in American men [5]. Isoflavones were also detected in 88% of the urine of American pregnant women [64], in all Israeli adults [65], and in all German adolescents [66] of a study cohort. Finally, it must be pointed out here that the human populations most exposed nowadays are infants or children on soy-based infant formula [67]. These formulas were advised to children with lactose intolerance mainly in the 2000s. In the USA and according to authors, 17–30% of infants received these formulas in the last past years. Table 5 shows data recorded in different countries by several authors.

In their paper [67], Badger and co-workers also showed that infant exposure to soy isoflavones is a modern practice. They explained the exposure to estrogens of infants fed with soy-based infant formula is dramatically different from that of Asian children enjoying traditional Asian soy intake. Traditional soy consumption in Asia was based on solid foodstuffs (tofu, natto, miso, tempeh), which were not suitable for babies. Traditionally, these foodstuffs were prepared following recipes including prolonged cooking, simmering, or soaking in water and most probably contained much less isoflavones than the modern industrial products. Therefore, the isoflavone exposure calculated in [67] was nearly nil before 12 months in Asian children but rose progressively thereafter. By comparison, in Western babies fed with soy-based infant formulas, the exposure rate is high as soon as soy formula intake begins, which rises and continues to rise progressively until food diversification. Exposure levels are currently 10–20 times higher than those inducing an estrogenic effect in women, as shown on breast reactivity [74–76], menstrual cycle lengthening [57, 77], and impaired sperm production [78–80]. The plausible consequences of such exposures will be discussed later.

3.2 Coumestans

Coumestrol as a phytoalexin is essentially produced by pulses following fungal attacks. Therefore, and because human foodstuffs are usually better controlled, this substance is more likely to be present in animal food rather than in human diet.

Although, it is not excluded that it could be present in food supplements such as those based on alfalfa. In England the study of Clarke and co-workers [81] failed to detect any COUM in the samples analyzed. In France, in 2011, according to the EAT2 study [82], the consumer exposure was considered to be at least 1,891 ng/day. However, the authors tend to think that this exposure is underestimated since all the contributors could not be analyzed in the EAT2 study. Recently a protein supplement based on alfalfa was authorized by EFSA although the supplier was able to measure milligrams of COUM in his extracts, i.e., 78 mg/kg [83]. The supplier also showed an estrogenic effect in mice and showed a large variability of phytoestrogen content from one batch to another. If such a supplement is to be used widely by the European consumers as it is proposed, the mean exposure to COUM will probably rise dramatically. However, in South Korea, it was reported in 2009 that the mean level of exposure was 0.3 mg per capita per day due to a local specific foodstuff consumption like soybean sprout and arrowroot [59].

3.3 Lignans

As already mentioned, lignans are not estrogenic in plants. The molecules present in grains and in vegetables are eventual precursors of estrogenic enterolignans depending on the composition and efficiency of the human gut flora. Therefore, the enterolignan exposure is difficult to assess because not all precursor sources are identified yet [49] and because not all consumers are able to produce them after ingestion. According to Zamora-Ros and co-workers [84], in European populations, lignans were the most abundant contributor of phytoestrogen intakes when the global intake is low. This study reports a low lignan intake (1.02 mg/day) in Mediterranean countries and even lower in Italy (0.67 mg/day). It also reported a higher intake (1.26–1.60 mg/day) in non-Mediterranean countries. The study also compared their data with those obtained in the Netherlands (1.24 mg/day), in Sweden (0.50–2.81 mg/day), and Finland (1.22 mg/day). Still according to [84], in other Western countries such as Mexico, the USA, and Canada, lignan intakes were considered to be much lower (from 0.35 to 0.86 mg/day). However, when pharmacokinetic studies are performed in humans, the enterolignan levels recorded in the blood of ENL producers are never null [85]. Moreover, in urine and in serum, of citizen of Western countries, they are of the same range concentrations with isoflavones [86, 87]. As an example according to [86] in the USA (Albany), the urine lignan concentrations range from 0.99 to 1230 ng/mL and were found in 94% of the population. In parallel, still in the same study, isoflavone urine levels range from 1.33 to 1290 ng/mL. The detection rate reached 100% for DAID in women. In the UK and according to [87], the enterolignans in urine sample ranged from 0 to 10,229 μg/mmol creatinine and isoflavones (DAID, GEN and equol) excreted in urine ranged from 0 to 1,442.6 μg/mmol creatinine. This shows that the sources of enterolignan precursors are far from being identified and that the exposure is only approached and most probably underestimated. In addition, this lignan intake cannot

be readily assimilated to a phytoestrogen exposure because the gut metabolism differs between consumers [88].

3.4 Resorcylic Acid Lactones

Because of a careful screening of plant matter, usually the exposure to the mycoestrogens, ZEN, α-ZOL, and β-ZOL in the European populations are considered to be low. More precisely based on a bio-monitoring approach linking the scarce pharmacokinetic data of ZEN in human and data obtained on urinary levels [89], it appeared that zearalenone daily exposure is most probably below the tolerable daily intake (TDI) of 0.25 μg/kg body weight in European study cohorts. However, some individuals from Haiti and in African countries, where corn is a major food source, may have an exposure that exceeds the TDI. Table 6 from [54] gives exposure calculations in different categories of European consumers.

4 Effects in Plants

4.1 Isoflavones

Isoflavones in plants act as phytoalexin [21]. They are antimicrobial and may have a role in plant protection. As an example, the antifungal activity of lupin isoflavones was demonstrated [90]. In soybean cultivars, an increase in isoflavone concentrations was shown to be a specific response to the attack of a saprophytic fungi *Mucor ramosissimus*. In the soybean strains resistant to the fungus, isoflavones (including glyceollins I, II, and III, glycinol, glyceocarpin, GEN, isoformononetin, and *N*-acetyltyramine) were induced by the fungal attack, all compounds possessing antifungal activity with the exception of GEN [91]. A contamination of a soy strain with the fungus *Diaporthe phaseolorum f.* sp. *Meridionalis* induced the accumulation of isoflavones (GEN, DAID), pterocarpans (glyceolins), and flavones (apigenin and luteolin) via a nitric oxide synthase pathway [92]. To go on with interactions of the plants with microorganisms, there is some evidence that the symbiotic relationship between *Rhizobium lupine* and *Lupinus albus* stimulates an increase in the production of prenylated isoflavones in the root nodules [93]. These prenylated isoflavones possess in vivo activities against a number of other *Rhizobium* species. Zhang and Smith [94] also showed that GEN plays a major role in the establishment of the symbiosis between *Bradyrhizobium japonicum*, the arbuscular mycorrhyzas such as *Glomus mosseae*, and their host, i.e., soy (*Glycine max*). Genistein is the soy recognition molecule inducing the greatest plant-to-bacterium signal. In fact, the binding of GEN to *B. japonicum* activates many of the bacteria nod genes.

There is solid proof that farming practices directly influence the levels of isoflavones in soy. As a matter of fact, irrigation was shown to enhance isoflavone content in soybean by as much as 2.5-fold [95]. A deficit in nitrogen fertilizer increases the estrogenic activity of a clover pasture [96]. On the other hand, a

Table 6 Daily exposure of European citizen to zearalenone as calculated by EFSA in ng/kg/b.w [54]

Age class	Summary statistics of exposure to zearalenone (ng/kg/b.w. per day)					
	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
Mean dietary exposure in total population						
Infants[a]	3.3	87	6.4	87	9.4	88
Toddlers	9.3	51	13	83	23	100
Older children	5.7	29	11	44	22	75
Adolescents	3.6	17	6.1	26	12	42
Adults	2.4	14	4.3	18	7.2	29
Elderly	2.0	13	3.4	16	6.4	26
Very elderly	2.3	12	2.9	16	7.1	29
95th percentile exposure in total population[b]						
Infants	33	[c]	[c]	[c]	[c]	217[d]
Toddlers	24	104	31	182	50	277
Older children	9.9	59	22	80	42	124
Adolescents	7.5	38	15	53	26	76
Adults	4.7	28	9.5	35	14	54
Elderly	3.5	25	7.5	31	12	42
Very elderly	7.0	26	7.7	35	13	47

b.w., body weight; *LB*, lower-bound; *UB*, upper-bound (lower-bound, values below the detection limit are extrapolated to 0; upper-bound, values below the detection limit are extrapolated to detection limit)

[a]Estimates based on only two dietary surveys

[b]The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust [54], and therefore they should not be considered in the risk characterization. Those estimates were not included in this table

[c]Not calculated

[d]Estimates are based on one dietary survey only

supplementation in the same fertilizer decreases the occurrence of isoflavones with estrogenic activity in a clover pasture [96]. A study where soy cultivars (*Glycine max*) were bred for 3 years demonstrated that levels of isoflavones were related to both environmental and genetic characteristics and could be susceptible to selection [97]. The genomic regions implicated in this process have been identified [98]. Vyn and co-workers [99] showed that GEN, DAID, and glycitein contents in soy are correlated with potassium in roots and leaves and with crop management using potassium-rich fertilizers. Lindner in [23] gave an evolutionist theory about the ability of Leguminosae to produce estrogenic isoflavones. Indeed, it appears that isoflavonoid compounds are synthesized by the plants in response to bacterial or fungal attacks or to water stress. Estrogenic isoflavones can then be considered as protective compounds. In the case of overgrazing of a pasture by mammalian predators, the production of great quantities of estrogenic compounds which could impair predator reproduction would result in the reduction of the predator pressure. Finally, because estrogenic isoflavones are also specific attractants

for symbiotic bacteria of the *Rhizobium* gender and of mycorrhizal fungi of the *Glomus* gender, and because these bacteria can fix the atmospheric nitrogen, human selection against the production of isoflavones in pulses would result in the loss of one of their greatest interests in crop management.

5 Effects in Humans and Animals

5.1 Beneficial Effects

5.1.1 Beneficial Effects in Animals

Estrogens are used in cattle as anabolic agents. Therefore, some studies attempted to figure out if a phytoestrogen supplementation can enhance cattle or poultry growth and meat quality [100]. Beside a potential interest in antioxidant content of meat from animal raised on estrogenic food [100], it was seen that a DAID supplementation in the diet increased marbling meat score in steers [101]. If the meat quality was significantly improved considering several parameters, the carcass bone proportion was also greater probably reflecting a positive effect of phytoestrogens on osteoblast function. In another study [102] and at low doses, i.e., 300 and 400 mg/day, DAID was also shown to enhance immune function in late-lactation cows under heat stress. However, the DAID supplementation does not strictly mimic a legume intake since in all the roughages based classically on pulses, DAID or its precursor formononetin is present together with other compounds either estrogenic per se (GEN) or precursors of these estrogenic isoflavones (biochanin A) playing in interaction.

There is no positive effect reported for lignans, COUM, or ZEN on farmed animals although ENL was found in milk of cows fed with flaxseed or forages rich in ENL precursors [103].

5.1.2 Menopause in Women

Isoflavones

Menopausal symptoms encompass hot flushes, night sweats, vaginal dryness, mammary density, and mood fluctuations. Menopause is not a disease. In Western countries, menopausal symptoms were treated for more than 20 years using hormone replacement therapy (HRT). However, several studies including the Women's Health Initiative (WHI) showed that deleterious effects of the US treatments on breast or endometrial cancers and those on cardiovascular diseases may overcome their benefits [104]. More than 543 epidemiological studies were performed to examine if the consumption of soy and isoflavones with estrogenic activities may prevent hot flushes. This effect can be suspected since it is known that hot flushes and night sweats are controlled by the hypothalamus preoptic area implicated in the body core temperature regulation. In addition, the neurones in this region have estradiol membrane receptors involved in body temperature and energy homeostasis [105]. This area induces shiverings or sweatings when it records a body temperature over

a low and an upper threshold. The amplitude of the neutral zone situated between these thresholds is under serotonin and noradrenergic control. 17β-E_2 is known to induce the local synthesis of these two neuromediators as well as their respective receptors [106]. The recent meta-analysis of Chen and co-workers [107] showed a reduction in hot flushes with isoflavone intake. The study is based on 15 randomized clinical trials comparing phytoestrogens vs. placebo for which the mean age of the subjects ranged from 48 to 60.1 years. The number of participants was at least 30 and up to 252. The study duration ranged from 3 to 12 months. The meta-analysis of the 7 studies reporting the Kupperman index gathering 11 menopausal symptoms showed no significant effects of phytoestrogens as compared to placebo. However, the meta-analysis of ten studies showed a significant reducing effect of phytoestrogens on hot flush frequency compared to placebo. The meta-analysis of the five studies that planned to report side effects of phytoestrogens did not show any significant difference between the two groups. Menopausal symptoms are known to be influenced by psychological elements. Therefore, a placebo effect exists. Nevertheless, in accordance with an estrogenic effect in women especially when estradiol receptors are present, the phytoestrogen effect can be observed.

Coumestrol

In women some data exist linking the use of some herbal medicine, some of them containing COUM with other substances with or without estrogenic activities [108]. This is the case for clover or alfalfa preparations. However, menopausal symptoms are influenced by psychological factors [109], and some meta-analyses failed to really correlate isoflavones and/or COUM with a decrease of menopausal symptoms [110]. For COUM its level in the preparation is generally low even if it can also be highly variable. In addition, little is known about its serum bioavailability. Therefore, its effect is very difficult to truly associate to any observation.

Lignans

Only eight randomized, double-blind, placebo-controlled clinical trials were found trying to prove the effectiveness of flaxseed on the management of menopausal symptoms [111–116]. None of them, whatever the amount of flaxseed or secoisolariciresinol diglucoside (SDG) tested, whatever the age and number of volunteers involved, was able to show an effect over placebo treatment. This could be expected at least for two reasons. First, enterolignan precursors are ubiquitously distributed in human diet, and therefore the placebo group also receives estrogenic enterolignans in rather variable amount. See Table 2 and the known sources of lignans. This, of course, reduces the power of the interventional studies. Second, only END or ENL can possibly exert estrogenic effect, which could explain hot flashes relief. However, only a small portion of the population harbors the gut flora able to produce these enterolignans in sufficient amount [117]. Therefore, the enterolignan effect should only be seen if women able to produce enterolignans were compared to women unable to produce them. This would require a selective procedure at the entrance of the clinical studies and enterolignan monitoring in blood to account for the dietary exposure through casual diet. None of the studies done until now did undergo such

protocol. Note that urine measurements may not be precise enough since an eliminated compound is not an active one and that there may be interindividual variations in the elimination pathways of enterolignans.

Zearalenone
Zearalenone and its metabolites α- and β-ZOL as mycotoxins have not been tested in clinical intervention on menopausal symptom relief.

5.1.3 Bone Health in Women

Isoflavones
Two meta-analyses converge in saying that isoflavone from soy may be protective on bone density in menopausal women but only at high doses, i.e., over 80 mg/day. The first one [118] considered only supplementation by soy isoflavone extracts (not soy protein or foods containing isoflavones) on bone mineral density (BMD) in menopausal women. It was based only on randomized controlled trials published in English, Japanese, or Chinese reporting the effects of soy isoflavone extracts on lumbar spine or hip BMD in menopausal women. Only 11 studies in total were found to match the given criteria. The meta-analysis included data from 1,240 menopausal women. It revealed that daily ingestion of an average of 82 mg of soy isoflavones (aglycone) for 6 to 12 months significantly increased spine BMD. Treatment duration, geographic origin, and basal BMD had a major influence on the effect observed. No significant effects on femoral neck, hip total, and trochanter BMD were found, while soy isoflavone extract supplements increased BMD feature that was restricted to the lumbar spine in menopausal women. The second meta-analysis [119] included randomized controlled trials (RCTs) examining the effect of a soy isoflavone supplementation in women for at least 1-year duration. The main outcomes were BMD changes from baseline at the lumbar spine, total hip, and femoral neck. Ten RCTs gathering 896 women were found to be eligible according to the study criteria. A mean dose of 87 mg of soy isoflavones for at least 1 year did not significantly affect BMD changes. However, when doses were stratified, it was shown that only a large dose over 80 mg/day of isoflavone tended to have a weak beneficial effect on spine BMD. The BMD was not increased but preserved.

Coumestrol
*N*o data seem to exist on the effect of COUM on menopausal women bone health. Only one study tested the effect of this compound in rodents [120], showing a preventive effect against bone loss in an ovariectomized rodent model. The estrogenic activity of COUM in vivo on bone cells has also been shown on the prevention of osteoclast differentiation [121] and the enhancement of osteoblast formation [122]. These are classical estrogenic effects.

Lignans
The only study which was found dealing with the association of lignans and bone health is likely to be insufficient to prove any effect [111]. It deals with an exposure

evaluated from a dietary questionnaire, and since median intake of total phytoestrogens was estimated at 876 μg/day, the study most probably underestimated the exposure. Phytoestrogen plasma assays should have been done for this study to really help in determining the enterolignan effects. Nevertheless, the authors said that enterolignans estimated from food intake irrespective of the gut flora efficiency were positively associated with bone density in postmenopausal women. However, this association became nonsignificant when dietary Ca^{2+} was added to the model. In light of this finding, further data are needed before any definitive conclusion was drawn out.

Zearalenone

Because of the toxicity of the molecule and of its metabolites α- and β-ZOL, no study was performed and published in humans. As for COUM, some data can confirm the estrogenic effects of ZEN and metabolites on bone cells in vitro [123] but also in vivo in rodent models [124]. Again the main outcomes are characteristic of estrogenic effects, with a prevention of bone loss [125], although in some studies chromosome aberrations were also reported [126 and publications included].

5.2 Deleterious Effects

Looking at deleterious effects is a challenge since in several occasions it can be seen that the experts do not agree on what is adverse and what is safe. Before the 2010s hormonal changes were not always considered as adverse, and therefore two types of noneffective levels were defined for phytoestrogens: the no observable adverse effect level (NOAEL) and the no observable effect level (NOEL). Conventional toxicology relies upon measures of exposure that induce pharmacological or physiological adverse effects. Hence, the NOEL is the highest dose tested at which there is no measurable effect, and the NOAEL is the highest dose tested at which there is no measurable adverse effect. Then the definition of what is an adverse effect is crucial to consider. Nowadays, the concept of endocrine disruption has emerged, and all hormonal modifications especially those dealing with reproductive hormones can be considered as adverse. However, such adverse effects are usually observed on a long-term basis, and therefore multigenerational studies may sometimes be required to see an effect. For example, if chronic classical toxic effects are considered for GEN, the NOAEL on liver endpoint is 50 mg/kg/day. However, considering hormonal endpoints and according to McClain and co-workers, the NOEL is 5 mg/kg/day [6]. If hormonal effects are seen as endocrine-disrupting effects, then the NOAEL of GEN switches from 50 mg/kg/day to 5 mg/kg/day. This NOAEL is sustained by the recent studies of the National Toxicology Program (NTP) in the USA [4]. However, the NTP study also showed reduced birth weight and reduced weight at weaning of pups at all doses tested including 0.3 mg/kg/day in females and over generations. The authors concluded that if this effect is considered as a toxic effect, then the NOAEL is below 1.2 mg/kg/day in rat. It also showed a reduced anogenital distance reduction in pups of both sexes at various doses and in several

exposure conditions. Litter size was also impaired after several generation of exposure for the highest dose tested. These effects sign a physiological impairment which can have consequences on reproduction which is finely tuned by complex endocrine balances. Therefore, effects which could have been neglected in the past come to the front nowadays because science progresses. NOAEL and LOAEL (lowest observed adverse effect level) can be used to establish mean tolerable daily intake (MTDI). A TDI is an estimate of the amount of a substance that can be taken daily over a lifetime without appreciable health risk. TDIs are calculated on the basis of laboratory toxicity data to which uncertainty factors are applied. According to [127], reasonable uncertainty factors should be applied. When a NOAEL in rat is available in chronic study, this uncertainty should include a factor accounting for the difference of sensitivities among humans and a factor accounting for the relative sensitivities of animals compared to humans. In that case, the MTDI in human is 46 times lower than the NOAEL in rat. When only LOAEL are available, the uncertainty factor should also account for uncertainty in extrapolating from a low-risk level. Therefore, the global uncertainty factor between LOAEL and MTDI increases to 184. Here, the adverse effect can be considered to be a hormonal disruption. As TDIs are regarded as representing a tolerable intake for a lifetime, short-term exposure to levels exceeding the TDI is not a cause for concern, provided the individual's intake averaged over longer periods of time does not appreciably exceed the level set. The uncertainty factors used to establish a TDI provide assurance on the relative safety of the TDI. However, there should be concern if the TDI is substantially exceeded for long time periods. In order to have a quick overview of what can be said about the molecules retained here, we propose Table 7 which gathers NOAEL or LOAEL obtained in rat. It also gives an overview of the estimated exposure and a weight limit under which the exposure is due to overcome the MTDI. This table is partly deduced from that of Hendrich [128].

These data have to be considered with caution, since here again, science evolves and toxicological studies performed more than 10 years ago may not reach the standard of quality actually required. However, if they have not been reproduced recently, they should be considered as valid. The MTDI presented in this table are compared to the mean daily exposure expressed in mg/day. The values given here may be corrupted by many biases when they were estimated from food frequency questionnaires. First these questionnaires may not exactly reflect all population exposure, and second the large variability of the content in the phytoestrogen of interest may not be taken into account. Some food sources can be completely omitted as for isoflavones. Hence, as already mentioned, soy is nowadays ubiquitously present in industrially processed food, and the isoflavone intake is so common that they can be found in a large proportion of consumers' urine samples (100% in the US study by Mumford [5], 100% in the German study by Degen [66], 98% in the Israeli study by Berman [65]). Note that for lignans, no data of exposure were found in Asian consumers. This is due to the fact that many plants including plants used for personal care in Asia are sources of the estrogenic-enterolignan precursors. All these precursors are not known yet, and this has to be combined to the fact that not all the consumers harbor the competent gut flora for ENL production. Therefore, when the

Table 7 Summary of NOAEL or LOAEL of natural estrogenic substances together with the consumer estimated exposures and the limit at which the mean dietary tolerable intake can be overcome

Phytoestrogens	NOAEL in toxicological studies	References	Human MTDI (mg/kg)	Estimated daily mean intake (mg/day)	Country	References	Inferior weight limit for MDTI overcome
Isoflavones	5 mg/kg	[6]	0.11	2.7	USA (general pop)	[31]	24.55
				50	Japan (general pop)	[58]	454.55
				2.257	UK (general pop)	[84]	20.52
				1.12	France (women)	[84]	10.18
				0.1782	Spain (women)	[84]	1.62
				0.22	Greece (women)	[84]	2.00
				0.317	Italy (women)	[84]	2.88
				0.7675	Netherlands (women)	[84]	6.98
				0.46	Denmark (women)	[84]	4.18
				0.893	Sweden (women)	[84]	8.12
				1.285	Norway (women)	[84]	11.68
Coumestrol	4 mg/kg (LOAEL)	[129]	0.022[a]	0.01243	USA	[130]	0.57
				Trace	China	[60]	–
				0.06259	Europe (men, mean)	[84]	2.85
				0.04348	Europe (women, mean)	[84]	1.98
Lignans	100 mg/kg (LOAEL)	[131]	0.540[a]	0.155	USA	[132]	0.29
				nd	Asia	–	nd
				1.371	Europe (men, mean)	[84]	2.54
				1.207	Europe (women, mean)	[84]	2.24

(*continued*)

Table 7 (continued)

Phytoestrogens	NOAEL in toxicological studies	References	Human MTDI (mg/kg)	Estimated daily mean intake (mg/day)	Country	References	Inferior weight limit for MDTI overcome
Zearalenone	0.2 mg/kg	[133]	0.0001	0.000055	Europe mean (UB)	[54]	0.01
				0.000973	New Zealand (mean)	[134]	0.19
				0.000019	Canada (young men)	[135]	0.19
				0.000047	Canada (young children)	[135]	0.19
				0.000098	Canada (adults)	[135]	0.47
				0.0017	USA	[136]	17.00
				0.00153	France (adults UB)	[82]	15.30
				0.001155	France (children UB)	[82]	11.55

The inferior weight limit for MDTI overcome is obtained dividing the estimated daily intake by MTDI
MTDI mean tolerable daily intake
[a]extrapolated from the LOAEL; *nd*, not determined

effect of ENL is analyzed, the substance is assayed in the urine, but the correlation of the urine dose to a dietary intake is far to be simple. Finally, when the dose is low, the detection technique may not be reliable enough to get good results. Therefore, looking at the exposure to lignans in Asia, it was impossible to find any published data. The last column of the table gives a figure which materialized the inferior weight limit for MTDI overcome. It is calculated by dividing the estimated daily intake by the MTDI. As an example, for GEN in the USA, considering a mean daily intake of 2.7 mg in the general population, it appears that all people, whose body weight is lower than 24.55 kg, overcome the MTDI. Because this limit is considered as safe, the concern about this situation can arise only from its chronicity and from the degree of overtaking from the MTDI. The MTDI is a mean of the population exposure, and some people can be exposed to much higher doses. The situation in Japan could be considered as worrying since the calculation indicates that all people under 454.55 kg overcome the MTDI, considering the daily intake estimation. In that case, a fertility problem over generation should be investigated because it is seen in experimentally exposed rats.

5.2.1 Reproduction in Animals

Isoflavones

Isoflavones were considered for many years as estrogenic endocrine disruptors and studied as such by several authors [137–144]. These compounds can be present at high doses in estrogenic pastures based on certain clover species or on alfalfa. In sheep on these pastures, estrogenic isoflavones were shown to induce permanent estrus, heavy vaginal secretion, uterus prolapse, early abortion, reduced prolificity in reproductive ewes, and mammary fluid production in nulliparous ewes and castrated males. Castrated rams also exhibited abnormalities in their reproductive tracts, including enlargement of the prostate, the bulbourethral gland, and the membrane of the *vasa differentia*. In ewes affected by clover diseases, clover isoflavones were shown to delay LH secretion via a GnRH interaction [142]. This LH delay in secretion, which led to progesterone secretion impairment, was considered to be responsible for early abortion. More recently, a renewed interest emerged among scientist for the soy and isoflavone effects on reproduction of domestic animals. This is because it was recently figured out that isoflavone deleterious effects on cattle could have been missed out because soy was progressively incorporated to cattle food while genetic selection was undertook to improve production features including reproduction. Therefore, the antagonistic effects of both processes could have partly masked each other. As an example, it was shown that soy isoflavones can prevent in cows as in rodent the response of the *corpus luteum* to GnRH [145]. This result obtained, in vivo, is confirmed by data recently obtained ex vivo on Prim Holstein cows [146]. In addition, clover isoflavones were shown to block the early progesterone synthesis in Holstein heifers fed with a *Trifolium alexandrinum*-rich roughage after insemination [147]. This disruption was thought to be responsible for lesser ($P = 0.054$) conception rate and the greater ($P = 0.062$) percentage of heifers returning to estrus when compared to the control silage-fed heifers. As demonstrated

for COUM, GEN and DAID are able to increase the secretion of oxytocin by cow *corpus luteum* [148]. This can induce early abortion in these females and reduce global fertility and hence financial incomes to farmers relying either on veal or milk production. Recently, it was shown that isoflavones intake through Egyptian clover (*Trifolium alexandrinum*) as estrogenic roughage exerted a deleterious effect on fertility. Comparing the estrogenic roughage to the control diet based on maize silage, the % of Holstein heifers returning to estrus was 7.70 (1/13) under the control diet, and it was 38.46 (5/13) when cows ate clover roughage. Still in cows, it was shown that soy phytoestrogens increase prostaglandin secretion in cattle during estrous cycle and early pregnancy [149]. The number of cows involved in the trial was low. However, it appeared that soy-derived phytoestrogens and their metabolites disrupt reproductive efficiency and uterus function by increasing the ratio of prostaglandin F_2-alpha (PGF_2-α) to prostaglandin E_2 (PGE_2). Therefore, phytoestrogens led to high, nonphysiological production of luteolytic PGF_2-α in cattle during the estrous cycle and early pregnancy. The consequence of this increased PGF_2-α production is a higher failure rate of pregnancy. In addition, in vitro but at physiological doses, GEN, DAID, and equol were shown to inhibit bovine adrenal 3-beta-hydroxysteroid dehydrogenase directly involved into the synthesis of progesterone [150]. This inhibition seems to be possible in vivo considering the active doses of isoflavones in vivo. On top of the LH disruption, this direct effect may be a way by which isoflavones reduce progesterone secretion in early pregnancy. This can cause early abortion.

The phenomenon is generally enough to have led to endocrine disruption also observed in lower vertebrates of economic importance like birds or fish. Although the isoflavone effect in hens and laying hens does not seem to be deleterious, some long-term effects were recorded in ducks [151]. Noteworthy, ducklings were significantly smaller at hatching when exposed maternally to DAID supplementation. The difference in size compared to control animals disappeared at 4 weeks of age. It was accompanied by changes in the secretion of metabolic hormones and expression of growth-related genes. Although the negative effect of maternal DAID on embryonic growth could be eliminated 4 weeks after hatching, the long-term effect of maternal DAID on reproductive function was noted. Namely, it was an obvious downregulation of hypothalamic GnRH mRNA expression observed in ducklings maternally exposed to DAID. Fish in fish farms fed with a soy-containing diet can also exhibit altered reproductive performances or at least endocrine-disrupting features [152–154]. These effects were recently confirmed in goldfish considered as a model for other cyprinids [155] and also in other species of commercial interest like catfish or sea bass [156, 157]. Both estrogenic and thyroid functions involved in smoltification were shown to be impaired by soy phytoestrogens in the Atlantic salmon [158].

Coumestrol

Coumestrol was shown to exhibit deleterious effects on grazing animal reproduction [159]. Coumestrol was also involved in the clover disease affecting ewe reproduction in New Zealand in the late 1940s, and these endocrine disruptions still attract

scientific interest [160]. According to Adams [161], cows are more sensitive to COUM than to isoflavones most probably due to differences in metabolism when compared to ewes. As an example, COUM was shown to increase the production of oxytocin by cows decreasing the ratio of progesterone to oxytocin. This endocrine modification induces a higher failure rate to pregnancy. Still in the same study, COUM was shown to decrease PGE_2 secretion. These endocrine-disrupting effects can increase the risk of early abortion in this species [162] and therefore can affect the % of females returning to estrus as well as the calving to calving interval. In mare, COUM exerts endocrine-disrupting effects on chronic exposure leading to ovulation impairment [163]. The authors conclude to a potential infertility syndrome due to estrogenic silage (clover and alfalfa) in mares. The first reports of the deleterious effects of COUM in rat were produced in 1970 [164]. Then the Whitten's group published several coherent data showing the estrogenic effects of dietary COUM on the rat puberty and cycle [165], on uterus development [166], on the embryo implantation [167], and on the pituitary and hypothalamus hormone disruptions [167]. They also pointed out the remaining effects after the end of exposure [168] as well as sexual behavior impairments [169]. Other studies showed reproductive adverse effects on males' semen production which could be explained by a precocious estrogenic effect affecting the pituitary hormones and steroid secretion [170]. According to Hendrich [128], there is no NOAEL for COUM, and the LOAEL is 4 mg/kg/b.w./day.

Lignans

There is few data on the role of estrogenic enterolignans in domestic animals. These lignans can be present in significant amount especially when linseeds are used to modify the meat quality toward a greater proportion of omega-3 polyunsaturated fatty acid (PUFAs). According to [171], ENL concentration is increased in follicular fluid from cows fed with flaxseed-rich diet, although the serum levels are not affected. These increased enterolignan concentrations are correlated with increased concentrations of estradiol locally addressing the question of an effect of ENL on follicular steroid biosynthesis. Still in cattle, [172] showed that ENL deriving from a flaxseed-enriched diet or given alone in parallel of specific mixtures of PUFAs can decrease PGE_2 and $PGF_2\alpha$ concentrations in endometrial, stromal, and epithelial cells. This reduction was associated with lower mRNA abundance of the PG synthase genes in stromal cells. An omega-3-enriched PUFA mixture increased the effect of ENL compared to ENL alone and PUFA mixture rich in omega-3 alone. The authors concluded that considering the known luteolytic properties of $PGF_2\alpha$, a reduction in endometrial $PGF_2\alpha$ secretion would favor the establishment and maintenance of pregnancy. Enterolignans were found in the early 1980s in human and cattle semen. In both cases, their concentrations were shown to be 2.5 times higher than the corresponding plasmas [173]. In rat they were found to alter pregnancy outcome and reproductive development [131].

Resorcylic Acid Lactones

Zearalenone and ZOL are known as endocrine-disrupting agents from fungi since the early 1960s [51]. In cattle, ZEN was shown to lower the conception rate of the heifers [174]. In addition, ZEN contaminating sugar beet pellets was considered to be responsible for a reduced embryo transfer efficiency from a dairy farm experiencing low success rates of embryo transfer [175]. More recently, thanks to better detection techniques, it was shown that low ZEN contamination (below the allowed limit in Japan) was able to modify anti-Mullerian hormone levels in cow showing an effect in the ovarian antral-follicle populations in cows [176]. In swine the deleterious effects of ZEN on reproduction have been studied scientifically as early as in the 1970s [177]. It was later shown that concentrations of 25, 50, or 100 ppm of 95% purified ZEN fed to groups of healthy, multiparous sows during pre-estrus or throughout the gestation period (or both) produced multiple reproductive deficiencies [178]. These disorders included infertility, constant estrus, pseudopregnancy, diminished fertility, reduced litter size, offspring's small size, offspring malformations, juvenile hyperestrogenism, and probably fetal resorption. Sows' reproductive organs exhibited lesions, and their uterus, uterine duct, and cervix showed marked epithelial changes, i.e., squamous metaplasia. Similar features were found in the vagina and mammary glands. Transgenerational effects were also documented in sows [179], and ZEN was shown to reduce the quantity of healthy follicles, which probably lead to premature oocyte depletion in adulthood. In rat toxicity studies were undertook using ZEN (in corn oil) at doses of 0, 1, 2, 4, or 8 mg/kg/b.w. in order to determine the NOAEL for in utero development [180]. The fetal and pups' body weights were decreased in a dose–response manner in both sexes. Fetal development was delayed in all treated groups and linked to maternal toxicity. Zearalenone delayed skeletal ossification at 4 and 8 mg/kg. Fetal anogenital distance was increased in all treated groups, and fetal viability was decreased at 8 mg/kg. The weight of several maternal organs was modified at 4 and 8 mg/kg doses. Gonadotrophins were only marginally affected. Prolactin was significantly increased at 8 mg/kg. Estradiol dose dependently decreases at 2, 4, and 8 mg/kg. Therefore, ZEN was maternally toxic and fetotoxic but not teratogenic. Based on the dose-related maternal and fetal toxicity in all treated groups, the NOAEL for reproductive and teratogenic effects was less than 1 mg/kg.

5.2.2 Reproduction in Human

Isoflavones

Fertility: Unfortunately, as regards the human fertility, no clear-cut data are currently available about the effect of the modern exposure to estrogenic isoflavones. In 2014, an epidemiologic study performed on Adventist populations [181] sorted into six groups of isoflavone consumers showed that in young women (26 years old on average), the highest exposure of >50 mg/day was associated with a significant decrease in the number of birth. The same isoflavone intake was also correlated to an increased risk in women over 50 years of never being pregnant. Fertility also depends on the ability of men to produce efficient gametes. Noteworthy, sperm

production is a long process, starting from spermatogonial recruitment, continuing by spermatogenesis in the testis, and ending by the capacitating steps into *rete testis* and epididymis. In men the first step in the testis lasts for about 74 days [182]. Sperm presence in the *rete testis* and epididymis can vary from 2 days up to 18 days. It is only after sperm has undergone all steps that it can be ejaculated for fertilization and studied for its ability to fecundate. Therefore, the whole process from production to ejaculation can be considered as lasting about 3 months [182]. Consequently, whenever the effects of any endocrine-disrupting compound need to be studied on sperm production in men, exposure to those compounds should exceed 3 months to avoid any effect remaining undetected. As the capacitating process in the epididymis requires adequate thermal conditions, environmental conditions can exert bias on the final sperm quality. It is well-known that sperm production is influenced by both androgens and estrogens and that environmental endocrine disruptors (androgens, antiandrogens, estrogens, antiestrogens) can disturb sperm production in quality and in quantity [183]. As certain interventional studies on sperm production and quality like those of [184] or [185] were, unfortunately, performed over a short duration (less than 50–60 days), those studies cannot be retained when arguing about the effects of isoflavones on full spermatozoa production. Interindividual variation in sperm production and release, over the study period, is also a confounding factor that is not always taken into account and which can explain unexpected results [186]. In addition, the exposing dose should be in the dietary range. The data given in [1] indicate that, if a deleterious effect should be confirmed regarding sperm production and fertility, this should be addressed at a demographic level with data from industrial soy-eating countries and traditional soy-eating countries being treated separately. Noteworthy, the two countries that are most exposed to isoflavones because they have been eating industrial soy since the 1960s are South Korea and Japan. IndexMundi in 2017 indicates that the total fertility rate being the average number of children that would be born to a woman over her lifetime is 1.41 for Japan and 1.25 for Korea. These figures are lower than those recorded in all other developed countries with the exception of China territories. In France the total fertility rate is 2.07, and it is 1.87, 1.44, and 1.89 in the USA, Germany, and the UK, respectively. Noteworthy and still from the same sources, the contraceptive prevalence rate for women between 15 and 49 in Japan is only 33% when it is 80% in South Korea, 88% in France, 74.1% in the USA, 66% in Germany, and 84% in the UK. In addition, in both Asian countries consuming industrial soy, the demography regressed since 1983, i.e., one generation after soy-processing industrialization. This is also concomitant with the massive introduction of plant protection products in crop production all over the world. Moreover, in all studies that did show a correlation between decreased sperm quality and isoflavone intake, it was never possible to definitively exclude the interacting effects of other steroidal estrogens (in the case of obese men [78]) or of other endocrine disruptors like plant protection products [5, 79, 80]. In addition in [5], Mumford showed a positive interaction of sperm abnormalities with BMI. This indicates that the deleterious action of isoflavones on sperm production is probably influenced by other endocrine disruptors, present or not in the environment, and by endogenous estrogens.

Plausible consequences of early exposure: From a physiological point of view, a significant exposure to estrogens of babies under 6 months of age comes with its inevitable load of consequences [187]. During that period, differentiating processes involving estrogens and androgens are still at work, and steroid receptors are present in the different target tissues. These steroids, which were shown to potentiate isoflavone actions, are well-known to be disturbed in animals [4, 188] or in vitro [189] by isoflavones. The study from Strom et al. [190] showed that early isoflavone exposure via soy-based infant formula led to an endocrine impact with ulterior consequence on menstrual cycle impairment. It should be noted that cycle impairments were noticed in the rats of the NTP study [4] that examined the effects of GEN on the reproductive physiology of rats. However, the subjects enrolled in [190] were too young to allow a fertility impact of early isoflavone exposure to be shown. Noticeably, the sperm quality and production of men exposed to early soy were not assessed either. The number of stillborns in the group of women fed with soy (3 out of 74 births) was not significantly higher than the number of stillborns in the cow milk-fed group (0 out of 149 births), because only a small number of women had given birth at the time of the study. In fact Gilchrist and co-workers [191] discovered that 4-month-old boys fed with soy-based infant formula (S-BIF) from 3 weeks of age had a lower testis volume than that of their counterparts fed with breast milk ($p < 0.03$). Their testes volume was lower but not significantly than that of boys fed with cow's milk. This can be explained by a decrease of LH and testosterone neonatal production due to isoflavone intake. This is sustained by the study of Sharpe et al. on paired twin marmosets fed with either breast milk or soy-based infant formula prepared for human infants [192]. The soy-fed baby marmosets had lower testosterone plasma levels than their twin brother fed with cow's milk formula. In marmosets the exposure was lower than in boys exclusively fed with S-BIF, and no differences were seen in testes histology when the monkeys reached adulthood [193]. Adgent and co-workers reported a masculinization of the play and toy preferences in girls fed with S-BIF from 3 weeks to 6 months of age [194]. The observation was done at 42 months of age and tended to vanish at 57 months apparently due to social pressure. This observation can be linked to the masculinization effects of estrogens on the sexually dimorphic area of the hypothalamus during neonatal exposure (see [11] for further details). In addition and still according to the same team, a subtle acceleration of menarche could be provoked by an early exposure to estrogenic isoflavones via S-BIF [195]. These results although not fully convincing could also be linked to data published by Kim and co-workers [196] associating high serum concentrations of DAID ($P = 0.0202$), GEN ($P = 0.0021$), and total isoflavones ($P = 0.0009$) with central precocious puberty in young South Korean girls. As already mentioned, South Korea is in 2017 the country with the world's lowest total fertility rate [197]. More recently, it was shown that S-BIF can epigenetically modify the promoter of the PRR5L exon 1 gene [198]. The role of this gene is not yet fully understood, but it seems to regulate cell migration via an interaction with mTORC2 and PKC-delta. The study essentially points out that early exposure to isoflavones can have noticeable consequences later in life. The reason why reproductive deleterious effects linked to early isoflavones intake were

not yet observed at population level is essentially because the appropriate studies have not so far been performed [68]. A longitudinal study, which could be the best approach, is unlikely to show significant effects, if it does not involve a huge number of subjects, especially during childhood reproductive dormancy [199]. In [190] Strom et al. were focused on too early a stage, before both adult men and women really want to have children, and the study was unable, therefore, to help in determining whether early soy feeding could induce a fertility problem. One consequence is that, even if no alarming effects have been reported so far, it is not because they do not exist but rather because they may be expressed long after the initial causal exposure. Accordingly, the effect of this initial exposure may be masked by other psychological, social, economic, or even chemical disrupting factors, inducing large interindividual variability. If a reproductive effect really exists, it should be seen in those populations which are largely exposed to isoflavones through industrial modern soy food and not in those consuming traditional soy. Because it is now recognized that there can be interactions with other anthropogenic endocrine disruptors [2] like bisphenol A or glyphosate [200], the effects of isoflavone exposure can be potentiated by other environmental chemicals. Careful monitoring, which would allow these effects to be separated, should be undertaken in order to discover what is really happening. The expected effects could induce lower sperm production in men and an increased incidence of abnormal menstrual cycle, of early or late abortion, and of larger uterine fibroids in women exposed during their infancy [190, 201]. According to [198], a higher risk of uterine cancer may be observed. If the percentage of infants fed with soy-based infant formula in a given population was monitored for many years, then it could be possible to check whether that percentage is maintained or increased in male adults consulting for fertility problems.

Physiological effects of actual exposure: Nutritional doses of modern soy isoflavones (45 mg/day) have estrogenic effects in women, which can lead to a modest lengthening of the menstrual cycle [57, 77]. Noteworthy, when the diet is fully monitored to avoid the presence of hidden isoflavones, the menstrual cycle of American premenopausal women can be significantly lengthened by 2 days [57]. This lengthening is due to a retardation of the LH surge at mid-cycle. The same consequence is also observed in ewes eating clover rich in isoflavones [142]. This phenomenon was also observed in Japanese women. In that case, the mean menstrual cycle length is 30 days as opposed to 28 days in the West [77], with the mean modern isoflavone intake in Japan and Korea currently being evaluated at between 45 and 60 mg [58]. In this context, Nagata and co-workers [77] showed that supplementing the current Japanese diet with 50 mg of extra isoflavones from soy juice can lead to a menstrual cycle length of 32 days. Although this lengthening may possibly be protective against estrogen-dependent cancers, it can also reduce, however, the opportunity of ovulation in fertile women. This may well contribute to reducing the reproductive efficiency of a given population. In addition, as shown in domestic animals, it can be hypothesized that modern dietary intake of isoflavones can impair embryo implantation and induce early abortion. In women so far, only luteal phase deficiency was associated with isoflavone intake among healthy eumenorrheic women. It is a modest correlation with an adjusted odds ratio (aOR) of 1.38 (95%

CI: 0.99, 1.92), $P = 0.06$, because the subpopulation studied was small. Because luteal phase deficiency can lead to miscarriages and impaired fertility, it may be responsible for a progressive demographic regression at the population level. Moreover, according to [202], high soy intake for long durations can have even more significant effects. In this study, the authors reported that high soy intake was responsible for abnormal bleeding in women under norethisterone (contraceptive pills). This means that their large soy intake was able to transform a contraceptive treatment based on progesterone into an estro-progesterone form of contraception. A similar case involving a 32-year-old woman who drank a liter of soy juice, 3 times a week, after her basketball training, was also observed by our team. Her cycle impairment regressed when soy intake was stopped. In [202], the authors also alerted against uterine fibroids and repeated endometriosis features in the three cases they followed. They reported that all the problems regressed when soy intake was stopped.

Vaginal and endometrial health: Both the vaginal mucosa and endometrium develop under estradiol stimulation and therefore bear all the known estradiol receptors: ERα, ERβ, GPER, as well as ERRs [203–205]. The estrogenic effects of isoflavones were first shown on the uterotrophic test and even before on the uterus maturation of the New Zealand ewes on clover pasture. Therefore, negating an effect of estrogenic isoflavones on the endometrium makes no sense. However, in animals, isoflavones effects were reported either in reproductive females or in female freshly ovariectomized. In all cases, this means that estradiol receptors were available to mediate an estrogenic effect of isoflavones. In humans, most of the studies were performed in menopausal women. Most often their distance to menopause was largely variable, and the availability of ERs in the vagina and endometrium, although known to decrease after the arrest of ovarian function [205, 206], was not checked. Therefore, no study showed a significant effect on the endometrium especially in Western postmenopausal women [207], and very few studies were able to show an effect on the vagina in postmenopausal women. Among them is that of Lima and co-workers [208] which showed a significant estrogenic effect of a vaginal gel containing isoflavones on vaginal dryness, on dyspareunia, and on maturing index of vaginal cells. The last endpoint evolves similarly in the isoflavone group and in the conjugated equine estrogen group [208]. Interestingly, a study performed in Japanese students by Watanabe and co-workers [209] dealt with the supplementation on normal Japanese diet with either 20 or 40 mg isoflavones. Considering the mean urinary levels at baseline, the basal isoflavone exposure was most probably close to 25 mg/day. In their study, Watanabe found that extra isoflavone lengthen the menstrual cycle by 2 days and lengthen menstruations and bleedings in a dose-dependent manner. They observed that not all students react equally to the treatment. On the blood samples collected from three volunteers, they did not show any significant modification of steroid or gonadotrophin hormones. This suggests a direct effect of isoflavones on the endometrial mucosa. To conclude, isoflavone effects on the vagina and uterus of premenopausal women must not be rejected although they require additional work for evidence. In postmenopausal women, a vaginal effect of isoflavones may be achieved, while an endometrial effect has never been clearly shown despite a large number on trials.

Coumestrol

Although there is a great deal of studies reporting the effect of COUM on rodent genital tract, there is little data in women. Only in epidemiology studies COUM is sometimes investigated. Each time the level of intake is usually low (a few μg per day) and can be omitted in front of other phytoestrogens considering its poor availability and its large distribution in human fluid. As an example, in the study by Xia [80], showing a deleterious effect of phytoestrogens in Chinese men presenting idiopathic infertility, the 19th percentile exhibiting the largest impairment presented 619.36, 408.86, and 504.90 μg/g of excreted DAID, equol, and GEN, respectively, while they excreted 4.04 μg/g of COUM. The total excreted isoflavones was then 1533.12 μg/g. This is more than 350 times higher than the dose of COUM.

Lignans

Because not all humans can produce the estrogenic metabolites of dietary lignans, only studies based on either blood or urine measurements can be taken into account while considering the effects of enterolignans on reproduction. Hence, urinary levels reflect an elimination process that reduces the blood bioavailability. Therefore, whenever possible plasma or serum levels should be preferred. Considering these restrictions, the study by Tang and co-workers [210] indicated that when sorted in four quartiles (i.e., 16.54, 85.53, 325.91, 891.7 μg/g of urine) in pregnant women at delivery, birth outcomes were significantly modified. The higher the enterolignans' urinary levels, the lower the gestation length. There is also a tendency toward lower birth weight with END. The decrease in gestation length can be explained by a precocious induction of oxytocin receptor production increasing the action of oxytocin at the time of labor delivery. This has been shown for estrogens, not yet for enterolignans [211]. The smaller birth weight can be linked to a vasoconstriction of placental vessels due to estrogens which are not harvested by the alpha-fetoprotein because their affinity for this protein is lower than that for the estradiol receptor [212]. In addition, it was also shown in American men that urinary END and ENL were associated with subtle deterioration of spermatozoa quality parameters in a dose–response manner [5]. However, it should be kept in mind that lignans are biomarkers associated with grain, vegetable, and fruit consumption. All these items can be contaminated by plant protection products, and this effect may reflect at least a synergistic effect between several endocrine disruptors. Contrarily to isoflavones, enterolignans are associated with better reproductive incomes in humans [213]. Likewise, Mumford and co-workers showed an association of urinary ENL and shorter time to pregnancy. This may be due to the specific effect of enterolignans on the ERα [45].

Zearalenone

There is little convincing information about the effects of ZEN in humans because such an effect could only be recorded on long-lasting exposure if an effect has to be correlated with a serum or urine level. Nevertheless, ZEN was pointed out in the occurrence of precocious puberty, in Italy [214] and Puerto Rico [215], although levels were not actually assessed in any biological fluid. In Hungary [216], an

increased incidence of early thelarche was reported in patients with serum ZEN levels of 18.9–103 μg/mL. In 2010 it was published that in Viareggio (Tuscany), the incidence of precocious puberty was 22 to 29 times higher than in the neighboring areas [217]. Among 63 cases, 6 of them had high serum ZEN (933.7 ± 200.3 pg/mL) and α-ZOL (106.5 ± 1.9 pg/mL). But only 5 of 36 patients with early thelarche presented detectable levels of mycoestrogens. In 2003, ZEN was associated with "endemic enlargement disease" in China [218]. All grains analyzed were contaminated by mold, and 34% exhibited *Fusarium* species and COUM. More recently it was found that mycoestrogens were detectable in a large proportion of the urine of the 163 girls, aged 9 and 10 years, participating in the Jersey Girl Study and enrolled in the survey by Bandera et al. [219]. The subjects presenting the highest values were also being significantly of shorter stature and less likely to have reached the onset of breast development. This may sign an estrogenic effect on the pituitary gland with resulting anti-FSH effect. Such effects were reported in rodents [220] and more recently in pigs [221].

6 Questions

6.1 Estrogen-Dependent Cancers

6.1.1 Isoflavones

There has been a long-lasting controversy about the positive or negative effect of isoflavones on breast, endometrial, or prostate cancers all considered being more or less estrogen-dependent. Their incidence is also lower in Asia.

Endometrium

As far as endometrial cancers are concerned, in 2015, the EFSA opinion [222] indicated that no definitive opinion could be given on any deleterious effects of isoflavones on uterine cancer. However, the study only concerns healthy peri- and postmenopausal women, because it was based on the scientific literature in which volunteers are recruited following specific inclusion criteria, and such criteria do not, therefore, exactly reflect the global population. No case studies were performed to see an effect on endometrial cancer, and therefore neither the quality of the population nor its number was designed to demonstrate an effect. According to [222], there are very few studies triggering specifically perimenopausal women, and the number of studies was insufficient to conclude on this specific population. No conclusion could be rigorously drawn out on perimenopausal women because they cannot be assimilated to postmenopausal women. Noteworthy, their production of endogenous estrogens was still active, and estradiol receptors were still present at the target tissues. On the opposite, there is a progressive decrease in endometrium estradiol-receptor bioavailability in postmenopausal women [206]. Therefore, a lack of action of isoflavones in the uterus of these women is expected, because the estrogenic effects of isoflavones require an interaction with the estradiol receptors. On top of other things, this tissue bears both alpha and beta subtypes of ERs [206], and

isoflavones affinity is greater for the beta subtype [223, 224]. As the activation of the ER-beta was shown to negate the proliferative action of the ERα [225], via its AF-1 function [226], normal soy intake is not likely to affect the rate of endometrial cancer of healthy postmenopausal women.

Prostate

For prostate cancer, there is also a controversy. If the incidence of prostate cancer is lower in Asian population, the postmortem tumor detection indicates that the frequency of prostate cancer is equivalent between Asia and the West [227]. To add to the controversy, Nakamura and co-workers [228] recently developed a patient-derived prostate cancer xenograft model. They grafted clinical prostatectomy samples into nude athymic mice fed with 0 or 2 or 10 mg/kg genistein. For these doses, they showed dose-dependent increases in lymph nodes and secondary organ metastases (liver, lungs). They also showed the aggregation of invasive malignant cells in the secondary organs of the genistein-treated groups and not in the control. Their data suggest that the effect of genistein may depend on the kind of prostate tumor considered. A recent meta-analysis [229], performed on 21 case-control and 2 cohort studies, found that GEN and DAID may, in some cases, be associated with decreased risk of prostate cancer. However, when studies were sorted out according to the way isoflavone intakes were assessed, it appeared that the risk was not reduced when isoflavones were assayed in the serum or urine, although it was reduced when GEN and DAID exposure was assessed via food questionnaires. Because this last method is less accurate than the assay in biological fluid, the validity of these results remains questionable.

Breast

Although things are still unclear for endometrial or prostate cancers, there are now enough data to conclude that both positive and negative effects can occur considering isoflavone effects on estrogen-dependent breast cancer. Sufficient data now exist to consider that isoflavone through soy intake may be preventive against the occurrence of breast cancer [230–232] playing its role during the initial phase of cancer development [1]. However, there are also many data indicating that estrogenic isoflavones exert a proliferative effect on breast estrogen-dependent cancer cell lines. These data now exist in vitro [233], in vivo in animals [234], and in women [235].

The mechanism by which cancer prevention occurs is not yet clear and may be related to different mechanisms including early cell differentiation [236, 237], oncosuppressor expression [238], or pro-oncogene depletion [239]. Unfortunately, not all the protective mechanisms were so far recorded in vivo, using plausible dietary doses. Nevertheless, this preventive effect of isoflavones is in accordance with the demonstrations made by Lamartiniere on rats exposed to dimethylbenz(a)anthracene (DMBA) in which the preventive effect of GEN appears during the initial phase [240]. This protective effect is also sustained by the study of the National Toxicology Program [241] examining the carcinogenic effect of GEN in rats. Hence, they found that females from the first generation exposed to 5 and 100 ppm of GEN from

conception exhibited lower incidences of combined mammary adenoma and adenocarcinoma than the control or than the 500 ppm group. The differences were not statistically significant, but they may support the idea that a low exposure from conception would protect against mammary tumor incidence. Of course, reducing the occurrence of cancer cells via inhibiting their occurrence by any pathway during the initial step would result in a decreased incidence of cancer at the population level.

However, most established breast cancers rely on the activation of the alpha estradiol receptor subtype cross talking with the IGF receptor [242, 243]. This means that 17β-E_2 and other estrogens act as growth factors of the tumors [40, 244, 245]. At least three studies showed an estrogenic effect of soy isoflavones at modern dietary doses (45–60 mg/day) on the mammary gland of premenopausal women [74–76]. The three studies were undertaken for short durations and showed a positive impact of the isoflavone dietary intake on estrogenic biomarkers (pS2, nipple aspirate apolipoprotein D, progesterone receptor). In premenopausal women, an interaction of isoflavones with endogenous 17β-E_2 could not be excluded. Therefore, this effect cannot be extrapolated to postmenopausal women. This does not, however, negate the estrogenic effect of 45–60 mg of isoflavones per day in premenopausal women. Such active doses in premenopausal women are sustained by other physiological data concerning the menstrual cycle length duration, as explained before. Unfortunately, when they can exert their estrogenic effect, isoflavones increase cell proliferation speed **on already established** estrogen-dependent cancer cells [233]. This was also shown in vivo in implanted nude mice [234]. This is also sustained by the study of Shike et al. [235] in women with a declared breast cancer. Examining the proliferative effect of soy isoflavones in women diagnosed with breast cancer is both very difficult and ethically disputable, which would explain why only one study [235] has been published so far. The study is not fully conclusive because of a short treatment duration (between 7 and 30 days) and a low compliance with the treatment. Therefore, the biomarkers followed probably reflect a large interindividual response to a large interindividual variation in exposure. Therefore, in [235], the authors relied on isoflavone plasma levels to sort the treated subjects and then went on to perform genomic analysis on proliferation markers. The results they obtained in vivo with plasma forms (i.e., conjugated forms) of isoflavones are consistent with the in vitro data obtained by many other authors, who showed an estrogenic effect of isoflavones on breast cancer cells (i.e., proliferative effect) when used in the μM range (0.2 to 5) [246]. Shike's study indicates that both in vivo and in humans the circulating forms of isoflavones can, as already shown, have estrogenic effects [74–76]. Hence, at modern dietary doses, they can act as growth factors for estrogen-dependent tumors. Although the studies in nude mice implanted with human breast cancer cells cannot help in determining an active dose, the NTP study [241], which showed a significant increase in adenocarcinomas in the mammary and pituitary gland of rats exposed to 500 ppm GEN from conception to sampling, is particularly important. This classical toxicology study was designed to define the mean tolerable daily intake in humans. In that case, the LOAEL is 100 ppm (5 mg/kg/day for rats), and the NOAEL is 5 ppm in the diet (1.2 mg/kg/day for rats).

Finally, some breast cancer cells can be triple negative for ER, PR, and Her2/neu. They are considered to be resistant to hormone therapy based on antiestrogens or on anti-aromatase and therefore to have particularly negative prognosis [247]. It was shown in some occasions that, in the absence of ER, breast cancer cells such as MDA-MB-231 did not proliferate under isoflavone treatments [248]. This triple-negative cell line only bears trace amounts of GPER [249]. However, some triple-negative cancer cell lines such as HCC1806 were shown to have a large proportion of GPER in their membranes [249]. Because of that although they were first considered as estrogen independent, they can proliferate under estrogen stimulation [250]. GPER can mediate rapid 17β-E_2-induced non-genomic signaling pathways. In addition, by the effect of transactivation of epidermal growth factor (EGF) receptors, GPER induces mobilization of intracellular calcium (Ca^{2+}) stores and activation of mitogen-activated protein kinase and PI3K signaling pathways [247]. Furthermore, when present in triple-negative cancer cells, GPER seems to be with poor clinical outcome. It has been shown to upregulate cyclins and BCL-2 genes favoring cell proliferation and survival. Therefore, GPER possibly plays an important role in the carcinogenesis process. The GPER-induced proliferation can also be blocked in vivo by inactivating GPER [251] with siRNA, using GPER antagonist like G-15 [252] or even better estriol [251], opening a new way for breast cancer therapy. In Egypt, where breast cancers represent 17.5% of all malignant tumors, it was observed that these mammary cancers were more aggressive than that encountered in the West [247]. In this country, it was shown by immunostaining that 65% of archival formalin-fixed paraffin-embedded cases of invasive ductal carcinoma were GPER positive. GPER as a membrane receptor of the rhodopsin-like family is activated by low doses of many xenoestrogens including bisphenol A (BPA) [253]. As mentioned earlier, GEN and DAID are ligands of GPER with affinity 2 to 3 times higher than that of BPA. The IC_{50} for GEN is 133 nM, which is only ten times higher than that of estradiol [253], and GPER pathway activation is achieved using 0.2 μM. This dose can be obtained in human blood with dietary soy intake. Equally, DAID is able to induce GPER pathways at doses as low as 0.1 μM [254]. Therefore, the effects of phytoestrogens on breast cancer should take into account the GPER pathway as another target for proliferation induction. Lastly, data on the effect of isoflavones on breast cancer cell lines through the estrogen-related receptors (ERR) α, β, or γ are presently too scattered to conclude on a clinical effect. When tested, the doses of isoflavones required for the ERR pathway activation do not fit with a dietary isoflavone intake [255].

6.1.2 Coumestrol

Because this substance is only anecdotally present in human food at least in Western developed countries, its effects on estrogen-dependent cancers are only scarcely examined. There is no interventional study that is strictly controlled, and there are a few observational studies mentioning COUM as a parameter which have been taken into account because it was above the assay detection limit in biological samples. In 2006, Hedelin and co-workers showed that COUM together with a precise Snp present in the promoter of the ERβ subtype was correlated with a strong

significance to a decreased risk of prostate cancer [256]. However, this correlation should be taken with caution since the COUM urine levels measured are close to the detection limit.

6.1.3 Lignans

Two major problems linked to the exposure evaluation of the estrogenic enterolignans can explain the paucity of clear data gathered on enterolignans and estrogen-dependent diseases. As already mentioned, not all sources of lignans are yet characterized in food [257], and secondly not all human being can efficiently transform lignan precursors into estrogenic enterolignans [258].

Prostate

It is in the late 1980s that lignans were correlated to reduced risk of prostate cancer [259]. Despite this early hope, in 2010, Saarinen and co-workers made a point on population and intervention studies dealing with enterolignans and prostate cancer and found no clear correlations at the population level [260]. In population studies even when ENL was followed in plasma and/or urine, a clear association with prostate cancer incidence, progression, or risk could be pointed out. However, three studies dealing with the administration of flaxseed in men with a diagnosed prostate cancer did show a decreased serum total PSA and proliferation rate of benign epithelium and a significant decrease in total testosterone and free androgen indices. Among men with Gleason score below 6, they reported decreased tumor proliferation index and increased tumor apoptotic scores when comparing the flaxseed group to historic controls. Finally, they also mentioned a significantly reduced tumor proliferation rates with flaxseed supplemented diets. Their conclusion was to say that enterolignans may have a protective effect on prostate cancer but that the normal dietary intake is currently insufficient to induce such protective effect. More recently the study by Azrad and co-workers [261] came to confirm that prostate cancer preoperating treatment with flaxseed supplement was associated with the decrease of several tumor biomarkers. Namely, there was a significant decrease in NFκB, Ki67, and VEGF. The authors conclude that flaxseed supplementation inhibits cancer cell growth and possibly reduces tumor angiogenesis in patients with prostate cancer.

Breast

Enterolignans have a greater affinity for the ERα receptor; however, ENL which is currently the most concentrated in human plasma was shown to better recruit the AF-2 transactivation factors and therefore to reduce the proliferative effects of compounds activating gene transcription through the AF-1 domain [45]. This in vitro mechanism was further confirmed in vivo on nude mice transplanted with MFC-7 cells [262]. In this study, ENL and END were tested alone and together with GEN. They were able to prevent the proliferation induced by GEN. Foods containing the precursors of enterolignans were also tested in the nude mice model [263]. The study showed that secoisolariciresinol diglucoside (SDG), the most

classical precursor of enterolignans in the scientific literature, was less efficient than sesamin in protecting against breast tumor proliferation. Sesamin was converted in vivo by the nude mice in unknown compound proportion that was able to increase significantly apoptosis of the cells. If the mice metabolism is involved in this effect, which is most plausible, then the result cannot be translated directly to human. Nevertheless, in some cases, studies showed that a higher intake of lignans was associated with lower risk of developing cancer. In several occasions, the receptor status of cancers was mentioned [264, 265]. The discrepancy between the studies can be explained by at least two factors: (1) not all women can produce enterolignans from their lignan precursors, and if no plasma or urine measurements are available, this induces confusion; (2) not all the sources of lignans are yet characterized, and this reduces the power of the analyses. To illustrate the first point, the study by Buck and co-workers [265] showed a better reduction in the breast cancer risk when data were collected based on an ENL measurement rather than on a lignan dietary intake. When the lignan intake was taken globally, the ER-negative tumors seemed to be more susceptible to a protective effect. Therefore, and because lignan intake was not systematically associated with estrogenic enterolignan production, the action may not be an estrogenic or antiestrogenic effect. Regarding an effect of enterolignans through the GPER pathway, there are too few such data to hypothesize any effect. However, several epidemiological studies gathered in the meta-analysis [266] showed overall that a decreased risk of death by breast cancer could be associated with serum ENL. This study performed on 2,182 patients divided the population into 4 quartiles based on their serum ENL levels. In each case, the highest quartile corresponding to serum levels over 70 nM was associated with lower risks. The endpoints which were monitored were all-cause mortality, breast cancer-specific mortality, distant disease-free survival, and risk of recurrence [266]. In this study and thanks to a large number of subjects, it was possible to see that enterolignans could have a positive effect per se, distinctive of a healthy lifestyle pattern. Finally, as for isoflavone, a preventive effect at the initial phase of breast cancer progression is plausible since it was shown that ENL at a dose of 10 mg/kg/day was able to decrease the occurrence and size of chemo-induced mammary tumors using DMBA in ovariectomized rat [267]. Because DMBA is able to transform a healthy cell in cancer cells, this effect of ENL triggers the initial phase of mammary cancer progression.

6.2 Other Side Effects

6.2.1 Thyroid

Links were made between soy consumption and alterations of fragile thyroid function. In the 1960s when the first soy-based infant formulas were commercialized, hypothyroidism goiters were observed [268] in infants and led to iodine supplementation in these formulas [269]. However, nowadays hypothyroid infants can still develop a goiter under soy-based infant formula, and their thyroid function is still difficult to manage under these soy-based formulas [270–272]. Generally speaking,

until now, whenever isoflavone effects were reported on hypothyroidism, it was always in persons with preexisting thyroid impairments [271, 273–275]. In parallel to these data is the case of a woman who was treated with Levothyrox for hypothyroidism. It showed that the intake of soy-based food supplements interacts with the drug absorption [274]. As a result, the doses required to balance her thyroid function had to be increased when soy-based food supplement was taken simultaneously with Levothyrox treatment. Consequently, in Europe, these medications are generally advised avoiding soy consumption. The mechanism of action is not fully characterized, but GEN was found to bind to thyroid hormone receptors [276] and to compete with triiodothyronine (T_3) on its receptor at concentrations of 1 μM which are dietary relevant. In addition, in the presence of iodide ions, genistein and daidzein blocked TPO-catalyzed tyrosine iodination by acting as alternate substrates in vivo [277]. This mechanism would explain the greater need of iodine for correct thyroid function in the presence of soy. Many other studies, however, showed no adverse effects from soy or isoflavones on thyroid function of healthy subjects [278–282]. In addition, in Japan, isoflavones are consumed at the highest rate on earth; until recently [283] no adverse effects were consistently reported. The exception [283] is the case of a 72-year-old woman with preexisting lymphocytic thyroiditis who was admitted to the hospital with acute hypothyroidism. It was due to the consumption of a health drink containing soy. Symptoms progressively disappeared after soy-based health drink arrest. However, the traditional Japanese diet is based on a large variety of seafoods which are rich in iodine. They may usually compensate for the effect of soy and/or isoflavones on an alteration of the thyroid function. Finally, for many years, it was unclear if any soy component or specifically isoflavones were responsible for the hypothyroid effect of soy. The study by Sathyapalan and co-workers [284] finally answered the question. When isoflavones were removed or added to soy, and fed to volunteers with preexisting mild hypothyroidism, the isoflavone supplementation clearly aggravated the hypothyroid symptoms. Taking all these data together, it seems that the adverse effects of soy isoflavones may occur only in persons with previous hypothyroidism. In that case, a balance in the thyroid function is more difficult to achieve medically.

6.2.2 Autoimmune Diseases

Autoimmune diseases occur when the immune system attacks and destroys the organs and tissues of its own host. Several autoimmune diseases occur with a higher frequency in women than in men [285]. Although links with several factors expressed by the X chromosomes have been related to the occurrence of autoimmune diseases [286], the role played by female sex steroids is currently under investigation. Hence, the incidence of autoimmune diseases is generally higher in premenopausal women than in children (girls) and decreases after menopause [287]. At the cell level, 17β-E_2 was shown to induce the expression of many different factors playing a role in the activation of autoantibodies. As an example, 17β-E_2 at physiological doses increases lymphocyte B and plasma dendritic cell differentiations in vitro [288, 289]. This process is inhibited by the pure antiestrogens ICI

182 780 or by tamoxifen. Plasma dendritic cell activation and IFNα production are enhance in vitro and in vivo by 17β-E_2 and reduced when the ERα receptor is reduced or blocked [289]. Autoimmune processes seem to be reduced during pregnancy, while progesterone is present in the body at high concentrations [290]. Testosterone also seems to exert a preventive effect [291]. For systemic lupus erythematosus (SLE), the sex ratio distortion is one of the highest with currently nine women and one man being hit by the disease in France [292]. In Japan, the ratio seems to be more in favor to men with eight women and two men being sick [287]. In addition, the disease evolves by flares which occurrence is still difficult to prevent because of unknown causes. Among other causes, environmental factors have been suggested to be involved [293, 294]. Dietary phytoestrogens, because of their estrogenic potencies and their mostly unknown sources of exposure in modern diet, are good candidates for further studies. In a transgenic mouse model of SLE, the MRL/Mp-lpr/lpr mouse GEN and DAID at nutritional doses were shown to aggravate the SLE-induced nephropathy and to induce earlier mortality when compared to a diet devoid of soy [295]. Noteworthy, at pharmacological doses, isoflavones seem to have the opposite effect [296]. Fort and co-workers [297] examined the incidence of autoimmune diseases in children fed with soy-based infant formula compared to their counterpart fed with other formulas in their infancy. They found out that the frequency of feedings with soy-based milk formulas in early life was significantly higher in children with autoimmune thyroid diseases (prevalence 31%) as compared with their siblings (prevalence 12% $p < 0.01$) and healthy nonrelated control children (prevalence 13%, $p < 0.02$). More recently working in rodent, Tran and co-workers [298] showed that GEN, DAID, or glycitein and a soy extract suppressed iodine uptake and stimulated the production of autoimmunogen in rat thyrocytes in vitro. However, cells were from rat, and the efficient doses were higher than 1 μM. Therefore, the transposition to the clinical situation should be taken with caution. Finally, Portman and co-workers [299] recently showed an association between soy and the incidence of Kawasaki disease (KD) in children. A significant increased KD risk was observed in children for total isoflavone intake (OR, 2.33; CI 95%, 1.37–3.96) and for genistein intake (OR, 2.46; CI 95%, 1.46–4.16), when comparing high-soy consumers vs. non-consumers. KD risk was also significantly increased in Asian-American children with the highest isoflavone consumption. Hence, total isoflavones were highly significantly correlated to an increased risk (OR, 7.29; CI 95%, 1.73–30.75), and this was also the case for genistein (OR, 8.33; CI 95%, 1.92–36.24) when compared to white children. The authors concluded that childhood dietary isoflavone consumption, but not maternal isoflavone intake during pregnancy and nursing, strongly relates to KD risk in an ethnically diverse US population. To conclude, the association of autoimmune disease with estrogens is clear, and progesterone and testosterone are most probably protective. Phytoestrogens being estrogenic, antiandrogenic [300], and able to reduce progesterone levels at dietary doses [301] can be good environmental candidate to solve the reason of unexplained autoimmune disease flares. However, additional studies are needed to ascertain this effect and therefore to go toward disease prevention.

7 Conclusion

Natural potent estrogens are ubiquitous in our environment. Resorcylic acid lactones and COUM, because of their strong known effects on rodent reproductive physiology, have been adequately monitored so far. Hence, the human exposure, except in specific circumstances, is not a cause of concern. Lignans, although leading to the eventual production of estrogenic compounds by the gut bacteria, are far less studied, and the exposure to the weak estrogen enterolignans is far from being correctly assessed. A large interindividual response is expected from the exposure to dietary lignans. However, as far as we can approach them with our current tools, these compounds act as SERMs, and their effects appear to be more beneficial than adverse. Their effects on reproduction appear to be positive, and they were shown to reduce the proliferation on estrogen-dependent breast cancer cell lines. Their exposure being associated with grain, fruit, and vegetable intakes does not seem to suppress the benefit of a diet rich in these foodstuffs. Inducing more concerns is the case of isoflavones. Their estrogenic effects in vitro range between ENL on one hand and COUM and ZEN on the other hand. Their bioavailability is the highest of all compounds listed here. Many effects, them being beneficial or having adverse effects, have been reported so far for these compounds. However, based on the assumption that they had always been part and parcel of the human diet, they were, until now, essentially considered as inactive. This discrepancy vanishes if it is admitted that the isoflavone exposure is modern and essentially due to changes in the cooking process of soy. Then the compounds can be considered for what they are, i.e., estrogenic compounds, possibly exerting endocrine-disrupting effects in synergy with other anthropoid compounds recently liberated in the environment. The actual data tend to indicate that their most pronounced deleterious effect is observed on reproduction. Their deleterious effects on already established estrogen-dependent cancers seem to occur at higher doses based on toxicology studies. These effects are counterbalanced by a preventive effect probably due to a favorable interaction at the initial step of breast cancer progression. More mechanistic data are required to ascertain this mechanism. Therefore, although more research is still required to take the best part of these compounds, the question of their endocrine-disrupting effect should be taken into consideration. Because the history shows that isoflavone can be reduced easily in food, this should be done for both human and domestic animals to reduce the exposure to endocrine disruptors and improve farmers' incomes.

References

1. Fernandez-Lopez A, Lamothe V, Delample M, Denayrolles M, Bennetau-Pelissero C (2016) Removing isoflavones from modern soyfood: why and how? Food Chem 210:286–294
2. Hicks KD, Sullivan AW, Cao J, Sluzas E, Rebuli M, Patisaul HB (2016) Interaction of bisphenol A (BPA) and soy phytoestrogens on sexually dimorphic sociosexual behaviors in male and female rats. Horm Behav 84:121–126

3. Bennetau-Pelissero C (2017) Positive or negative effects of isoflavones: toward the end of a controversy: response to the letter from Dr Messina and Dr Badger following the publication of the paper by Fernandez-Lopez A, Lamothe V, Delample M, Denayrolles M and Bennetau-Pelissero C. entitled: removing isoflavones from modern soyfood: why and how? Food Chem 225:293–301
4. National Toxicology Program (2008) Multigenerational reproductive study of genistein (Cas No. 446-72-0) in Sprague-Dawley rats (feed study). Natl Toxicol Program Tech Rep Ser 539:1–266
5. Mumford SL, Kim S, Chen Z (2015) Urinary phytoestrogens are associated with subtle indicators of semen quality among male partners of couples desiring pregnancy. J Nutr 145:2535–2541
6. McClain MR, Wolz E, Davidovich A, Pfannkuch F, Edwards JA, Bausch J (2006) Acute, subchronic and chronic safety studies with genistein in rats. Food Chem Toxicol 44 (1):56–80
7. European Centre for Ecotoxicology and Toxicology of Chemicals (2002) Guidance on evaluation of reproductive toxicity data. Monograph no. 31. Brussels. ISSN-0773-6347-31
8. Carreau S, Bouraima-Lelong H, Delalande C (2011) Estrogens: new players in spermatogenesis. Reprod Biol 11(3):174–193
9. Rochira V, Granata AR, Madeo B, Zirilli L, Rossi G, Carani C (2005) Estrogens in males: what have we learned in the last 10 years? Asian J Androl 7:3–20
10. Katzenellenbogen JA, Katzenellebogen BS, Tatee T, Robertson DW, Landvatter SW (1980) The chemistry of estrogens and antiestrogens: relationships between structure, receptor binding and biological activity. In: McLachlan JA (ed) Estrogens in the environment. Elsevier Science, New York, p 33
11. Bennetau-Pelissero C (2013) Chapitre 77. Isoflavonoids and phytoestrogenic activity. In: Natural products 2013 phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes. Springer, Berlin/Heidelberg, pp 2381–2432. https://doi.org/10.1007/978-3-642-22144-6_80
12. Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Ström A, Treuter E, Warner M, Gustafsson JA (2007) Estrogen receptors: how do they signal and what are their targets. Physiol Rev 87(3):905–931
13. Bhavnani BR, Tam SP, Lu X (2008) Structure activity relationships and differential interactions and functional activity of various equine estrogens mediated via estrogen receptors (ERs) ERalpha and ERbeta. Endocrinology 149(10):4857–4870
14. Meitzen J, Luoma JI, Boulware MI, Hedges VL, Peterson BM, Tuomela K, Britson KA, Mermelstein PG (2013) Palmitoylation of estrogen receptors is essential for neuronal membrane signaling. Endocrinology 154(11):4293–4304
15. Adlanmerini M, Solinhac R, Abot A, Fabre A, Raymond-Letron I, Guihot AL, Boudou F, Sautier L, Vessières E, Kim SH, Lière P, Fontaine C, Krust A, Chambon P, Katzenellenbogen JA, Gourdy P, Shaul PW, Henrion D, Arnal JF, Lenfant F (2014) Mutation of the palmitoylation site of estrogen receptor α in vivo reveals tissue-specific roles for membrane versus nuclear actions. Proc Natl Acad Sci USA 111(2):E283–E290
16. Morrill GA, Kostellow AB, Gupta RK (2015) Transmembrane helices in “classical” nuclear reproductive steroid receptors: a perspective. Nucl Recept Signal 13:e003
17. Petrie WK, Dennis MK, Hu C, Dai D, Arterburn JB, Smith HO, Hathaway HJ, Prossnitz ER (2013) G protein-coupled estrogen receptor-selective ligands modulate endometrial tumor growth. Obstet Gynecol Int 2013:472720
18. Ren GY, Chen CY, Chen WG, Huang Y, Qin LQ, Chen LH (2016) The treatment effects of flaxseed-derived secoisolariciresinol diglycoside and its metabolite enterolactone on benign prostatic hyperplasia involve the G protein-coupled estrogen receptor 1. Appl Physiol Nutr Metab 41(12):1303–1310
19. Prossnitz ER, Arterburn JB, Sklar LA (2007) GPR30: a G protein-coupled receptor for estrogen. Mol Cell Endocrinol 265–266:138–142

20. Suetsugi M, Su L, Karlsberg K, Yuan Y-C, Chen S (2003) Flavone and isoflavone phytoestrogens are agonists of estrogen-related receptors. Mol Cancer Res 1:981–991
21. Boué SM, Burow ME, Wiese TE, Shih BY, Elliott S, Carter-Wientjes CH, McLachlan JA, Bhatnagar D (2011) Estrogenic and antiestrogenic activities of phytoalexins from red kidney bean (*Phaseolus vulgaris* L.) J Agric Food Chem 59(1):112–120
22. Kim HJ, Suh HJ, Kim JH, Kang SC, Park S, Lee CH, Kim JS (2010) Estrogenic activity of glyceollins isolated from soybean elicited with *Aspergillus sojae*. J Med Food 13(2):382–390
23. Lindner HR (1976) Occurrence of anabolic agents in plants and their importance. Qual Saf Suppl 5:151
24. Song T, Barua K, Buseman G, Murphy PA (1998) Rapid simultaneous determination of major isoflavones of *Pueraria lobata* and discriminative analysis of its geographical origins by principal component analysis. Am J Clin Nutr 68:1474S
25. Zhao C, Chan HY, Yuan D, Liang Y, Lau TY, Chau FT (2011) Rapid simultaneous determination of major isoflavones of *Pueraria lobata* and discriminative analysis of its geographical origins by principal component analysis. Phytochem Anal 22(6):503
26. He X, Blount JW, Ge S, Tang Y, Dixon RA (2011) A genomic approach to isoflavone biosynthesis in kudzu (*Pueraria lobata*). Planta 233(4):843
27. Rochfort S, Panozzo J (2007) Phytochemicals for health, the role of pulses. J Agric Food Chem 55(20):7981
28. Keinan-Boker L, Van der Schouw YT, De Kleijn MJJ, Jacques PF, Diederick E, Grobbee DE, Peeters PHM (2002) J Nutr 132:1319
29. Farnsworth NR, Bingel AS, Cordell GA, Crane FA, Fong HS (1975) Potential value of plants as sources of new antifertility agents I. J Pharm Sci 64(4):535–598
30. Farnsworth NR, Bingel AS, Cordell GA, Crane FA, Fong HS (1975) Potential value of plants as sources of new antifertility agents II. J Pharm Sci 64(5):717–754
31. Horn-Ross PL, Barnes S, Lee M, Coward L, Mandel JE, Koo J, John EM, Smith M (2000) Assessing phytoestrogen exposure in epidemiologic studies: development of a database (United States). Cancer Causes Control 11(4):289–298
32. Franke AA, Custer LJ (1994) High-performance liquid chromatographic assay of isoflavonoids and coumestrol from human urine. J Chromatogr B Biomed Appl 662(1):47–60
33. Knuckles BE, deFremery D, Kohler GO (1976) Coumestrol content of fractions obtained during wet processing of alfalfa. J Agric Food Chem 24(6):1177–1180
34. Valsta LM, Kilkkinen A, Mazur W, Nurmi T, Lampi AM, Ovaskainen ML, Korhonen T, Adlercreutz H, Pietinen P (2003) Phyto-oestrogen database of foods and average intake in Finland. Br J Nutr 89(Suppl 1):S31–S38
35. Adlercreutz H, Mazur W (1997) Phyto-oestrogens and western diseases. Ann Med 29:95–120
36. USDA-Iowa State University Isoflavones Database. www.nal.us-da.gov/fnic/foodcomp/Data/isoflav/isoflav.html
37. Fletcher RJ (2003) Food sources of phytoestrogens and their precursors in Europe. Br J Nutr 89(1):S39–S43
38. Pillow PC, Duphorne CM, Chang S, Contois JH, Strom SS, Spitz MR, Hursting SD (1999) Development of a database for assessing dietary phytoestrogen intake. Nutr Cancer 33:3–19
39. Francis CM, Millington AJ (1971) Presence of methylated coumestans in annual *Medicago* species; response to a fungal pathogen. Aust J Agric Res 22:75–80
40. Li Y, Luh CJ, Burns KA, Arao Y, Jiang Z, Teng CT, Tice RR, Korach KS (2013) Endocrine-disrupting chemicals (EDCs): in vitro mechanism of estrogenic activation and differential effects on ER target genes. Environ Health Perspect 121(4):459–466
41. Mallis LM, Sarkahian AB, Harris HA, Zhang MY, McConnell OJ (2003) Determination of rat oral bioavailability of soy-derived phytoestrogens using an automated on-column extraction procedure and electrospray tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 796(1):71–86
42. Bickoff EM, Loper GM, Hanson CH, Graham JH, Witt SC, Spencer RR (1967) Effect of common leafspot on coumestans and flavones in alfalfa. Crop Sci 7(3):259–261

43. Wong E, Latch GCM (1971) Effect of fungal diseases on phenolic contents of white clover. NZ J Agric Res 14(3):633–638
44. http://dietgrail.com/coumestrol/
45. Carreau C, Flouriot G, Bennetau-Pelissero C, Potier M (2008) Enterodiol and enterolactone, two major diet-derived polyphenol metabolites have different impact on ERalpha transcriptional activation in human breast cancer cells. J Steroid Biochem Mol Biol 110(1–2):176–185
46. Moreno-Franco B, García-González Á, Montero-Bravo AM, Iglesias-Gutiérrez E, Úbeda N, Maroto-Núñez L, Adlercreutz H, Peñalvo JL (2011) Dietary alkylresorcinols and lignans in the Spanish diet: development of the alignia database. J Agric Food Chem 59(18):9827–9834
47. Pianjing P, Thiantanawat A, Rangkadilok N, Watcharasit P, Mahidol C, Satayavivad J (2011) Estrogenic activities of sesame lignans and their metabolites on human breast cancer cells. J Agric Food Chem 59(1):212–221
48. Nicolle C, Manach C, Morand C, Mazur W, Adlercreutz H, Rémésy C, Scalbert A (2002) Mammalian lignan formation in rats fed a wheat bran diet. J Agric Food Chem 50(21):6222–6226
49. Peñalvo JL, Adlercreutz H, Uehara M, Ristimaki A, Watanabe S (2008) Lignan content of selected foods from Japan. J Agric Food Chem 56(2):401–409
50. Bhatnagar D, Yu J, Ehrlich KC (2002) Toxins of filamentous fungi. Chem Immunol 81:167–206
51. Stob M, Baldwin RS, Tuite J, Andrews FN, Gillette KG (1962) Isolation of an anabolic, uterotrophic compound from corn infected with *Gibberella zeae*. Nature 196:1318
52. Yazar S, Omurtag GZ (2008) Fumonisins, trichothecenes and zearalenone in cereals. Int J Mol Sci 9:2062–2090
53. Castelo MM, Sumner SS, Bullerman LB (1998) Stability of fumonisins in thermally processed corn products. J Food Prot 161:1030–1033
54. EFSA Panel on Contaminants in the Food Chain (2011) Scientific opinion on the risks for public health related to the presence of zearalenone in food. EFSA J 9(6):2197–2421
55. Kuhnle GG, Dell'Aquila C, Aspinall SM, Runswick SA, Mulligan AA, Bingham SA (2008) Phytoestrogen content of foods of animal origin: dairy products, eggs, meat, fish, and seafood. J Agric Food Chem 56(21):10099–10104
56. Liu Z, Li W, Sun J, Liu C, Zeng Q, Huang J, Yu B, Huo J (2004) Intake of soy foods and soy isoflavones by rural adult women in China. Asia Pac J Clin Nutr 13(2):204–209
57. Cassidy A, Bingham S, Setchell KD (1994) Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr 60(3):333–340
58. Wada K, Tsuji M, Tamura T, Konishi K, Kawachi T, Hori A, Tanabashi S, Matsushita S, Tokimitsu N, Nagata C (2015) Soy isoflavone intake and stomach cancer risk in Japan: from the Takayama study. Int J Cancer 137(4):885–892
59. Surh J, Kim M-J, Koh E, Young-Kyung L, Kim Y-KL, Kwon H (2009) Estimated intakes of isoflavones and coumestrol in Korean population. Int J Food Sci Nutr 57(5–6):325–344
60. Hu XJ, Song WR, Gao LY, Nie SP, Eisenbrand G, Xie MY (2014) Assessment of dietary phytoestrogen intake via plant-derived foods in China. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 31(8):1325–1335
61. Agence française de sécurité sanitaire des aliments (2005) Sécurité et bénéfices des phytoestrogènes apportés par l'alimentation: recommandations. Publisher, AFSSA, 2005. ISBN, 2110954434, 9782110954435, 440 pages
62. Carmichael SL, Gonzalez-Feliciano AG, Ma C, Shaw GM, Cogswell ME (2011) Estimated dietary phytoestrogen intake and major food sources among women during the year before pregnancy. Nutr J 10:105–114
63. Bai W, Wang C, Ren C (2014) Intakes of total and individual flavonoids by US adults. Int J Food Sci Nutr 65(1):9–20
64. Fleck SC, Churchwell MI, Doerge DR, Teeguarden JG (2016) Urine and serum biomonitoring of exposure to environmental estrogens II: soy isoflavones and zearalenone in pregnant women. Food Chem Toxicol 95:19–27

65. Berman T, Goldsmith R, Göen T, Spungen J, Novack L, Levine H, Amitai Y, Shohat T, Grotto I (2013) Urinary concentrations of environmental contaminants and phytoestrogens in adults in Israel. Environ Int 59:478–484
66. Degen GH, Blaszkewicz M, Shi L, Buyken AE, Remer T (2011) Urinary isoflavone phytoestrogens in German children and adolescents–a longitudinal examination in the DONALD cohort. Mol Nutr Food Res 55(3):359–367
67. Badger TM, Ronis MJ, Hakkak R, Rowlands JC, Korourian S (2002) The health consequences of early soy consumption. J Nutr 132(3):559S–565S
68. McCarver G, Bhatia J, Chambers C, Clarke R, Etzel R, Foster W, Hoyer P, Leeder JS, Peters JM, Rissman E, Rybak M, Sherman C, Toppari J, Turner K (2011) NTP-CERHR expert panel report on the developmental toxicity of soy infant formula. Birth Defects Res B Dev Reprod Toxicol 92(5):421–468
69. Hoey L, Rowland IR, Lloyd AS, Clarke DB, Wiseman H (2004) Influence of soya-based infant formula consumption on isoflavone and gut microflora metabolite concentrations in urine and on faecal microflora composition and metabolic activity in infants and children. Br J Nutr 91(4):607–616
70. Irvine CH, Shand N, Fitzpatrick MG, Alexander SL (1998) Daily intake and urinary excretion of genistein and daidzein by infants fed soy- or dairy-based infant formulas. Am J Clin Nutr 68(Suppl 6):1462S–1465S
71. Genovese MI, Lajolo FM (2002) Isoflavones in soy-based foods consumed in Brazil: levels, distribution, and estimated intake. J Agric Food Chem 50(21):5987–5993
72. Fonseca ND, Villar MP, Donangelo CM, Perrone D (2014) Isoflavones and soyasaponins in soy infant formulas in Brazil: profile and estimated consumption. Food Chem 143:492–498
73. Bennetau-Pelissero C, Sauvant P, Peltre G, Auriol P, Rocca A, Rancé F (2004) Phyto-oestrogènes du soja: problèmes posés chez le nourrisson allergique au lait de vache et consommant des formules à base de soja. Cah Nutr Diét 39(1):24–32
74. Petrakis NL, Barnes S, King EB, Lowenstein J, Wiencke J, Lee MM, Miike R, Kirk M, Coward L (1996) Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women. Cancer Epidemiol Biomark Prev 5(10):785–794
75. McMichael-Phillips DF, Harding C, Morton M, Roberts SA, Howell A, Potten CS, Bundred NJ (1998) Effects of soy-protein supplementation on epithelial proliferation in the histologically normal human breast. Am J Clin Nutr 68(Suppl 6):1431S–1435S
76. Hargreaves DF, Potten CS, Harding C, Shaw LE, Morton MS, Roberts SA, Howell A, Bundred NJ (1999) Two-week dietary soy supplementation has an estrogenic effect on normal premenopausal breast. J Clin Endocrinol Metab 84(11):4017–4024
77. Nagata C, Takatsuka N, Inaba S, Kawakami N, Shimizu H (1998) Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. J Natl Cancer Inst 90(23):1830–1835
78. Chavarro JE, Toth TL, Sadio SM, Hauser R (2008) Soy food and isoflavone intake in relation to semen quality parameters among men from an infertility clinic. Hum Reprod 23(11):2584–2590
79. Toshima H, Suzuki Y, Imai K, Yoshinaga J, Shiraishi H, Mizumoto Y, Hatakeyama S, Onohara C, Tokuoka S (2012) Endocrine disrupting chemicals in urine of Japanese male partners of subfertile couples: a pilot study on exposure and semen quality. Int J Hyg Environ Health 215(5):502–506
80. Xia Y, Chen M, Zhu P, Lu C, Fu G, Zhou X, Chen D, Wang H, Hang B, Wang S, Zhou Z, Sha J, Wang X (2013) Urinary phytoestrogen levels related to idiopathic male infertility in Chinese men. Environ Int 59:161–167
81. Clarke DB, Lloyd AS, Lawrence JM, Brown JE, Storey L, Raats M, Rainsbury RM, Culliford DJ, Bailey-Horne VA, Parry BM (2013) Development of a food compositional database for the estimation of dietary intake of phyto-oestrogens in a group of postmenopausal women previously treated for breast cancer and validation with urinary excretion. Br J Nutr 109(12):2261–2268

82. Anses working group (2011) Étude de l'alimentation totale française 2 (EAT 2) Tome 1 Contaminants inorganiques, minéraux, polluants rganiques persistants, mycotoxines, phyto-estrogènes. Anses Edition Scientifique. 346 pages
83. EFSA (2009) Opinion on the safety of 'Alfalfa protein concentrate' as food. EFSA J 997:1–19
84. Zamora-Ros R, Knaze V, Luján-Barroso L, Kuhnle GG, Mulligan AA, Touillaud M, Slimani N, Romieu I, Powell N, Tumino R, Peeters PH, de Magistris MS, Ricceri F, Sonestedt E, Drake I, Hjartåker A, Skie G, Mouw T, Wark PA, Romaguera D, Bueno-de-Mesquita HB, Ros M, Molina E, Sieri S, Quirós JR, Huerta JM, Tjønneland A, Halkjær J, Masala G, Teucher B, Kaas R, Travis RC, Dilis V, Benetou V, Trichopoulou A, Amiano P, Ardanaz E, Boeing H, Förster J, Clavel-Chapelon F, Fagherazzi G, Perquier F, Johansson G, Johansson I, Cassidy A, Overvad K, González CA (2012) Dietary intakes and food sources of phytoestrogens in the European prospective investigation into cancer and nutrition (EPIC) 24-hour dietary recall cohort. Eur J Clin Nutr 66(8):932–941
85. Kuijsten A, Arts IC, van't Veer P, Hollman PC (2005) The relative bioavailability of enterolignans in humans is enhanced by milling and crushing of flaxseed. J Nutr 135(12):2812–2816
86. Kunisue T, Tanabe S, Isobe T, Aldous KM, Kannan K (2010) Profiles of phytoestrogens in human urine from several Asian countries. J Agric Food Chem 58(17):9838–9846
87. Grace PB, Taylor JI, Low YL, Luben RN, Mulligan AA, Botting NP, Dowsett M, Welch AA, Khaw KT, Wareham NJ, Day NE, Bingham SA (2004) Phytoestrogen concentrations in serum and spot urine as biomarkers for dietary phytoestrogen intake and their relation to breast cancer risk in European prospective investigation of cancer and nutrition-norfolk. Cancer Epidemiol Biomark Prev 13(5):698–708
88. Jin JS, Hattori M (2010) Human intestinal bacterium, strain END-2 is responsible for demethylation as well as lactonization during plant lignan metabolism. Biol Pharm Bull 33(8):1443–1447
89. Mally A, Solfrizzo M, Degen GH (2016) Biomonitoring of the mycotoxin Zearalenone: current state-of-the art and application to human exposure assessment. Arch Toxicol 90(6):1281–1292
90. Tahara S, Ingham JL, Nakahara S, Mizutani J, Harborne JB (1984) Fungitoxic dihydrofuranoisoflavones and related compounds in white lupin, *Lupinus albus*. Phytochemistry 23:1889–1900
91. Garcez WS, Martins D, Garcez FR, Marques MR, Pereira AA, Oliveira LA, Rondon JN, Peruca AD (2000) Effect of spores of saprophytic fungi on phytoalexin accumulation in seeds of frog-eye leaf spot and stem canker-resistant and -susceptible soybean (Glycine max L.) cultivars. J Agric Food Chem 48:3662–3665
92. Modolo LV, Cunha FQ, Braga MR, Salgado I (2002) Nitric oxide synthase-mediated phytoalexin accumulation in soybean cotyledons in response to the *Diaporthe phaseolorum* f. sp. meridionalis elicitor. Plant Physiol 130:1288–1297
93. Gagnon H, Grandmaison J, Ibrahim RK (1995) Phytochemical and immunocytochemical evidence for the accumulation of 2′-hydroxylupalbigenin in lupin nodules and bacteroids. Mol Plant-Microbe Interact 8:131–137
94. Zhang F, Smith DL (1995) Preincubation of *Bradyrhizobium japonicum* with genistein accelerates nodule development of soybean at suboptimal root zone temperatures. Plant Physiol 108(3):961–968
95. Bennett JO, Yu O, Heatherly LG, Krishnan HB (2004) Accumulation of genistein and daidzein, soybean isoflavones implicated in promoting human health, is significantly elevated by irrigation. J Agric Food Chem 52:7574–7579
96. Rossiter RC (1969) Physiological and ecological studies on the oestrogenic isoflavones in subterranean clover (*T. subterraneum* L.) VII. Effects of nitrogen supply. Aust J Agric Res 20(6):1043–1051
97. Mebrahtu T, Mohamed A, Wang CY, Andebrhan T (2004) Analysis of isoflavone contents in vegetable soybeans. Plant Foods Hum Nutr 59:55–61

98. Kassem MA, Meksem K, Iqbal MJ, Njiti VN, Banz WJ, Winters TA, Wood A, Lightfoot DA (2004) Definition of soybean genomic regions that control seed phytoestrogen amounts. J Biomed Biotechnol 2004:52–60
99. Vyn TJ, Yin X, Bruulsema TW, Jackson CJ, Rajcan I, Brouder SM (2002) Potassium fertilization effects on isoflavone concentrations in soybean [Glycine max (L.) Merr.] J Agric Food Chem 50:3501–3506
100. Jiang ZY, Jiang SQ, Lin YC, Xi PB, Yu DQ, Wu TX (2007) Effects of soybean isoflavone on growth performance, meat quality, and antioxidation in male broilers. Poult Sci 86(7):1356–1362
101. Zhao XH, Yang ZQ, Bao LB, Wang CY, Zhou S, Gong JM, Fu CB, Xu LJ, Liu CJ, Qu M (2015) Daidzein enhances intramuscular fat deposition and improves meat quality in finishing steers. Exp Biol Med (Maywood) 240(9):1152–1157
102. Liu DY, He SJ, Liu SQ, Tang YG, Jin EH, Chen HL, Li SH, Zhong LT (2014) Daidzein enhances immune function in late lactation cows under heat stress. Anim Sci J 85(1):85–89
103. Gagnon N, Côrtes C, da Silva D, Kazama R, Benchaar C, dos Santos G, Zeoula L, Petit HV (2009) Ruminal metabolism of flaxseed (Linum usitatissimum) lignans to the mammalian lignan enterolactone and its concentration in ruminal fluid, plasma, urine and milk of dairy cows. Br J Nutr 102(7):1015–1023
104. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J, Writing Group for the Women's Health Initiative Investigators (2002) Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 288(3):321–333
105. Roepke TA, Bosch MA, Rick EA, Lee B, Wagner EJ, Seidlova-Wuttke D, Wuttke W, Scanlan TS, Rønnekleiv OK, Kelly MJ (2010) Contribution of a membrane estrogen receptor to the estrogenic regulation of body temperature and energy homeostasis. Endocrinology 151(10):4926–4937
106. Archer DF, Sturdee DW, Baber R, de Villiers TJ, Pines A, Freedman RR, Gompel A, Hickey A, Hunter MS, Lobo RA, Lumsden MA, MacLennan AH, Maki P, Palacios S, Shah SD, Villaseca P, Warren M (2011) Menopausal hot flushes and night sweats: where are we now? Climacteric 14:515–528
107. Chen MN, Lin CC, Liu CF (2015) Efficacy of phytoestrogens for menopausal symptoms: a meta-analysis and systematic review. Climacteric 18(2):260–269
108. Thompson LU, Boucher BA, Cotterchio M, Kreiger N, Liu Z (2007) Dietary phytoestrogens, including isoflavones, lignans, and coumestrol, in nonvitamin, nonmineral supplements commonly consumed by women in Canada. Nutr Cancer 59(2):176–184
109. Ferrari A (2009) Soy extract phytoestrogens with high dose of isoflavones for menopausal symptoms. J Obstet Gynaecol Res 35(6):1083–1090
110. Gold EB, Leung K, Crawford SL, Huang MH, Waetjen LE, Greendale GA (2013) Phytoestrogen and fiber intakes in relation to incident vasomotor symptoms: results from the study of Women's Health Across the Nation. Menopause 20(3):305–314
111. Dodin S, Lemay A, Jacques H, Légaré F, Forest JC, Mâsse B (2005) The effects of flaxseed dietary supplement on lipid profile, bone mineral density, and symptoms in menopausal women: a randomized, double-blind, wheat germ placebo-controlled clinical trial. J Clin Endocrinol Metab 90:1390–1397
112. Lewis JE, Nickell LA, Thompson LU, Szalai JP, Kiss A, Hilditch JR (2006) A randomized controlled trial of the effect of dietary soy and flaxseed muffins on quality of life and hot flashes during menopause. Menopause 13:631–642
113. Simbalista RL, Sauerbronn AV, Aldrighi JM, Arêas JA (2010) Consumption of a flaxseed-rich food is not more effective than a placebo in alleviating the climacteric symptoms of postmenopausal women. J Nutr 140:293–297
114. Lucas EA, Wild RD, Hammond LJ, Khalil DA, Juma S, Daggy BP, Stoecker BJ, Arjmandi BH (2002) Flaxseed improves lipid profile without altering biomarkers of bone metabolism in postmenopausal women. J Clin Endocrinol Metab 87:1527–1532

115. Colli MC, Bracht A, Soares AA, de Oliveira AL, Bôer CG, de Souza CG, Peralta RM (2012) Evaluation of the efficacy of flaxseed meal and flaxseed extract in reducing menopausal symptoms. J Med Food 15(9):840–845
116. Pruthi S, Qin R, Terstreip SA, Liu H, Loprinzi CL, Shah TR, Tucker KF, Dakhil SR, Bury MJ, Carolla RL, Steen PD, Vuky J, Barton DL (2012) A phase III, randomized, placebo-controlled, double-blind trial of flaxseed for the treatment of hot flashes: North Central Cancer Treatment Group N08C7. Menopause 19(1):48–53
117. Clavel T, Doré J, Blaut M (2006) Bioavailability of lignans in human subjects. Nutr Res Rev 9(2):187–196
118. Taku K, Melby MK, Takebayashi J, Mizuno S, Ishimi Y, Omori T, Watanabe S (2010) Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials. Asia Pac J Clin Nutr 19(1):33–42
119. Liu J, Ho SC, Su YX, Chen WQ, Zhang CX, Chen YM (2009) Effect of long-term intervention of soy isoflavones on bone mineral density in women: a meta-analysis of randomized controlled trials. Bone 44(5):948–953
120. Dodge JA, Glasebrook AL, Magee DE, Phillips DL, Sato M, Short LL, Bryant HU (1996) Environmental estrogens: effects on cholesterol lowering and bone in the ovariectomized rat. J Steroid Biochem Mol Biol 59(2):155–161
121. Kanno S, Hirano S, Kayama F (2004) Effects of the phytoestrogen coumestrol on RANK-ligand-induced differentiation of osteoclasts. Toxicology 203(1–3):211–220
122. Kanno S, Hirano S, Kayama F (2004) Effects of phytoestrogens and environmental estrogens on osteoblastic differentiation in MC3T3-E1 cells. Toxicology 196(1–2):137–145
123. Zong S, Zeng G, Fang Y, Peng J, Zou B, Gao T, Zhao J (2016) The effects of α-zearalanol on the proliferation of bone-marrow-derived mesenchymal stem cells and their differentiation into osteoblasts. J Bone Miner Metab 34(2):151–160
124. Abdelhamid AM, Kelada IP, Ali MM, el-Ayouty SA (1992) Influence of zearalenone on some metabolic, physiological and pathological aspects of female rabbits at two different ages. Arch Tierernahr 42(1):63–70
125. Zong S, Wei B, Xiong C, Zhao Y, Zeng G (2012) The role of α-zearalanol in reversing bone loss induced by ovarian hormone deficiency in rats. J Bone Miner Metab 30(2):136–143
126. Ayed Y, Ayed-Boussema I, Ouanes Z, Bacha H (2011) *In vivo* and *in vivo* induction of chromosome aberrations by alpha- and beta-zearalenols: comparison with zearalenone. Mutat Res 726(1):42–46
127. Kodell RL, Gaylor DW (1999) Combining uncertainty factors in deriving human exposure levels of noncarcinogenic toxicants. Ann N Y Acad Sci 895:188–195
128. Hendrich S (2009) Chapter 17 Phytoestrogens and phytosterols In endocrine disrupting chemicals in food. I Shaw Edt. Elsevier, Woodhead Publishing Series in Food Science, Technology and Nutrition. Copyright © 2009 Woodhead Publishing Limited. pp 437–458. https://doi.org/10.1533/9781845695743.4.437. https://www.sciencedirect.com/science/article/pii/B9781845692186500173
129. Galey FD, Mendez LE, Whitehead WE, Holstege DM, Plumlee KH, Johnson B (1993) Estrogenic activity in forages: diagnostic use of the classical mouse uterine bioassay. J Vet Diagn Investig 5(4):603–608
130. Greendale GA, Huang MH, Leung K, Crawford SL, Gold EB, Wight R, Waetjen E, Karlamangla AS (2012) Dietary phytoestrogen intakes and cognitive function during the menopausal transition: results from the Study of Women's Health Across the Nation Phytoestrogen Study. Menopause 19(8):894–903
131. Tou JC, Chen J, Thompson LU (1998) Flaxseed and its lignan precursor, secoisolariciresinol diglycoside, affect pregnancy outcome and reproductive development in rats. J Nutr 128(11):1861–1868
132. McCann SE, Hootman KC, Weaver AM, Thompson LU, Morrison C, Hwang H, Edge SB, Ambrosone CB, Horvath PJ, Kulkarni SA (2012) Dietary intakes of total and specific lignans are associated with clinical breast tumor characteristics. J Nutr 142(1):91–98

133. Yang R, Wang YM, Zhang L, Zhao ZM, Zhao J, Peng SQ (2016) Prepubertal exposure to an oestrogenic mycotoxin zearalenone induces central precocious puberty in immature female rats through the mechanism of premature activation of hypothalamic kisspeptin-GPR54 signaling. Mol Cell Endocrinol 437:62–74
134. Maragos CM (2010) Zearalenone occurrence and human exposure. World Mycotoxin J 3(4):369–383
135. Kuiper-Goodman T, Scott PM, Watanabe H (1987) Risk assessment of the mycotoxin zearalenone. Regul Toxicol Pharmacol 7(3):253–306
136. JEFCA panel: Eriksen GS, Pennington J, Schlatter J, Alexander J, Thuvander A (2000) Safety evaluation of certain food additives and contaminants 53rd report. Who Food Additives Series: 44
137. Lindner HR (1967) Study of the fate of phyto-oestrogens in the sheep by determination of isoflavones and coumestrol in the plasma and adipose tissue. Aust J Agric Res 18:305–333
138. Shutt DA (1967) Interraction of genistein with estradiol in the reproductive tract of the ovariectomized mouse. J Endocrinol 37:231–232
139. Shutt DA, Braden AWH (1968) The significance of equol in relation to the estrogenic responses in sheep ingesting clover with a high formononetin content. Aust J Agric Res 19:545–553
140. Leavitt WW, Meismer DM (1968) Sexual development altered by non-steroidal œstrogens. Nature 218:181–182
141. Shutt DA, Cox RI (1972) Steroid and phyto-estrogen binding to sheep uterine receptors *in vivo*. J Endocrinol 52:299–310
142. Findlay JK, Buckmaster JM, Chamley WA, Cumming IA, Hearnshaw H, Goding JR (1973) Release of luteinising hormone by œstradiol 17β and a gonadotrophin-releasing hormone in ewes affected with clover disease. Neuroendocrinology 11:57–66
143. Setchell KDR, Gosselin SJ, Welsh MB, Johnston JO, Balisteri WF, Kramer LW, Dresser BL, Tarr MJ (1987) Dietary estrogens. A probable cause of infertility and liver disease in captive cheetahs. Gastroenterology 93:225–233
144. Pelissero C, Le Menn F, Kaushik S (1991) Estrogenic effect of dietary soya bean meal on vitellogenesis in cultured Siberian sturgeon *Acipencer baeri*. Gen Comp Endocrinol 83:447–457
145. Piotrowska KK, Woclawek-Potocka I, Bah MM, Piskula MK, Pilawski W, Bober A, Skarzynski DJ (2006) Phytoestrogens and their metabolites inhibit the sensitivity of the bovine corpus luteum to luteotropic factors. J Reprod Dev 52(1):33–41
146. Cools S, Van den Broeck W, Vanhaecke L, Heyerick A, Bossaert P, Hostens M, Opsomer G (2014) Feeding soybean meal increases the blood level of isoflavones and reduces the steroidogenic capacity in bovine corpora lutea, without affecting peripheral progesterone concentrations. Anim Reprod Sci 144(3–4):79–89
147. Hashem NM, El-Azrak KM, Sallam SM (2016) Hormonal concentrations and reproductive performance of Holstein heifers fed *Trifolium alexandrinum* as a phytoestrogenic roughage. Anim Reprod Sci 170:121–127
148. Mlynarczuk J, Wrobel MH, Kotwica J (2011) The adverse effect of phytoestrogens on the synthesis and secretion of ovarian oxytocin in cattle. Reprod Domest Anim 46(1):21–28
149. Woclawek-Potocka I, Bah MM, Korzekwa A, Piskula MK, Wiczkowski W, Depta A, Skarzynski DJ (2005) Soybean-derived phytoestrogens regulate prostaglandin secretion in endometrium during cattle estrous cycle and early pregnancy. Exp Biol Med (Maywood) 230(3):189–199
150. Wong CK, Keung WM (1999) Bovine adrenal 3beta-hydroxysteroid dehydrogenase (E.C. 1.1.1. 145)/5-ene-4-ene isomerase (E.C. 5.3.3.1): characterization and its inhibition by isoflavones. J Steroid Biochem Mol Biol 71(5–6):191–202
151. Zhao R, Wang Y, Zhou Y, Ni Y, Lu L, Grossmann R, Chen J (2004) Dietary daidzein influences laying performance of ducks (*Anas platyrhynchos*) and early post-hatch growth of their hatchlings by modulating gene expression. Comp Biochem Physiol A Mol Integr Physiol 138(4):459–466

152. Pelissero C, Bennetau B, Babin P, Le Menn F, Dunogues J (1991) The estrogenic activity of certain phytoestrogens in the Siberian sturgeon *Acipenser baeri*. J Steroid Biochem Mol Biol 38(3):293–299
153. Bennetau-Pelissero C, Breton BB, Bennetau B, Corraze G, Le Menn F, Davail-Cuisset B, Helou C, Kaushik SJ (2001) Effect of genistein-enriched diets on the endocrine process of gametogenesis and on reproduction efficiency of the rainbow trout *Oncorhynchus mykiss*. Gen Comp Endocrinol 121(2):173–187
154. Latonnelle K, Le Menn F, Kaushik SJ, Bennetau-Pelissero C (2002) Effects of dietary phytoestrogens in vivo and in vitro in rainbow trout and Siberian sturgeon: interests and limits of the in vitro studies of interspecies differences. Gen Comp Endocrinol 126(1):39–51
155. Bagheri T, Imanpoor MR, Jafari V, Bennetau-Pelissero C (2013) Reproductive impairment and endocrine disruption in goldfish by feeding diets containing soybean meal. Anim Reprod Sci 139(1–4):136–144
156. Green CC, Kelly AM (2009) Effects of the estrogen mimic genistein as a dietary component on sex differentiation and ethoxyresorufin-*O*-deethylase (EROD) activity in channel catfish (*Ictalurus punctatus*). Fish Physiol Biochem 35(3):377–384
157. Pinto PIS, Estêvão MD, Andrade A, Santos S, Power DM (2016) Tissue responsiveness to estradiol and genistein in the sea bass liver and scale. J Steroid Biochem Mol Biol 158:127–137
158. Malison JA, Lima LC, Yuliana, Barry TP, Held JA (2005) Effects of genistein on growth, development and reproduction of rainbow trout *Onchorynchus mykiss* and Atlantic Salmon. http://www.soyaqua.org/reports/effects-genistein-growth-development-and-reproduction-rainbow-trout-onchorynchus-mykiss-and
159. Leavitt WW, Wright PA (1965) The plant estrogen, coumestrol, as an agent affecting hypophysial gonadotrophic function. J Exp Zool 160:319–328
160. Reed KFM (2016) Fertility of herbivores consuming phytoestrogen-containing *Medicago* and *Trifolium* species. Agriculture 6:35–64
161. Adams NR (1995) Detection of the effects of phytoestrogens on sheep and cattle. J Anim Sci 73(5):1509–1515
162. Młynarczuk J, Wróbel MH, Kotwica J (2013) Adverse influence of coumestrol on secretory function of bovine luteal cells in the first trimester of pregnancy. Environ Toxicol 28(7):411–418
163. Ferreira-Dias G, Botelho M, Zagrajczuk A, Rebordão MR, Galvão AM, Bravo PP, Piotrowska-Tomala K, Szóstek AZ, Wiczkowski W, Piskula M, Fradinho MJ, Skarzynski DJ (2013) Coumestrol and its metabolite in mares' plasma after ingestion of phytoestrogen-rich plants: potent endocrine disruptors inducing infertility. Theriogenology 80(6):684–692
164. Perel E, Lindner HR (1970) Dissociation of uterotrophic action from implantation-inducing activity in two non-steroidal oestrogens (coumestrol and genistein). J Reprod Fertil 21(1):171–175
165. Whitten PL, Naftolin F (1992) Effects of a phytoestrogen diet on estrogen-dependent reproductive processes in immature female rats. Steroids 57(2):56–61
166. Whitten PL, Russell E, Naftolin F (1992) Effects of a normal, human-concentration, phytoestrogen diet on rat uterine growth. Steroids 57(3):98–106
167. Whitten PL, Lewis C, Russell E, Naftolin F (1995) Potential adverse effects of phytoestrogens. J Nutr 125(Suppl 3):771S–776S
168. Whitten PL, Lewis C, Naftolin F (1993) A phytoestrogen diet induces the premature anovulatory syndrome in lactationally exposed female rats. Biol Reprod 49(5):1117–1121
169. Whitten PL, Lewis C, Russell E, Naftolin F (1995) Phytoestrogen influences on the development of behavior and gonadotropin function. Proc Soc Exp Biol Med 208(1):82–86
170. Awoniyi CA, Roberts D, Chandrashekar V, Veeramachaneni DN, Hurst BS, Tucker KE, Schlaff WD (1997) Neonatal exposure to coumestrol, a phytoestrogen, does not alter spermatogenic potential in rats. Endocrine 7(3):337–341

171. Zachut M (2015) Short communication: concentrations of the mammalian lignan enterolactone in preovulatory follicles and the correlation with intrafollicular estradiol in dairy cows fed extruded flaxseed. J Dairy Sci 98(12):8814–8817
172. Hallé C, Goff AK, Petit HV, Blouin R, Palin MF (2015) Effects of different n-6:n-3 fatty acid ratios and of enterolactone on gene expression and PG secretion in bovine endometrial cells. Br J Nutr 113(1):56–71
173. Dehennin L, Reiffsteck A, Jondet M, Thibier M (1982) Identification and quantitative estimation of a lignan in human and bovine semen. J Reprod Fertil 66(1):305–309
174. Weaver GA, Kurtz HJ, Behrens JC, Robison TS, Seguin BE, Bates FY, Mirocha CJ (1986) Effect of zearalenone on the fertility of virgin dairy heifers. Am J Vet Res 47(6): 1395–1397
175. Shappell NW, Mostrom MS, Lenneman EM (2012) E-screen evaluation of sugar beet feedstuffs in a case of reduced embryo transfer efficiencies in cattle: the role of phytoestrogens and zearalenone. In Vitro Cell Dev Biol Anim 48(4):216–228
176. Fushimi Y, Takagi M, Monniaux D, Uno S, Kokushi E, Shinya U, Kawashima C, Otoi T, Deguchi E, Fink-Gremmels J (2015) Effects of dietary contamination by zearalenone and its metabolites on serum anti-müllerian hormone: impact on the reproductive performance of breeding cows. Reprod Domest Anim 50(5):834–839
177. Harwig J, Munro IC (1975) Mycotoxins of possible importance in diseases of Canadian farm animals. Can Vet J 16(5):125–141
178. Chang K, Kurtz HJ, Mirocha CJ (1979) Effects of the mycotoxin zearalenone on swine reproduction. Am J Vet Res 40(9):1260–1267
179. Schoevers EJ, Santos RR, Colenbrander B, Fink-Gremmels J, Roelen BA (2012) Transgenerational toxicity of zearalenone in pigs. Reprod Toxicol 34(1):110–119
180. Collins TF, Sprando RL, Black TN, Olejnik N, Eppley RM, Alam HZ, Rorie J, Ruggles DI (2006) Effects of zearalenone on *in utero* development in rats. Food Chem Toxicol 44(9):1455–1465
181. Jacobsen BK, Jaceldo-Siegl K, Knutsen SF, Fan J, Oda K, Fraser GE (2014) Soy isoflavone intake and the likelihood of ever becoming a mother: the Adventist Health Study-2. Int J Womens Health 6:377–384
182. Thibault C, Levasseur MC (2001) La reproduction chez les mammifères et l'homme. Editions Quae, Paris, 928 pages
183. Jeng HA (2014) Exposure to endocrine disrupting chemicals and male reproductive health. Front Public Health 2:55–67
184. Mitchell JH, Cawood E, Kinniburgh D, Provan A, Collins AR, Irvine DS (2001) Effect of a phytoestrogen food supplement on reproductive health in normal males. Clin Sci (Lond) 100(6):613–618
185. Beaton LK, McVeigh BL, Dillingham BL, Lampe JW, Duncan AM (2010) Soy protein isolates of varying isoflavone content do not adversely affect semen quality in healthy young men. Fertil Steril 94(5):1717–1722
186. Casini ML, Gerli S, Unfer V (2006) An infertile couple suffering from oligospermia by partial sperm maturation arrest: can phytoestrogens play a therapeutic role? A case report study. Gynecol Endocrinol 22(7):399–401
187. Skakkebaek NE (2016) A brief review of the link between environment and male reproductive health: lessons from studies of testicular germ cell cancer. Horm Res Paediatr 86 (4):240–246
188. Faber KA, Hughes CL (1991) The effect of neonatal exposure to diethylstylbestrol, genistein and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. Biol Reprod 45:649–653
189. Mueller JK, Heger S (2014) Endocrine disrupting chemicals affect the gonadotropin releasing hormone neuronal network. Reprod Toxicol 44:73–84
190. Strom BL, Schinnar R, Ziegler EE (2001) Exposure to soy-based formula in infancy and endocrinological and reproductive outcomes in young adulthood. JAMA 286(7):807–814

191. Gilchrist JM, Moore MB, Andres A, Estroff JA, Badger TM (2010) Ultrasonographic patterns of reproductive organs in infants fed soy formula: comparisons to infants fed breast milk and milk formula. J Pediatr 156:215–220
192. Sharpe RM, Martin B, Morris K, Greig I, McKinnell C, McNeilly AS, Walker M (2002) Infant feeding with soy formula milk: effects on the testis and on blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity. Hum Reprod 17(7):1692–1703
193. Tan KA, Walker M, Morris K, Greig I, Mason JI, Sharpe RM (2006) Infant feeding with soy formula milk: effects on puberty progression, reproductive function and testicular cell numbers in marmoset monkeys in adulthood. Hum Reprod 21(4):896–904
194. Adgent MA, Daniels JL, Edwards LJ, Siega-Riz AM, Rogan WJ (2011) Early-life soy exposure and gender-role play behavior in children. Environ Health Perspect 119(12):1811–1816
195. Adgent MA, Daniels JL, Rogan WJ, Adair L, Edwards LJ, Westreich D, Maisonet M, Marcus M (2012) Early-life soy exposure and age at menarche. Paediatr Perinat Epidemiol 26(2):163–175
196. Kim J, Kim S, Huh K, Kim Y, Joung H, Park M (2011) High serum isoflavone concentrations are associated with the risk of precocious puberty in Korean girls. Clin Endocrinol 75(6):831–835
197. Index Mundi (2017) Korea, south demographics profile 2017. CIA World Factbook Updated on July 9, 2017. https://www.indexmundi.com/south_korea/demographics_profile.html
198. Harlid S, Adgent M, Jefferson WN, Panduri V, Umbach DM, Xu Z, Stallings VA, Williams CJ, Rogan WJ, Taylor JA (2017) Soy formula and epigenetic modifications: analysis of vaginal epithelial cells from infant girls in the IFED study. Environ Health Perspect 125(3):447–452
199. Andres A, Cleves MA, Bellando JB, Pivik RT, Casey PH, Badger TM (2012) Developmental status of 1-year-old infants fed breast milk, cow's milk formula, or soy formula. Pediatrics 129(6):1134–1140
200. Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J (2013) Glyphosate induces human breast cancer cells growth via estrogen receptors. Food Chem Toxicol 59:129–136
201. Upson K, Harmon QE, Baird DD (2016) Soy-based infant formula feeding and ultrasound-detected uterine fibroids among young African-American women with no prior clinical diagnosis of fibroids. Environ Health Perspect 124:769–775
202. Chandrareddy A, Muneyyirci-Delale O, McFarlane SI, Murad OM (2008) Adverse effects of phytoestrogens on reproductive health: a report of three cases. Complement Ther Clin Pract 14(2):132–135
203. Mylonas I, Jeschke U, Shabani N, Kuhn C, Kriegel S, Kupka MS, Friese K (2005) Normal and malignant human endometrium express immunohistochemically estrogen receptor alpha (ER-alpha), estrogen receptor beta (ER-beta) and progesterone receptor (PR). Anticancer Res 25(3A):1679–1686
204. Tica AA, Tica OS, Georgescu CV, Pirici D, Bogdan M, Ciurea T, Mogoantă SŞ, Georgescu CC, Comănescu AC, Bălşeanu TA, Ciurea RN, Osiac E, Buga AM, Ciurea ME (2016) GPER and ERα expression in abnormal endometrial proliferations. Romanian J Morphol Embryol 57(2):413–418
205. Cavallini A, Dinaro E, Giocolano A, Caringella AM, Ferreri R, Tutino V, Loverro G (2008) Estrogen receptor (ER) and ER-related receptor expression in normal and atrophic human vagina. Maturitas 59(3):219–225
206. Mylonas I, Jeschke U, Shabani N, Kuhn C, Kunze S, Dian D, Friedl C, Kupka MS, Friese K (2007) Steroid receptors ERα, ERβ, PR-A and PR-B are differentially expressed in normal and atrophic human endometrium. Histol Histopathol 22:169–176
207. Liu J, Yuan F, Gao J, Shan B, Ren Y, Wang H, Gao Y (2016) Oral isoflavone supplementation on endometrial thickness: a meta-analysis of randomized placebo-controlled trials. Oncotarget 7(14):17369–17379

208. Lima SM, Yamada SS, Reis BF, Postigo S, Galvão da Silva MA, Aoki T (2013) Effective treatment of vaginal atrophy with isoflavone vaginal gel. Maturitas 74(3):252–258
209. Watanabe S, Terashima K, Sato Y, Arai S, Eboshida A (2000) Effects of isoflavone supplement on healthy women. Biofactors 12(1–4):233–241
210. Tang R, Chen M, Zhou K, Chen D, Yu J, Hu W, Song L, Hang B, Wang X, Xia Y (2015) Prenatal lignan exposures, pregnancy urine estrogen profiles and birth outcomes. Environ Pollut 205:261–268
211. Garreau B, Vallette G, Adlercreutz H, Wähälä K, Mäkelä T, Benassayag C, Nunez EA (1991) Phytoestrogens: new ligands for rat and human alpha-fetoprotein. Biochim Biophys Acta 1094(3):339–345
212. Vannuccini S, Bocchi C, Severi FM, Challis JR, Petraglia F (2016) Endocrinology of human parturition. Ann Endocrinol (Paris) 77(2):105–113
213. Mumford SL, Sundaram R, Schisterman EF, Sweeney AM, Barr DB, Rybak ME, Maisog JM, Parker DL, Pfeiffer CM, Louis GM (2014) Higher urinary lignan concentrations in women but not men are positively associated with shorter time to pregnancy. J Nutr 144(3):352–358
214. Fara GM, Del Corvo G, Bernuzzi S, Bigatello A, Di Pietro C, Scaglioni S, Chiumello G (1979) Epidemic of breast enlargement in an Italian school. Lancet 2:295–297
215. Saenz de Rodriguez CA, Bongiovanni AM, Conde de Borrego L (1985) An epidemic of precocious development in Puerto Rican children. J Pediatr 107:393–396
216. Szuets P, Mesterhazy A, Falkay G, Bartok T (1997) Early thelarche symptoms in children and their relations to Zearalenon contamination in foodstuffs. Cereal Res Commun 25:429–436
217. Massart F, Saggese G (2010) Oestrogenic mycotoxin exposures and precocious pubertal development. Int J Androl 33:369–376
218. Fugh-Berman A (2003) "Bust enhancing" herbal products. Obstet Gynecol 101:1345–1349
219. Bandera EV, Chandran U, Buckley B, Lin Y, Isukapalli S, Marshall I, King M, Zarbl H (2011) Urinary mycoestrogens, body size and breast development in New Jersey girls. Sci Total Environ 409(24):5221–5227
220. Arispe SA, Adams B, Adams TE (2013) Effect of phytoestrogens on basal and GnRH-induced gonadotropin secretion. J Endocrinol 219(3):243–250
221. He J, Wei C, Li Y, Liu Y, Wang Y, Pan J, Liu J, Wu Y, Cui S (2017) Zearalenone and alpha-zearalenol inhibit the synthesis and secretion of pig follicle stimulating hormone via the non-classical estrogen membrane receptor GPR30. Mol Cell Endocrinol 461:43. https://doi.org/10.1016/j.mce.2017.08.010
222. EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food) (2015) Scientific opinion on the risk assessment for peri- and post-menopausal women taking food supplements containing isolated isoflavones. EFSA J 13:4246 (342 pp)
223. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139(10):4252–4263
224. Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engström O, Ljunggren J, Gustafsson JA, Carlquist M (1999) Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. EMBO J 18(17):4608–4618
225. Gougelet A, Mueller SO, Korach KS, Renoir JM (2007) Oestrogen receptors pathways to oestrogen responsive elements: the transactivation function-1 acts as the keystone of oestrogen receptor (ER)beta-mediated transcriptional repression of ERalpha. J Steroid Biochem Mol Biol 104(3–5):110–122
226. Abot A, Fontaine C, Raymond-Letron I, Flouriot G, Adlanmerini M, Buscato M, Otto C, Bergès H, Laurell H, Gourdy P, Lenfant F, Arnal JF (2013) The AF-1 activation function of estrogen receptor α is necessary and sufficient for uterine epithelial cell proliferation in vivo. Endocrinology 154(6):2222–2233
227. Kimura T (2012) East meets west: ethnic differences in prostate cancer epidemiology between East Asians and caucasians. Chin J Cancer 31(9):421–429

228. Nakamura H, Wang Y, Kurita T, Adomat H, Cunha GR, Wang Y (2011) Genistein increases epidermal growth factor receptor signaling and promotes tumor progression in advanced human prostate cancer. PLoS One 6(5):e20034
229. Zhang Q, Feng H, Qluwakemi B, Wang J, Yao S, Cheng G, Xu H, Qiu H, Zhu L, Yuan M (2017) Phytoestrogens and risk of prostate cancer: an updated meta-analysis of epidemiologic studies. Int J Food Sci Nutr 68(1):28–42
230. Woo HD, Park S, Oh K, Kim HJ, Shin HR, Moon HK, Kim J (2014) Diet and cancer risk in the Korean population: a meta- analysis. Asian Pac J Cancer Prev 15(19):8509–8519
231. Nagata C, Mizoue T, Tanaka K, Tsuji I, Tamakoshi A, Matsuo K, Wakai K, Inoue M, Tsugane S, Sasazuki S Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan (2014) Soy intake and breast cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. Jpn J Clin Oncol 44(3):282–295
232. Chi F, Wu R, Zeng YC, Xing R, Liu Y, Xu ZG (2013) Post-diagnosis soy food intake and breast cancer survival: a meta-analysis of cohort studies. Asian Pac J Cancer Prev 14(4):2407–2412
233. Carreau C, Flouriot G, Bennetau-Pelissero C, Potier M (2009) Respective contribution exerted by AF-1 and AF-2 transactivation functions in estrogen receptor alpha induced transcriptional activity by isoflavones and equol: consequence on breast cancer cell proliferation. Mol Nutr Food Res 53(5):652–658
234. Wu Q, Yang Y, Yu J, Jin N (2012) Soy isoflavone extracts stimulate the growth of nude mouse xenografts bearing estrogen-dependent human breast cancer cells (MCF-7). J Biomed Res 26(1):44–52
235. Shike M, Doane AS, Russo L, Cabal R, Reis-Filho JS, Gerald W, Cody H, Khanin R, Bromberg J, Norton L (2014) The effects of soy supplementation on gene expression in breast cancer: a randomized placebo-controlled study. J Natl Cancer Inst 106(9):189–201
236. Hilakivi-Clarke L, Andrade JE, Helferich W (2010) Is soy consumption good or bad for the breast? J Nutr 140(12):2326S–2334S
237. Messina M, Hilakivi-Clarke L (2009) Early intake appears to be the key to the proposed protective effects of soy intake against breast cancer. Nutr Cancer 61(6):792–798
238. de Assis S, Warri A, Benitez C, Helferich W, Hilakivi-Clarke L (2011) Protective effects of prepubertal genistein exposure on mammary tumorigenesis are dependent on BRCA1 expression. Cancer Prev Res (Phila) 4(9):1436–1448
239. Li M, Zhang Z, Hill DL, Chen X, Wang H, Zhan R (2005) Genistein, a dietary isoflavone, down-regulates the MDM2 oncogene at both transcriptional and posttranslational levels. Cancer Res 65(18):8200–8208
240. Lamartiniere CA, Moore JB, Brown NM, Thompson R, Hardin MJ, Barnes S (1995) Genistein suppresses mammary cancer in rats. Carcinogenesis 16(11):2833–2840
241. National Toxicology Program (2008) Toxicology and carcinogenesis studies of genistein (Cas no. 446-72-0) in Sprague-Dawley rats (feed study). Natl Toxicol Program Tech Rep Ser 55:1–240
242. Chen M, Rao Y, Zheng Y, Wei S, Li Y, Guo T, Yin P (2014) Association between soy isoflavone intake and breast cancer risk for pre- and post-menopausal women: a meta-analysis of epidemiological studies. PLoS One 9(2):e89288
243. Fagan DH, Yee D (2008) Crosstalk between IGF1R and estrogen receptor signaling in breast cancer. J Mammary Gland Biol Neoplasia 13(4):423–429
244. van Duursen MB, Nijmeijer SM, de Morree ES, de Jong PC, van den Berg M (2011) Genistein induces breast cancer-associated aromatase and stimulates estrogen-dependent tumor cell growth in *in vivo* breast cancer model. Toxicology 289(2–3):67–73
245. Andrade JE, Ju YH, Baker C, Doerge DR, Helferich WC (2015) Long-term exposure to dietary sources of genistein induces estrogen-independence in the human breast cancer (MCF-7) xenograft model. Mol Nutr Food Res 59:413–423

246. Choi SY, Ha TY, Ahn JY, Kim SR, Kang KS, Hwang IK, Kim S (2008) Estrogenic activities of isoflavones and flavones and their structure-activity relationships. Planta Med 74 (1):25–32
247. Aiad HA, Wahed MM, Asaad NY, El-Tahmody M, Elhosary E (2014) Immunohistochemical expression of GPR30 in breast carcinoma of Egyptian patients: an association with immunohistochemical subtypes. APMIS 122(10):976–984
248. Pan H, Zhou W, He W, Liu X, Ding Q, Ling L, Zha X, Wang S (2012) Genistein inhibits MDA-MB-231 triple-negative breast cancer cell growth by inhibiting NF-κB activity via the Notch-1 pathway. Int J Mol Med 30(2):337–343
249. Girgert R, Emons G, Gründker C (2012) Inactivation of GPR30 reduces growth of triple-negative breast cancer cells: possible application in targeted therapy. Breast Cancer Res Treat 134(1):199–205
250. Prossnitz ER, Barton M (2014) Estrogen biology: new insights into GPER function and clinical opportunities. Mol Cell Endocrinol 389(1–2):71–83
251. Girgert R, Emons G, Gründker C (2014) Inhibition of GPR30 by estriol prevents growth stimulation of triple-negative breast cancer cells by 17β-estradiol. BMC Cancer 14:935
252. Imesch P, Samartzis EP, Dedes KJ, Fink D, Fedier A (2013) Histone deacetylase inhibitors down-regulate G-protein-coupled estrogen receptor and the GPER-antagonist G-15 inhibits proliferation in endometriotic cells. Fertil Steril 100(3):770–776
253. Thomas P, Dong J (2006) Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. J Steroid Biochem Mol Biol 102(1–5):175–179
254. Kajta M, Rzemieniec J, Litwa E, Lason W, Lenartowicz M, Krzeptowski W, Wojtowicz AK (2013) The key involvement of estrogen receptor β and G-protein-coupled receptor 30 in the neuroprotective action of daidzein. Neuroscience 238:345–360
255. Teng CT, Beames B, Alex Merrick B, Martin N, Romeo C, Jetten AM (2014) Development of a stable cell line with an intact PGC-1α/ERRα axis for screening environmental chemicals. Biochem Biophys Res Commun 444(2):177–181
256. Hedelin M, Bälter KA, Chang ET, Bellocco R, Klint A, Johansson JE, Wiklund F, Thellenberg-Karlsson C, Adami HO, Grönberg H (2006) Dietary intake of phytoestrogens, estrogen receptor-beta polymorphisms and the risk of prostate cancer. Prostate 66(14):1512–1520
257. Ward HA, Kuhnle GG, Mulligan AA, Lentjes MA, Luben RN, Khaw KT (2010) Breast, colorectal, and prostate cancer risk in the European prospective investigation into cancer and nutrition-Norfolk in relation to phytoestrogen intake derived from an improved database. Am J Clin Nutr 91(2):440–448
258. Clavel T, Henderson G, Alpert CA, Philippe C, Rigottier-Gois L, Doré J, Blaut M (2005) Intestinal bacterial communities that produce active estrogen-like compounds enterodiol and enterolactone in humans. Appl Environ Microbiol 71(10):6077–6085
259. Adlercreutz H (1990) Western diet and western diseases: some hormonal and biochemical mechanisms and associations. Scand J Clin Lab Invest 201(Suppl):3–23
260. Saarinen NM, Tuominen J, Pylkkänen L, Santti R (2010) Assessment of information to substantiate a health claim on the prevention of prostate cancer by lignans. Forum Nutr 2(2):99–115
261. Azrad M, Vollmer RT, Madden J, Dewhirst M, Polascik TJ, Snyder DC, Ruffin MT, Moul JW, Brenner DE, Demark-Wahnefried W (2013) Flaxseed-derived enterolactone is inversely associated with tumor cell proliferation in men with localized prostate cancer. J Med Food 16(4):357–360
262. Power KA, Saarinen NM, Chen JM, Thompson LU (2006) Mammalian lignans enterolactone and enterodiol, alone and in combination with the isoflavone genistein, do not promote the growth of MCF-7 xenografts in ovariectomized athymic nude mice. Int J Cancer 118(5):1316–1320

263. Truan JS, Chen JM, Thompson LU (2012) Comparative effects of sesame seed lignan and flaxseed lignan in reducing the growth of human breast tumors (MCF-7) at high levels of circulating estrogen in athymic mice. Nutr Cancer 64(1):65–71
264. Touillaud MS, Thiébaut AC, Fournier A, Niravong M, Boutron-Ruault MC, Clavel-Chapelon F (2007) Dietary lignan intake and postmenopausal breast cancer risk by estrogen and progesterone receptor status. J Natl Cancer Inst 99(6):475–486
265. Buck K, Zaineddin AK, Vrieling A, Linseisen J, Chang-Claude J (2010) Meta-analyses of lignans and enterolignans in relation to breast cancer risk. Am J Clin Nutr 92(1):141–153
266. Seibold P, Vrieling A, Johnson TS, Buck K, Behrens S, Kaaks R, Linseisen J, Obi N, Heinz J, Flesch-Janys D, Chang-Claude J (2014) Enterolactone concentrations and prognosis after postmenopausal breast cancer: assessment of effect modification and meta-analysis. Int J Cancer 135(4):923–933
267. Saarinen NM, Huovinen R, Wärri A, Mäkelä SI, Valentín-Blasini L, Sjöholm R, Ammälä J, Lehtilä R, Eckerman C, Collan YU, Santti RS (2002) Enterolactone inhibits the growth of 7,12-dimethylbenz(a)anthracene-induced mammary carcinomas in the rat. Mol Cancer Ther 1(10):869–876
268. Shepard TH, Pyne GE, Kirschvink JF, McLean MC, USAR (1960) Soybean goiter – report of three cases. N Engl J Med 262:1099–1103
269. Chorazy PA, Himelhoch S, Hopwood NJ, Greger NG, Postellon DC (1995) Persistent hypothyroidism in an infant receiving a soy formula: case report and review of the literature. Pediatrics 96(1 Pt 1):148–150
270. Doerge DR, Sheehan DM (2002) Goitrogenic and estrogenic activity of soy isoflavones. Environ Health Perspect 110(Suppl 3):349–353
271. Conrad SC, Chiu H, Silverman BL (2004) Soy formula complicates management of congenital hypothyroidism. Arch Dis Child 89(1):37–40
272. Fruzza AG, Demeterco-Berggren C, Jones KL (2012) Unawareness of the effects of soy intake on the Management of Congenital Hypothyroidism. Pediatrics 130(3):e699–e702
273. Ripp JA (1961) Soybean-induced goiter. Am J Dis Child 102:106–109
274. Bell DS, Ovalle F (2001) Use of soy protein supplement and resultant need for increased dose of levothyroxine. Endocr Pract 7(3):193–194
275. Jabbar MA, Larrea J, Shaw RA (1997) Abnormal thyroid function tests in infants with congenital hypothyroidism: the influence of soy-based formula. J Am Coll Nutr 16(3):280–282
276. Hofmann PJ, Schomburg L, Köhrle J (2009) Interference of endocrine disrupters with thyroid hormone receptor-dependent transactivation. Toxicol Sci 110(1):125–137
277. Divi RL, Chang HC, Doerge DR (1997) Anti-thyroid isoflavones from soybean: isolation, characterization, and mechanisms of action. Biochem Pharmacol 54(10):1087–1096
278. Bruce B, Messina M, Spiller GA (2003) Isoflavone supplements do not affect thyroid function in iodine-replete postmenopausal women. J Med Food 6(4):309–316
279. Milerová J, Cerovská J, Zamrazil V, Bílek R, Lapcík O, Hampl R (2006) Actual levels of soy phytoestrogens in children correlate with thyroid laboratory parameters. Clin Chem Lab Med 44(2):171–174
280. Bitto A, Polito F, Atteritano M, Altavilla D, Mazzaferro S, Marini H, Adamo EB, D'Anna R, Granese R, Corrado F, Russo S, Minutoli L, Squadrito F (2010) Genistein aglycone does not affect thyroid function: results from a three-year, randomized, double-blind, placebo-controlled trial. J Clin Endocrinol Metab 95(6):3067–3072
281. Li J, Teng X, Wang W, Chen Y, Yu X, Wang S, Li J, Zhu L, Li C, Fan C, Wang H, Zhang H, Teng W, Shan Z (2011) Effects of dietary soy intake on maternal thyroid functions and serum anti-thyroperoxidase antibody level during early pregnancy. J Med Food 14(5):543–550
282. Mittal N, Hota D, Dutta P, Bhansali A, Suri V, Aggarwal N, Marwah RK, Chakrabarti A (2011) Evaluation of effect of isoflavone on thyroid economy & autoimmunity in oophorectomised women: a randomised, double-blind, placebo-controlled trial. Indian J Med Res 133(6):633–640

283. Nakamura Y, Ohsawa I, Goto Y, Tsuji M, Oguchi T, Sato N, Kiuchi Y, Fukumura M, Inagaki M, Gotoh H (2017) Soy isoflavones inducing overt hypothyroidism in a patient with chronic lymphocytic thyroiditis: a case report. J Med Case Rep 11(1):253–258
284. Sathyapalan T, Manuchehri AM, Thatcher NJ, Rigby AS, Chapman T, Kilpatrick ES, Atkin SL (2011) The effect of soy phytoestrogen supplementation on thyroid status and cardiovascular risk markers in patients with subclinical hypothyroidism: a randomized, double-blind, crossover study. J Clin Endocrinol Metab 96(5):1442–1449
285. Rubtsova K, Marrack P, Rubtsov AV (2015) Sexual dimorphism in autoimmunity. J Clin Invest 125(6):2187–2193
286. Bianchi I, Lleo A, Gershwin ME, Invernizzi P (2012) The X chromosome and immune associated genes. J Autoimmun 38(2–3):J187–J192
287. Ohta A, Nagai M, Nishina M, Tomimitsu H, Kohsaka H (2013) Age at onset and gender distribution of systemic lupus erythematosus, polymyositis/dermatomyositis, and systemic sclerosis in Japan. Mod Rheumatol 23(4):759–764
288. Peeva E, Venkatesh J, Diamond B (2005) Tamoxifen blocks estrogen-induced B cell maturation but not survival. J Immunol 175(3):1415–1423
289. Laffont S, Seillet C, Guéry JC (2017) Estrogen receptor-dependent regulation of dendritic cell development and function. Front Immunol 8:108
290. Hughes GC, Choubey D (2014) Modulation of autoimmune rheumatic diseases by oestrogen and progesterone. Nat Rev Rheumatol 10(12):740–751
291. Gold SM, Voskuhl RR (2009) Estrogen and testosterone therapies in multiple sclerosis. Prog Brain Res 175:239–251
292. Arnaud L, Fagot JP, Mathian A, Paita M, Fagot-Campagna A, Amoura Z (2014) Prevalence and incidence of systemic lupus erythematosus in France: a 2010 nation-wide population-based study. Autoimmun Rev 13(11):1082–1089
293. Chighizola C, Meroni PL (2012) The role of environmental estrogens and autoimmunity. Autoimmun Rev 11(6–7):A493–A501
294. Wang F, Roberts SM, Butfiloski EJ, Morel L, Sobel ES (2007) Acceleration of autoimmunity by organochlorine pesticides: a comparison of splenic B-cell effects of chlordecone and estradiol in (NZBxNZW)F1 mice. Toxicol Sci 99(1):141–152
295. Zhao JH, Sun SJ, Horiguchi H, Arao Y, Kanamori N, Kikuchi A, Oguma E, Kayama F (2005) A soy diet accelerates renal damage in autoimmune MRL/Mp-lpr/lpr mice. Int Immunopharmacol 5(11):1601–1610
296. Hong Y, Wang T, Huang C, Cheng W, Lin B (2008) Soy isoflavones supplementation alleviates disease severity in autoimmune-prone MRL-lpr/lpr mice. Lupus 17(9):814–821
297. Fort P, Moses N, Fasano M, Goldberg T, Lifshitz F (1990) Breast and soy-formula feedings in early infancy and the prevalence of autoimmune thyroid disease in children. J Am Coll Nutr 9(2):164–167
298. Tran L, Hammuda M, Wood C, Xiao CW (2013) Soy extracts suppressed iodine uptake and stimulated the production of autoimmunogen in rat thyrocytes. Exp Biol Med (Maywood) 238(6):623–630
299. Portman MA, Navarro SL, Bruce ME, Lampe JW (2016) Soy isoflavone intake is associated with risk of Kawasaki disease. Nutr Res 36(8):827–834
300. Mahmoud AM, Yang W, Bosland MC (2014) Soy isoflavones and prostate cancer: a review of molecular mechanisms. J Steroid Biochem Mol Biol 140:116–132
301. Lu LJ, Anderson KE, Grady JJ, Nagamani M (2001) Effects of an isoflavone-free soy diet on ovarian hormones in premenopausal women. J Clin Endocrinol Metab 86(7):3045–3052

Jaboticaba: Chemistry and Bioactivity

41

Natália Crialeison Balbo Vall Ribeiro, Andressa Mara Baseggio, and Vicki Schlegel

Contents

Abstract

The jaboticaba is a fruit native to Brazil that grows in the wild throughout the country but is also cultivated on a low-scale basis by small farmers. Research is currently being reported that jaboticaba is a rich source of bioactive compounds, particularly the phenolic compounds. For example, high levels of the anthocyanins, cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside, and ellagitannins/ellagic acid are the predominant phenols present in jaboticaba and reside primar-

N. C. B. V. Ribeiro · A. M. Baseggio
Department of Food Science, Faculty of Food Engineering, University of Campinas, Campinas, Brazil

V. Schlegel (✉)
Department of Food Science and Technology, University of Nebraska Lincoln, Lincoln, NE, USA
e-mail: vschlegel3@unl.edu

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_24

ily in the peel and seeds of the fruit. These substances have been linked to multiple health benefits, including the prevention and/or mitigation of oxidation, inflammation, atherosclerosis risk factors, cancer, and conditions involved with metabolic syndrome. The fruit, or substances therein, has also been shown to enhance the immune system and gut microbiome. Therefore, the objective of this manuscript is to review the health-promoting properties exerted by jaboticaba or the compounds that reside in the fruit demonstrated throughout the literature, with an emphasis on the phenols.

Keywords

Jaboticaba · Bioactive agents · Phenols · Flavonoids · Anthocyanins · Ellagic acid · Gallic acid · Health benefits · Dietary bioactivity · Ellagitannins

List of Abbreviations

ATP	Adenine triphosphate (ATP)
CD40	Cluster of differentiation 40 protein
CD83	Cluster of differentiation 83 protein
CD86	Cluster of differentiation 86 protein
DNA	Deoxyribonucleic acid
GI	Gastrointestinal
H_2O_2	Hydrogen peroxide
hBD-	Human beta defensin 2
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HIV	Human immunodeficiency virus
iNOS	Inducible nitric oxide synthase
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LOX-1	Lectin-like oxidized low-density lipoprotein receptor
MetS	Metabolic syndrome
MMP	Matrix metalloproteinase
NO	Nitric oxide
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SLP1	Secretory leukocyte protease inhibitor
TC	Total cholesterol
w/w	Weight/weight

1 Introduction

The jaboticaba (*Plinia* sp.) is an edible tropical fruit native to Brazil that grows wildly throughout the country with the Minas Gerais and São Paulo states reporting the highest yields. This tiny black berry belongs to the family, Myrtaceae, which is

the same family as the guava tree. The fruit has a thin, smooth skin and a moisty white, translucent squash-like center that is sweet and slightly acidic [1]. Chemical characterization of the jaboticaba, particularly the peel, has revealed the presence of multiple health-promoting compounds, including phenolic compounds (e.g., flavonoids and phenolic acids) and insoluble and soluble fiber (e.g., pectin) [2]. More specifically, ellagic acid and its derivatives are important phenolic molecules present in jaboticaba due to their powerful antioxidative properties [3]. Other flavonoid compounds predominant in the jaboticaba peel are the anthocyanins, cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside. Anthocyanins are a class of flavonoid pigments that are also powerful antioxidants but have other multiple cellular stresses and disease-preventing properties [4–6]. Finally, pectin is a heteropolysaccharide composed of galacturonic acid, arabinose, galactose, and rhamnose, which contributes to its high capacity for forming gel, thereby aiding in the digestive health. A decrease in macronutrient absorption, specially fats and sugars, potentially leading to glycaemia, or improving the plasma lipid profile, is yet another benefit of pectin intake [7]. As a climacteric fruit, the maturation process continues postharvest, resulting in the activation of many metabolic pathways that degrade or modify many of the previously cited beneficial compounds with the anthocyanins being the most affected [2, 8].

2 Distribution and Botanical Characterization

The jaboticaba is cultivated by small farmers on a low-scale basis but is ubiquitously distributed throughout Brazil, especially in the Atlantic Forest biome [2, 9]. The jaboticaba tree has novel characteristics that include white blossoms located on both the trunk and branches that develop into the berry [10] (Fig. 1). In addition, the fruit matures rapidly (between 40 and 60 days); this aspect is related to the scientific name of the first jaboticaba species discovered in Brazil (*Plinia cauliflora*). The jaboticaba has a spherical shape that is 2.0–3.5 cm in diameter and contains one to four seeds. The pulp is mainly white and sweet, while the peel is dark purple when mature [10, 11] (Fig. 1). The color of the jaboticaba peel is due to the presence of high levels of anthocyanin pigments, primarily cyanidin 3-*O*-glucoside and delphinidin-3-*O*-glucoside. Two other jaboticaba species are also indigenous to the Brazilian territory, i.e., *Plinia cauliflora* ("Jabuticaba assú," in popular language) and *Plinia jaboticaba* ("Jabuticaba sabará," in popular language). Differences between species are evident in the tree characteristics. The *Plinia cauliflora* is a small tree (3–6 m in height) with long leaves (2–6 cm in length), while the *Plinia jaboticaba* is a taller tree (6–9 m in height) with smaller leaves (2–4 cm in length) [11]. The *Plinia jaboticaba* is the most widely cultivated and recognized species that grows mainly in the states of Minas Gerais and São Paulo [2]. Jaboticaba postharvest shelf life is relatively short due to its high moisture levels along with a fragile skin requiring mild industrial processes. Jaboticaba perishability also affects the functional features of the berry.

Fig. 1 Jaboticaba tree showing berries on trunk and branches [10]

3 Nutritional Composition of Jaboticaba

As stated previously, several studies have reported that the jaboticaba is a rich source of chemically diverse nutrients. For example, carbohydrates are the most abundant macronutrient followed by proteins and lipids [9, 12–17] (Table 1). High concentrations of fructose are located in the pulp [13], while higher fiber levels (soluble and insoluble) are present in the peel [14]. A wide variety of minerals are also present in jaboticaba with the predominant being potassium, magnesium, and phosphorus [13, 15] (Table 1). Moreover, whole fresh jaboticaba contain high levels of vitamin C [16] (Table 1). It must be emphasized that non-nutrients (as bioactive compounds) of the jaboticaba are currently receiving intensive interest, especially those that reside in the peel. These compounds are the secondary metabolites produced by the plant that provide protection against external hazards, such as disease, seed/insert pressure, and various types of bacterial/yeast infections. Similarly, these compounds have shown multiple human health benefits [12, 18–21]. Table 2 provides a summary of seminal research conducted on the health-promoting properties, while these and other studies are described in more details throughout the document on the fruit

Table 1 Different species of jaboticaba: chemical characterization

Species	Origin	Macronutrients (g/100 g)				Micronutrients (mg/100 g)		Ref.
		CHO[a]	PTN[b]	LIP[c]	Dietary fiber	Specific minerals	Specific vitamins	
Whole fruit								
Plinia cauliflora	São Paulo	78.2	1.02 ± 0.01	0.55 ± 0.01		K = 1320 Mg = 120	–	[13]
Plinia jaboticaba	São Paulo	76.5	0.94 ± 0.02	0.40 ± 0.10		K = 1000 Mg = 100	–	[13]
Plinia jaboticaba	Minas Gerais	90.1 ± 0.01	5.0 ± 0.10	1.8 ± 0.00	38.2 ± 0.06	K = 700.7 ± 81.20 Mg = 72.3 ± 7.60	Ascorbic acid = 8.6 ± 0.20	[9]
Plinia cauliflora	Santa Catarina	77.64	0.78 ± 0.00	0.53 ± 0.17	9.26 ± 0.03	–	–	[17]
Plinia cauliflora		–	–	–	–	–	Ascorbic acid=238 ± 2.2	[16]
Jaboticaba peel								
Plinia jaboticaba	São Paulo	62.75	7.31 ± 0.17	2.71 ± 0.17	28.79 ± 0.37	–	–	[12]
Plinia jaboticaba	São Paulo	72.06 ± 0.25	6.85 ± 0.05	2.94 ± 0.02	33.86 ± 1.38	–	–	[14]

[a]CHO = Total carbohydrate content
[b]PIN = Total protein content
[c]LIP = Total lipid content

Table 2 Bioactive compounds identified in jaboticaba

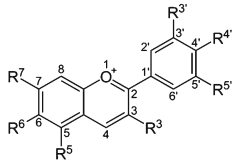
Species	Fruit part	Main bioactive compounds and concentration	Health effects	Ref.
Plinia jaboticaba	Peel	Cyanidin-3-*O*-glucoside (1541 ± 45.51 mg/100 g) and Delphinidin-3-*O*-glucoside (634.75 ± 1.83 mg/100 g)	In vitro: High antioxidant capacity, antiproliferative activity against tumor cells	[18]
Plinia jaboticaba	Peel	Gallic acid 4.03 mg/100 g	*Sprague-Dawley rats* fed with high-fat diet: ↓ lipid peroxidation in the brain	[19]
		Ellagic acid 348.08 mg/100 g	↑ antioxidant defenses in the liver	
		Quercetin 4.82 mg/100 g		
Plinia jaboticaba	Peel	Gallic acid 177.76 ± 2.26 μg/g	*Wistar rats* fed with high-fat diet ↑ antioxidant defenses in liver and plasma	[12]
		Ellagic acid 1581.61 ± 135.50 μg/g		
		Cyanidin-3-*O*-glucoside 34,242 ± 594.70 μg/g		
Plinia jaboticaba	Peel	Cyanidin-3-*O*-glucoside 1113.38 ± 19.37 mg/100 g	*Swiss* mice fed with high-fat diet: Improvement of cognitive function and prevention of insulin resistance and fat weight gain	[20]
		Ellagic acid 710.10 ± 33.77 mg/100 g		
Plinia jaboticaba	Peel	Cyanidin-3-*O*-glucoside 2866 ± 40.1 mg/100 g	In vitro: ↑ total antioxidant capacity (especially ellagitannins)	[21]
		Delphinidin-3-*O*-glucoside 356.3 ± 1.0 mg/100 g	In vivo: ↑ serum antioxidant capacity in humans	
		Ellagic acid 142.8 ± 11.7 mg/100 g		

and/or its components. Moreover, Fig. 2 shows the structure of the main phenolic components present in the jaboticaba. However, readers are referred to [10] for a comprehensive illustration of the multiple structures of the phenol compounds with only the primary phenols present in jaboticaba presented in Table 3.

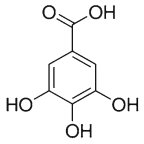
4 Health Benefits of Jaboticaba or Its Components

4.1 Antioxidative Properties

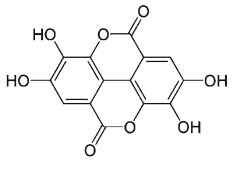
The term "oxidative stress" is defined as imbalance in metabolism that leads to the accumulation of reactive oxygen species and/or reactive nitrogen species (ROS/RNS) in the cells [21]. Excessive levels of free radicals are able to overwhelm the endogenous antioxidant capacity, resulting in oxidation damage to structural, transport, and regulatory molecules, such as proteins, DNA, and lipids [21]. If left unchecked, oxidative stress can lead to the onset of chronic degenerative diseases



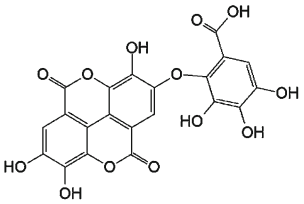
A. Anthocyanins: Basic structure.
Delphinidin-3-O-glucoside: R3'=OH, R4'=OH, R5'=OH, R3=O-Glucoside, R5=OH, R6=H, R7=OH, R8=H
Cyanidin-3-glucoside: R3'=OH, R4'=OH, R5'=H, R3=O-Glucoside, R5=OH, R6=H, R7=OH, R8=H



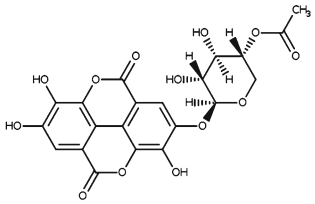
B. Gallic acid



C1. Ellagic acid
(Basic Structure)



C2. Valoneic acid dilactone
Conjugate of ellagic acid



C3. Ellagic acid-pentose
Conjugate of ellagic acid

Fig. 2 Common small phenols present in jaboticaba

that include coronary heart disease and cancer, as reviewed in [18]. Dietary antioxidants have been shown to protect or mitigate oxidative stress by scavenging the free molecules and/or binding reactive metal ions [22, 23]. However, the phenolic antioxidants are more likely to aid in the activation or upregulation of antioxidative enzymes, such as superoxide dismutase or catalase, or inhibit or downregulate oxidative enzymes, such as xanthine oxidase or inducible nitric oxide synthase (iNOS) [24–27].

Several studies have demonstrated the potent antioxidant properties of the jaboticaba. For example, the antioxidant capacity of extracts obtained from seven Brazilian fruits was evaluated using the 1,1-diphenyl-2-picrylhydrazyl radical test and the β-carotene-linoleic acid-coupled oxidation assay [28]. While all the fruits exhibited high antioxidative capacity, jaboticaba produced the highest activity for both tests. From a potential human health perspective, Leite et al. [29] analyzed the antioxidant status of rats fed with a high-fat-inducing obesity diet but supplemented with freeze-dried jaboticaba peel (at 1, 2, and 4% (w/w)). Compared to the animals fed with the high-fat diet only, the supplemented diets resulted in lower serum saturated fatty acids, higher plasma antioxidant defenses, decreased

Table 3 Other phenols reported in jaboticaba

Phenol[a]	Classification
Hexahydroxydiphenoyl group	Ellagitannin
Hexahydroxydiphenoyl-galloyl-glucose	Ellagitannin
Casuariin	Ellagitannin
Casuarinin	Ellagitannin
Tellimagrandin I	Ellagitannin
Tellimagrandin II	Ellagitannin
Pedunculagin	Ellagitannin
Casuaricitin	Ellagitannin
Iso-oenothein	Ellagitannin
Oenothein C	Ellagitannin
Pedunculagin	Ellagitannin
Casuaricitin	Ellagitannin
2-*O*-(3,4-Dihydroxybenzoyl)-2,4,6-trihydroxyphenyl acetic acid jaboticaba	Depside
Syringin	Phenolic acid aglycone
Syringin-*O*-glucoside	Phenolic acid glucoside
O-Coumaric acid	Phenolic acid aglycone
Protocatechuic acid	Phenolic acid aglycone
Methyl protocatechuic acid	Methylated phenolic acid
Peonidin	Anthocyanin methoxy derivative
Peonidin-3-*O*-glycoside	Anthocyanin glucoside
Isoquercitrin	Flavonoid glucoside
Quercimeritrin	Flavonoid conjugate
Myricetin	Flavonoid aglycone
Quercetin	Flavonoid aglycone
Rutin	Flavonoid glucoside

[a]Information obtained from [11] but tabulated instead of illustrated

lipid peroxidation of the liver and brain, and higher antioxidative status of the kidneys, depending on peel dosage. Moreover, Calloni et al. [30] reported that extracts from the jaboticaba peel were able to reduce H_2O_2-induced mitochondrial oxidative stress in human lung fibroblast cells by mitigating the decrease in the activity of the mitochondrial complex 1 protein and ATP levels. The researchers proposed that this berry may provide protection to the mitochondria when exposed to dysfunctional conditions. Martins de Sá [31] showed that fermented jaboticaba beverages (red, rose, and white) exerted a significant vasorelaxant effect on isolated arteries, which was inversely associated with the antioxidative capacity of a given beverage. The red drink produced the highest antioxidative activity and the lowest vasorelaxant response followed by the rose and then the white fermented beverages. While other studies have also shown jaboticaba as a rich source of antioxidative agents, the above-discussed reports provide a representation of the related

experiments currently being conducted [18, 32–34] with the majority attributing the phenols as the responsible components in protecting against oxidation, especially the anthocyanins.

The basic structure of flavonoids consists of two phenolic rings (Fig. 1) with a center heterocyclic ring bonded to either hydrogen, water, hydroxyl groups, carbohydrates, or organic acids at carbon sites on the rings. The various type of hydroxyl binding to the rings and position of the rings is specific to a particular flavonoid class [35]. Individual anthocyanins are distinguished from other flavonoids by the number and position of hydroxyl and methoxy groups on its basic structure and also by the type and the number of bound sugars or acids (Fig. 1) [5]. Importantly, anthocyanins are the only flavonoid able to exist as a cation or anion [8], resulting in color changes from deep purple to red depending on the pH. Among the multiple in vitro and in vivo studies that have been conducted to understand the antioxidative benefits of anthocyanins [36, 37], the following studies are discussed to provide support, in part, to the hypothesis that anthocyanins are the dominant antioxidative protective agents in jaboticaba. Using rats subjected to hepatic ischemia-reperfusion to produce oxidative stress after consumption of an anthocyanin supplemented diet, Tsuda et al. [38] determined that the consumption cyanidin-3-glucoside and its aglycone form produced an antioxidant response similar to that of α-tocopherol, specifically in preserving the liposomes, liver, and erythrocyte membranes. Ramirez-Tortosa et al. [39] also utilized a rat model, but the animals presented with a vitamin E deficiency making the animal susceptible to oxidative stress. Yet, when treated with an anthocyanin extract supplementation, the plasma antioxidative capacity increased relative to the control. As a result, the damage caused by the vitamin E deficiency, lipid peroxidation, and DNA damage was reduced. Based on the French Paradox, this study was conducted on the anthocyanins present in red wine. Importantly, the researchers determined that dietary anthocyanins were able to protect against the risks associated with oxidative stress.

Ellagic acid may or may only slightly contribute to the antioxidant protective properties of jabocatica. While this molecule is able to scavenge free radicals [40] and other characteristics in terms of protection against lipid peroxidation and DNA damage and increasing the activities of the antioxidative enzymes that include super oxide dismutase, catalase, and glutathione peroxidase in the presence of an oxidant using cell culture models [41, 42], its antioxidant protection in human health is sporadic, especially compared to other phenols. Meyer et al. [43] showed that five different phenols were able to inhibit copper-catalyzed low-density lipoprotein (LDL). The flavan-3-ol, catechin, exhibited the highest antioxidant protection followed by the anthocyanin, cyanidin, which was similar to caffeic acid, followed by quercetin and finally ellagic acid. The differences were attributed to the structural dissimilarities of the compounds. Interestingly, when the phenols were combined, an additive response was obtained with the notable exception of ellagic acid, which exerted a substantial antagonistic response. Yet, ellagic acid was able to ameliorate the effect against negative effects of cisplatin, a cytotoxic agent drug used in chemotherapy, using a rat model administered with this drug through gavage delivery. Turk et al. [44] showed that rats supplemented with ellagic acid were

presented with decreased plasma, sperm, and testicular T-bar levels (a secondary oxidation indicator). The ellagic acid supplement also activated the activity of catalase and glutathione peroxidase.

4.2 Immune System Health and Anti-inflammatory Properties

The immune system is a complex network composed of a variety of molecules, cells, tissues, and organs that act to defend their host against multiple invading infectious agents or mechanistic dysregulations [45]. When the immune system is exposed to these external/internal stresses, the inflammation response is initiated, which is the body's way of dealing with localized tissue damage and infection, and thus is critical to our survival. As such, maintaining a robust immune system is essential to human health. According to recent studies, the diet plays an important role in either adversely or positively regulating the immune system. For example, researchers determined that chronic, or silent, inflammation stimulates a self-perpetuating immunological responses that are linked to nutrient overload intake, which, in turn, can lead to multiple health risks [46–48]. On the other hand, certain foods or their components can prevent the onset of harmful cellular stressors or diseases that enhance and strengthen the immune system by acting upon or preventing chronic inflammation.

Chan et al. [49] completed a study using monocyte-induced dendritic cells treated with gallic acid (a phenolic acid ubiquitous to many foods, including jaboticaba) followed by a cocktail of pro-inflammatory cytokines. The results showed that various markers involved in the maturation of dendritic cells were suppressed by gallic acid, such as the stimulatory proteins, CD40/CD83 and CD80/CD86 responsible for activating antigen-presenting cells and T cells via co-stimulation, respectively [49].

Moreover, consuming ellagic acid-rich fruits and vegetables may aid in maintaining oral homeostasis as this phenolic acid can also enhance the integrity of epithelial oral cells, as reviewed in more detail by Dale [50]. Ellagic acid consists of two resonant phenolic rings, each bound to para-hydroxyl groups and two heterocyclic [51] (Fig. 2). This compound has been shown to be particularly potent in protecting the immune system from a variety of onslaughts. A study completed by Fakhry et al. [52] used oral epithelial cells to determine that ellagic acid increased the expression of beta-defensin 2 and antileukoproteinase. Beta-defensin 2 consists of an antimicrobial factor produced by neutrophils that attaches gram-negative microorganisms [53]. Alternatively, antileukoproteinase protects epithelial cells from serine proteases, which if left unchecked, degrade peptide bonds of proteins, thereby contributing to damages to mucosal cells. Such a response to ellagic acid contributes to a healthy immune response as strong mucosal cells promote physical and chemical barriers against pathogens. The physical barrier consists of cell-to-cell attachments, while the chemical barrier is constructed by antimicrobial peptides,

cytokines, and chemokines [53]. Another important factor related to the health of mucosal cells as mediated by ellagic acid includes the prevention of HIV transmission. The human β-defensin peptide, Hbd2, and the secretory leukocyte protease inhibitor, SLPI, have been recognized as mediators that not only enable the mucosal to defend against microbial infections but also inhibit adenoviral infections, such as HIV, as demonstrated by QuiÃones-Mateu et al. [54]. Yet another study completed by Promsong et al. [55] used ellagic acid-treated primary human gingival epithelial cells and showed that this bioactive agent (in concentrations of only μmoles) was able to increase the expression of Hbd2 and SLPI by 3.7- and 2.5-fold relative to the control (untreated cells). In addition, Modi et al. [56] showed that the ellagic acid inhibits HIV-1 integrase enzyme, which is responsible for integrating the virus DNA to the genetic content of the host DNA. As approximately 90% of HIV transmission occurs via mucosal cells, ellagic acid-rich foods, such as jaboticaba, may be yet another important preventative measure for contracting HIV by acting on the cited potential targets.

Additionally, anthocyanins have been linked to immune health, particularly via their anti-inflammatory properties. By enlisting female subjects (18–76 year, $n \sim 1250$) Jennings et al. [57] determined their intake of total flavonoids as well as individual subclasses, including the anthocyanins by using a frequency questionnaire. In terms of chronic inflammatory, the high-sensitivity C-reactive protein marker was monitored and showed that higher anthocyanin intake was associated with lower levels of this marker. In fact, no other associate with total or other flavonoid subclasses occurred with this inflammation marker. In yet another study using human subjects (31–315), Hassellund et al. [58] monitored the effects of anthocyanins on inflammation risk factors in prehypertensive male subjects (35–51 year, $n = 27$). The men were divided into two groups with one provided an anthocyanin supplement (640 mg) and the other a placebo for 4 weeks followed by a 4-week washout. No beneficial responses were detected in multiple inflammatory cytokines monitored, but endothelial dysfunction improved with the anthocyanin supplementation. It must be noted that the sample size was small in this study and trial duration short. Yet, Mena et al. [59] provided a review on the potential of anthocyanins, a powerful anti-inflammatory agent.

However, foods are not purified compounds acting on single targets but rather complex mixtures that may affect many biochemical pathways. Nonetheless, studies remain limited on the benefits of the whole jaboticaba fruit or the peel with following exception to our knowledge. Batista et al. [19] used Swiss mice to determine the effects of a high-fat diet supplemented with only 4% of jaboticaba peel on multiple markers for development of tauopathies, including hippocampal inflammatory markers, tumor necrosis factor-α, and interferon-γ. Firstly, the added peel supplement did not adversely affect either inflammatory markers as the animals fed with the high-fat supplemented diets presented with significantly similar values as those on the normal diet. Yet, compared to the mice fed with the high-fat diet only, these markers were 100% lower for the animals fed with the jaboticaba peel supplemented diet. Along with the anthocyanins and ellagic acids, the ellagitannins may also have

exerted a positive effect to mitigate inflammation exhibited the jaboticaba as supported by the following review [60].

4.3 Cancer Prevention and Mitigation Properties

Cancer is leading cause of death in many western countries, including the United States, second only to heart disease [61]. This disease is caused by uncontrolled cell proliferation leading to the formation of tumors. Tumors are complex structures that are composed of cells that have altered regulatory processes allowing a given tumor to grow in most types of tissues and/or organs. Proliferating cells (or neoplastic cells) are capable of shutting down the senescence pathway of a normal cell securing their perennial life [62, 63]. Furthermore, cancer cells release chemical signals that promote angiogenesis around the tumor structure, thereby supplying oxygen, blood, and other nutrients needed for growth. These remarkable cancer features are the primary reason this disease leads to high mortality rates [62, 63]. Additionally, proliferating cells reach a development point where they are able to combine signaling with taxis capacity and, as a consequence, metastasize to other tissues different from their original point of origin, which is yet another lethal characteristic associated with cancer [63, 64].

An estimated one-third of the cancer deaths are related to lifestyle that include diet factors [65]. Yet, diets rich in various natural systems, such as tomatoes, carrots, and berries, have exerted impressive effects in mitigating cancer [64], with jaboticaba providing potent anticancer properties among the berries [66]. A recent study conducted by Wang et al. [33] tested different sections of the fruit to identify the fractions that exerted most effective anticarcinogenic response. In order to accomplish this aim, human oral carcinoma cells obtained from the apical layer of the epithelial tissue were treated with extracts (aqueous or ethanoic) recovered from the seed, stem, and peel of the jaboticaba. The results showed that the water-extracted seed fraction provided the most potent effects in lowering cellular proliferation compared to the control by acting as inhibitors of apoptosis. Leite-Legatti et al. [18] reported that polar and nonpolar peel extracts of jaboticaba were able to provide effective antiproliferative properties against leukemia cells (K-562) and prostate cancer cells (PC-3), respectively, of the 11 different cancer cell types tested. It is evident from these two isolated studies that more research is needed to determine the anticancer potency of the whole fruit or its individual parts in vivo as well as to characterize the anticancer mechanism.

Nonetheless, several studies have reported on the anticancer mode of action exerted by the primary bioactive agents in jaboticaba credited (ellagic acid, gallic acid, anthocyanins, and ellagitannins) as shown by the following seminal reports. Firstly, ellagic acid has been shown to initiate a cascade of events in normal human lymphocytes that resulted in declining rates of DNA synthesis during the cell cycle. Also, the acid induced cell apoptosis by generating DNA molecule fragmentation, thereby preventing the neoplastic cells from proliferating [51]. Losso et al. [67] evaluated the antiproliferative activities of ellagic acid against eight different cell

lines with doses ranging from 10 to 100 mol/L. The most susceptible cells to the antiproliferative activity of ellagic acid were those derived from the colon (Caco-2), but the breast and prostate lines were also strongly impacted. As evidenced by microscopic examination of the cell gross morphology, ellagic acid prevented proliferation via induction of apoptosis. Moreover, lower ATP levels were produced, which is essential for cancer cell viability, indicating that ellagic acid exerts both a selective cytotoxicity and an anti-proliferation effect. Finally, ellagic acid was able to prevent fetal bovine stimulation of cell migration at the highest concentration used, suggesting that this compound may also mitigate metastasis. Edderkaoui et al. [68] determined that ellagic acid induced apoptosis in pancreatic cancer lines, which, in turn, prevented proliferation by approximately 20-fold at a concentration of 50 mmol/L. These results were associated with the depolarization of the mitochondria, release of cytochrome C, and activation of downstream caspase, which were attributed to the ellagic acid dose-dependent decrease of the binding activity of the transcription factor, nuclear factor kappa β. For more information about the ellagic acid effects in response to cancer and potential mechanisms, refer to Stoner and Muktar [69], Maas et al. [70], and Landete [60].

Secondly, as the largest phenolic compound present in jaboticaba, ellagitannins do not absorb into the bloodstream but rather are physiologically hydrolyzed to ellagic acid in the large intestine by the microbiota (refer to Sect. 4.5), which, in turn, is metabolized to smaller metabolites, such as the urolithins [71]. Urolithins A and C in turn have shown to inhibit HT-29 (colon cancer cells) cell proliferation via B0/G1 and G2/M arrest followed by the induction of apoptosis. Urolithins are also advantageous to cancer prevention as they are able to remain in the colon through enterohepatic circulation [72]. As an outcome to its metabolism into multiple but potent health-promoting metabolites, ellagitannins have been shown to have multi-targeted effects [73]. For example, Seeram et al. [74] showed that 25 miRNA, including the let family members, MiR-370, MiR-373, and miR526b, were likely targets of preventing proliferation and differentiation of Hep-G2 liver cancer cells. Indeed, other studies have been completed to show the chemopreventive properties of ellagitannins against prostate, colon, breast, oral/gastric, liver, cervical, lung, and skin cancer as reviewed by [72, 73].

Thirdly, current research has shown that gallic acid has exceptionally potent anticarcinogenic properties due to its ability to act on multiple targets. For example, gallic acid is able to prevent cancer by targeting angiogenesis, which is accomplished by concurrently inhibiting its initiation and by preventing additional neovessel growth after angiogenesis has already commenced [68–75]. More specifically, Lu et al. [75] determined that gallic acid was able to reduce glioma cell-mediated angiogenesis, in addition to inhibiting cell viability, proliferation, and invasion with U87 and U251 glioma cells as the cancer models. Using nude mice that were subcutaneously injected with the prostate cell lines 22Rv1 and DU145, Kaur et al. [76] reported that gallic acid consumption reduced the microvessel density in tumor xenografts as compared to the control. This study also showed that gallic acid decreased cell viability in a dose-dependent manner in both cell lines primarily by apoptosis induction. A recent study completed by Ho et al. [78] showed that the

exposure of squamous carcinoma cells to gallic acid resulted in inhibiting the activation of two matrix metalloproteinase (MMP) enzymes, MMP-2 and MMP-9, which are responsible for modulating cytokines and growth factors involved in angiogenesis as well as the invasion and migration of cancer cells [79]. As such the gallic acid may execute an important function against the tumor development. For more information on the anticancer effects of gallic acid, the reader is directed to excellent reviews presented by Verma et al. [77] and Locatelli et al. [80].

Lastly, the anthocyanins protect against cancer development due, in part, to their ability to bind directly to cell cycle regulator proteins, such as p53, p21, and cyclins [81, 82] and thus preventing cell proliferation. Additionally, these bioactive agents are able to increase the membrane potential of the mitochondrial membrane l and induce the accumulation of ROS resulting in cell apoptosis [83]. Other studies have shown that anthocyanins are able to downregulate the expression of MMP, which, in turn, decreases the ability of the tumor cell to degrade the extracellular matrix and invade new tissues [84, 85]. Anthocyanins have been shown to target the regulation of various cytokines involved in angiogenesis [86]. (Wang et al. [87] provides an in-depth review of the anticancer mode of action by anthocyanins.)

4.4 Atherosclerosis Prevention and Mitigation

Atherosclerosis is a condition caused by accumulating lipids and fibrous compounds in major arteries [88, 89], resulting in narrowing the arteries and thereby reducing blood flow to vital organs. This cardiovascular state is responsible for approximately 50% of reported fatal heart diseases, including cardiovascular disease [90]. Atherosclerosis is diagnosed by established biomarkers, including total cholesterol (TC) that is highly associated with the ratio of low-density lipoprotein (LDL) to high-density lipoprotein (HDL) levels. The higher the ratio, the more serious the condition as LDL is easily oxidized, which is a significant contributing factor to atherosclerosis [88]. Oxidized LDL molecules enhance the production of reactive oxygen species (ROS), decrease the formation of NO (nitric oxide, an endogenous antioxidant), and stimulate the endothelium to express adhesion molecules and chemokines, which in turn recruit monocytes and T cells to the arterial wall [91, 92] ultimately forming foam cells and perpetuating inflammation. Effective treatments must be able to decrease LDL cholesterol (LDL-C) and/or raise HDL cholesterol (HDL-C) in the bloodstream.

Natural alternatives to the commonly used treatments, mainly statin drugs, have been of intense interest for decades due, in part, to the side effects and cost of these drugs [93]. Because of the presence of several potent bioactive agents, jaboticaba is now being studied as a potential food or source of agents to protect against atherosclerosis, although research again remains limited on the fruit itself with the following exceptions. Batista et al. [94] evaluated the effects of obese rats fed with a high-fat diet (lard) supplemented with only 1, 2, and 4% dried jaboticaba peel on the lipid profiles present in serum, liver, and fecal material. Although hepatic and serum

lipid contents were not impacted by the peel, fecal triglyceride excretion increased, particularly for the animals fed with the 1 and 2% supplements. Most importantly, evaluation of lipid peroxidation of the liver showed that the 2% supplement was able to lower peroxide values by 28.35% compared to the positive control fed with the high-fat diet only. In contrast, Alezandro et al. [95] showed that total plasma cholesterol levels and triglycerides were reduced by 32% and 50%, respectively, in streptozotocin-induced diabetic rats fed with 1.0 and 2.0 g dry weight/kg of body weight of jaboticaba extracts. The difference in serum levels between the two studies may be due to the induction stressors used to elevate the lipids, with the dietary inducer being more resistant. Another report by Batista et al. [19], who again fed rats lard as the atherosclerosis inducer and freeze-dried jaboticaba supplements (2% and 4%), showed that that peel reduced serum saturated lipids, another risk factor for atherosclerosis [96].

Relative to specific bioactive agents present in jaboticaba, ellagic acid may be a primary candidate responsible for protecting against cardiac damage caused by atherosclerosis. Several studies have shown that this molecule acts directly on LDL oxidation responses by inhibiting the proliferation of aortic smooth muscle cells and decreasing the levels of induced ROS generation that, in turn, activate adhesion molecules [97–99]. To prevent ROS induction, ellagic acid downregulates the expression of the lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), which is also responsible for the expression of pro-inflammatory molecules, such as cytokines, and chemokines in the endothelial cells. Moreover, this phenolic compound suppresses the expression of iNOS, which catalyzes a pathway that produces nitric oxide (NO). Under normal conditions, NO regulates arterial wall homeostasis. However, when the production of NO is upregulated by the oxidized LDL, as in the case of the iNOS pathway, the NO molecules bind with tyrosine residues that are essential for the vascular tone, thereby remediating further complications to the endothelial dysfunction [100–102]. Ellagitannins most likely act in the same manner due to their hydrolysis to ellagic acid and the urolithins. For more information on vascular health in response to ellagitannins and its metabolites, refer to the [103].

In addition, Xia et al. [104] determined that anthocyanins exert a unique effect in mitigating the progression of endothelial dysfunction, namely, by reducing the risk for catastrophic ruptures by reducing or stabilizing plaque. In this study, animals prone to the formation of weakened plaques (apolipoprotein E-deficient mice) were treated with diets rich in anthocyanins. At the end of the 20-week feeding study, plaques decreased by 18% and were firmer in texture, and necrotic core was not enlarged compared to the control group. Although the mechanisms involved in this phenomenon are not clear, the authors proposed that the anthocyanins augmented the antioxidant levels inside the plaque leading to increased stability. Moreover, the action of the anthocyanins can be related to the lipid serum level improvement as the anthocyanin-fed animals presented with reduced plasma cholesterol, LDL, and triglyceride levels. As in most of the studies related to bioactive compounds, there remains a critical lack of information regarding the pathways involved in protecting

against atherosclerosis or related risk factors. Nonetheless, it is clear from several reviews [60, 105–107] that flavonoids and other types of phenolic molecule are able to prevent or ameliorate the atherosclerosis status.

Although not a phenolic compound, which this review has been the focus, pectin is particularly high in jabocatica and must be recognized as such, particularly on its effects on risks for atherosclerosis. Multiple studies that date back to the early 1960s have shown the advantageous impact of pectin consumption, which are reviewed in [108, 109]. The most commonly reported response is that pectin facilitates the excretion of cholesterol and by inhibiting bile acid absorption [110].

4.5 Gastrointestinal Health: Gut Microbiota Modulation

The symbiotic relationship between human health and the gastrointestinal (GI) microbiome is garnering intense research with diet playing a major role [111–114]. Although studies pertaining to gut microbiota health and jaboticaba have not been reported to our knowledge, related research has been conducted on the prevalent bioactive agents of this berry. Firstly, the ellagitannins and ellagic acid are partially absorbed by the intestinal tract as their bioavailability is low. However, the remaining unabsorbed compounds remain intact until each reaches the large intestine where it is metabolized by the bacterial population, resulting in smaller polyphenolic metabolites (Table 4) [115–118]. These metabolites have been reported to exhibit both anti-inflammatory and antiproliferative properties [118, 119], among other health-promoting properties [60]. In addition, ellagic acid has been shown to modulate the gut microbiome to a healthy state. For example, Selma et al. [120] compared the production of urolithin A, a metabolite of ellagic acid, and other GI metabolized components of human subjects fed an ellagic acid-rich extract of pomegranate extract. After analyzing the composition of their fecal material subsequent to consumption of the supplement, the researchers determined that only certain test individuals presented with elevated production of ellagic acid metabolites while levels of these agents were low in other subjects. Those subjects that produced high levels urolithin A also had elevated *Clostridium coccoides* populations. This difference in metabolite production and the simultaneous alteration in the microbiome composition suggest a strong relationship between the two factors [120]. Another study completed by Li et al. [121] analyzed the relationship between the production of urolithin isomers and the gut microbiota composition in response to duration of consumption of an ellagic-rich pomegranate extract. In the 1st week, urolithins were present in the stool of only 30% of the individuals. By the 4th week, 50% of the individuals who did not produce urolithin excreted significant amounts of ellagic acid. This difference provides additional evidence that ellagic acid is likely playing an important role in the alteration of the gut microbiota composition but is subject specific. The same study reported a change in the *Firmicutes*/*Bacteroidetes* ratio present in the fecal material of the test subjects after extraction administration. Those who were able to produce urolithin A

Table 4 Microbial metabolism of ellagitannins, ellagic acid, and anthocyanins

Ellagitannins	Ellagic acid	Anthocyanins
Ellagic acid	Nasutin A	m-Hydroxyphenyl propionic acid
–	Isonasutin	Isoferulic acid
–	Urolithin M-5	3,4-Dihydroxyphenylacetic acid
–	Urolithin M-6	Ferulic acid
–	Urolithin M-7	Catechol
–	Urolithin C	3,4-Dihydroxphenylpropionic acid
–	Urolithin D	m-Hydroxyphenylacetic acid
–	Urolithin A	Dihydroferulic acid
–	Urolithin B	Phloroglucinaldehyde
–	Isourolithin A	Vanillic acid
–	Isourolithin B	p-Hydroxybenzoic acid
–	–	Protocatechuic acid
–	–	Gallic acid
–	–	Tyrosol
–	–	Resorcinol
–	–	Pyrogallol
–	–	Caffeic acid
–	–	Homovanillic acid
–	–	Syringic acid
–	–	p-Coumaric acid

experienced a decrease in the *Firmicutes* population with a concomitant increase in bacteria from the genus *Prevotella*, which belongs to the phylum *Bacteroidetes*. The ratio between those two phyla has been shown to be closely related to lean and obese phenotypes with higher levels of *Bacteroides* and lower levels of formicates associated to lower body weight [122, 123]. This fact is useful in the development of functional foods, such as jaboticaba, as means to target obesity through gut microbiota modulation.

In the case of anthocyanins, multiple studies have been reported based on their ability to modulate the gut microbiota, as reviewed by Faria et al. [116]. Again, the majority of anthocyanin levels consumed are not absorbed by the upper GI tract, thereby reaching the lower intestinal microbiota. Similar to ellagitannins and ellagic acid, anthocyanins are biotransformed into smaller but multiple metabolites, which are then absorbed through the large intestine (Table 4). Once absorbed, these compounds have been shown to exert different biological properties compared to their parent counterparts [124]. Using an in vitro fecal fermentation approach, Hidalgo et al. [125] further reported that anthocyanins were able to modulate the gut microbiota. More specifically, malvidin-3-glucoside enhanced the growth of the putatively beneficial bacteria, *Bifidobacterium* spp. and *Lactobacillus* spp., but did not affect *Bactericides* spp. growth. However, the two predominant anthocyanins present in jaboticaba, delphinidin 3-glucoside and cyanidin 3-glucoside [18], were not studied in isolation in terms of their ability to modulate the gut microbiome.

Rather, they were combined with malvidin-3-glucoside, peonidin-3-glucoside, and petunidin-3-glucoside to monitor the combined response on the gut microbiota. The results showed even higher levels *Bifidobacterium* spp. and *Lactobacillus* spp. with latter growth even higher than the positive control, which used the proven prebiotic fructooligosaccharide [126]. The authors attributed these results to a synergistic effect exerted by the anthocyanins in combination. As such, it is reasonable to propose that the anthocyanins present in jaboticaba peel are capable of modulating the gut microbiota to a healthy community.

Pectin in jaboticaba may be able to modulate the gut microbiota to a health state. Jiang et al. [127] showed that apple-derived pectin supplement into a high-fat-inducing obesity diet prevented a decrease in the *Bacteroidetes* population and an increase in the *Firmicutes* phylum. Parkar et al. [128] investigated the effects of six different pectins obtained from kiwifruit on gut health in terms of influence on bacterial adhesion on intestinal epithelial cells (Caco-2 cells). The pectin which originated from the kiwifruit was more effective, enhancing the adhesion of *Lactobacillus rhamnosus* while decreasing the adhesion to *Salmonella typhimurium*. Still the adhesion of *Bifidobacterium bifidum* significantly increased in the presence of inulin and citrus pectin. Indeed, studies are needed on pectin present in jaboticaba to determine its effect on the microbiome as another study completed by Nazzaro et al. [129] further confirmed that pectin source affects gut microbe modulation.

4.6 Metabolic Syndrome: Obesity and Diabetes

Metabolic syndrome (MetS) is a cluster of conditions that involve risks for cardiovascular diseases and type 2 diabetes and is prevalent with obese state [130, 131]. Specific markers of MetS include excessive central adiposity, higher serum glucose levels, increased serum triglycerides and LDL-C, lower HDL-C, and high blood pressure [132–135]. (As the latter conditions have already been discussed previously, with the exception of central adiposity and serum glucose levels, this section will mainly focus only on health risks associated with obesity and diabetes.) According to the World Health Organization [133], obesity is defined an excessive accumulation of fat tissue that can cause various negative effects on the human body, such as chronic oxidative and pro-inflammatory states [134, 135]. Diabetes is defined as the dysregulation of glucose absorption and breakdown pathway leading to glucose resistance and further ketosis, tissue damage, and many other metabolic failures [135]. As this MetS is becoming a worldwide epidemic [136], researchers are now focusing their efforts on discovering bioactive compounds that could prevent or decrease the damages caused by these alarming clinical markers.

The relationship between cellular metabolic responses and the antioxidants present in the peel of the jaboticaba fruit is a main focus of several research studies. For example, in a recent study conducted by Lenquiste et al. [137], freeze-dried jaboticaba peel was administered at 1%, 2%, and 4% (w/w) of the diet as a supplement to

rats fed with a high-fat diet added to an AIN-93 M diet. Consumption of the high-fat diet + jaboticaba supplementation resulted in lower weight gain compared to the high-fat diet. Moreover, the antioxidant profile of the liver improved for rats fed with the high-fat + supplement diet, while those on the high-fat diet presented with significantly higher levels of lipid peroxidation. Meanwhile, serum insulin levels reduced by 47%, 57%, and 52% with respect to the animals fed with the 1%, 2%, and 4% freeze-dried jaboticaba peel supplemented diet. A previous study using freeze-dried jaboticaba peel supplement fed to a high-fat-induced obese rat model showed that incidence of type 2 diabetes was directly associated with plasma cholesterol ester levels and total short-chain fatty acids [19]. These factors are closely related to inflammation and insulin resistance [135]. In another study, Batista et al. [94] reported that high fat obesity induced animals fed three different concentrations of freeze-dried jaboticaba extracts (1%, 2%, and 4%) resulted in increased triglyceride output, decreased hyperinsulinemia, decreased hepatic inflammation, and improved insulin sensitivity. However, only the 4% diet exerted these benefits. The authors proposed that results were associated with a decrease in plasma short-chain fatty acids, which has been linked to a downregulation in the expression of lipogenic genes [83].

The anthocyanins present in jaboticaba may, in part, play a role in mitigating the risk factors of MetS by inhibiting the release of inflammatory adipokines secreted by the hypertrophic adipose tissue that contribute to insulin resistance as evidenced by Matsukawa et al. [138]; albeit only the anthocyanin, cyaniding-3-glucoside, was used. Still, this effect was attributed to the anthocyanin interacting with proteins involved in insulin signaling that induce a state of insulin resistance. Garcia-Diaz et al. [139] showed that anthocyanin-rich fractions obtained from blackberry and blueberry beverages were able to reduce intracellular fat accumulation when applied during the differentiation of 3T3-L1 adipocytes followed by the inhibition of lipolysis of the mature cells. Moreover, the blends partially restored the insulin-induced glucose uptake by the adipocytes. This effect was attributed to the interaction of the anthocyanins with proteins involved in insulin signaling that induce a state of insulin resistance. Using a mice model, Prior et al. [140] fed three groups increasing levels of fat that contributed to 10%, 45%, or 60% of their daily calories. The diets were supplemented with whole blueberries, strawberries, or purified anthocyanin extracts recovered from each of the fruits. The whole fruit, blueberry or strawberry, did not prevent the weight gain that accompanied the groups fed with the 45% and 60% high-fat caloric diets. Yet, consumption of the purified anthocyanins obtained in either fruits resulted in reduced obesity.

The above-discussed documents represent only a cross section of the numerous studies that have been reported on health benefits of anthocyanins as well as tannic, ellagic, and gallic acid on preventing or remediating MetS and/or its risk factors/conditions. Therefore, for more information on the possible mechanisms of action of these phytochemicals on MetS responses, the reader is referred to [141–143] for excellent reviews on this topic.

5 Conclusions

The jaboticaba fruit is an important crop indigenous to Brazil due, in part, to the numerous nutrients and phytochemicals that protect against multiple diseases currently afflicting western cultures. Despite its increasing importance, jaboticaba consumption is low in other parts of the world. This review provides information on jaboticaba, with an emphasis on their phenolic composition and links to human health benefits. Although our understanding of the latter health attributes is increasing, critical gaps in knowledge remain on the role that jaboticaba has on protecting against multiple disease risks or states. As jaboticaba contains multiple nutrients and other phytochemicals, they most likely exert these effects as synergists or additives within the complex berry system, but again research remains limited on the whole fruit with more data available on the peel. Such studies are particularly important considering that theses berries are consumed primarily as a whole product, with the market class and production location most likely affecting their compositional profiles and therefore its potential health effects.

However, the presented studies show the potential of the jaboticaba fruit as a highly effective functional food capable of providing multiple health benefits. Foods that have been branded as a functional food (or disease preventing/remediating foods), such as cranberries and grapes, have expanded approximately tenfold in the last decade, growing at three to four times the rate of conventional foods due to the increasing preference for natural interventions [144]. Based on this encouraging growth, expanding jaboticaba into the functional food category is expected to increase overall consumption and demand, which in turn can support and increase prices. By increasing the demand on the global scale for this currently underconsumed fruit, it is expected that a positive economic impact would affect all segments of the production and distribution chain.

References

1. Santos DT, Veggi PC, Meirele AA (2010) Extraction of antioxidant compounds from Jabuticaba (*Myrciaria cauliflora*) skins: yield, composition and economical evaluation. J Food Eng 10:23–31
2. Neves LC, da Silva VX, Benedette RM, Prill MADS, Vieites RL, Roberto SR (2008) Conservação de uvas "Crimson Seedless" e "Itália", submetidas a diferentes tipos de embalagens e dióxido de enxofre (SO2). Rev Bras Frutic 30:65–73
3. Abe LT, Lajolo FM, Genovese MI (2012) Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg). J Sci Food Agric 92:1679–1687
4. Zafra-Stone S, Yasmin T, Bagchi M, Chatterjee A, Vinson JA, Bagchi D (2007) Berry anthocyanins as novel antioxidants in human health and disease prevention. Mol Nutr Food Res 51:675–683
5. He J, Giusti MM (2010) Anthocyanins: natural colorants with health promoting properties. Annu Rev Food Sci Technol 1:163–187
6. Hagiwara A, Miyashita K, Nakanishi T, Sano M, Tamano S, Kadota T, Koda T, Nakamura M, Imaida K, Ito N (2001) Pronounced inhibition by a natural anthocyanin, purple corn color, of 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine (PhIP)-associated colorectal carcinogenesis in male F344 rats pretreated with 1, 2-dimethylhydrazine. Cancer Lett 171:17–25

7. Sriamornsak P (2011) Application of pectin in oral drug delivery. Exp Opin Drug Del 8:1009–1023
8. Francis FJ, Markakis PC (1989) Food colorants: anthocyanins. Crit Rev Food Sci Nutr 28:273–314
9. Inada KOP, Oliveira AA, Revorêdo TB, Martins ABN, Lacerda ECQ, Freire AS, Braz BF, Santelli RE, Torres AG, Perrone D, Monteiro MC (2015) Screening of the chemical composition and occurring antioxidants in jaboticaba (*Myrciaria jaboticaba*) and jussara (*Euterpe edulis*) fruits and their fractions. J Funct Foods 17:422–433
10. Obtained from https://en.wikipedia.org/wiki/Jabuticaba. Accessed on 31 Dec 201
11. Wu S-B, Long C, Kennelly EJ (2013) Phytochemistry and health benefits of jaboticaba, an emerging fruit crop from Brazil. Food Res Int 54:148–159
12. Lenquiste SA, Marineli RDS, Moraes ÉA, Dionísio AP, Brito ESD, Maróstica MR Jr (2015) Jaboticaba peel and jaboticaba peel aqueous extract shows in vitro and in vivo antioxidant properties in obesity model. Food Res Int 77:162–170
13. Alezandro MR, Dubé P, Desjardins Y, Lajolo FM, Genovese MI (2013) Comparative study of chemical and phenolic compositions of two species of jaboticaba (*Myrciaria jaboticaba* (Vell) Berg and *Myrciaria cauliflora* (Mart) O. Berg). Food Res Int 54:468–477
14. da Silva JK, Batista ÂG, Cazarin CBB, Dionísio AP, de Brito ES, Marques ATB, Maróstica MR Jr (2017) Functional tea from a Brazilian berry: overview of the bioactives compounds. LWT Food Sci Technol 76:292–298
15. Lima ADJB, Corrêa AD, Dantas-Barros AM, Nelson DL, Amorim ACL (2011) Sugars, organic acids, minerals and lipids in jaboticaba. Rev Bras Frutic 33:540–550
16. Rufino MDSM, Alves RE, de Brito ES, Pérez-Jiménez J, Saura-Calixto F, Mancini-Filho J (2010) Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem 121:996–1002
17. Gurak PD, De Bona GS, Tessaro IC, Marczak LDF (2014) Jaboticaba pomace powder obtained as a co-product of juice extraction: a comparative study of powder obtained from peel and whole fruit. Food Res Int 62:786–792
18. Leite-Legatti AV, Batista ÂG, Dragano NRV, Marques AC, Malta LG, Riccio MF, Eberlin MN, Machado ART, de Carvalho-Silva LB, Ruiz ALTG, de Carvalho JE, Pastore GM, Maróstica MR (2012) Jaboticaba peel: antioxidant compounds, antiproliferative and antimutagenic activities. Food Res Int 49:596–603
19. Batista ÂG, Lenquiste SA, Cazarin CBB, da Silva JK, Luiz-Ferreira A, Bogusz SB Jr, Souza RN, Augusto F, Prado MA, Maróstica MR Jr (2014) Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high fat diet induced obesity. J Funct Foods 6:450–461
20. Batista ÂG, Soares ES, Mendonça MCP, da Silva JK, Dionísio AP, Sartori CR, da Cruz-Höfling MA, Maróstica MR Jr (2017) Jaboticaba berry peel intake prevents insulin-resistance-induced tau phosphorylation in mice. Mol Nutr Food 61(10):1600952
21. Plaza M, Batista ÂG, Cazarin CBB, Sandahl M, Turner C, Östman E, Maróstica MR Jr (2016) Characterization of antioxidant polyphenols from *Myrciaria jaboticaba* peel and their effects on glucose metabolism and antioxidant status: a pilot clinical study. Food Chem 211:185–197
22. Miguel MG (2010) Antioxidant activity of medicinal and aromatic plants. A review. Flavour Frag J 25:291–312
23. Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15:7313–7352
24. Toyokuni S, Tanaka T, Kawaguchi W, Fang N, Ozeki RL, Akatsuka M, Hiai S, Okezie H, Aruoma OI, Bahorun T (2003) Effects of the phenolic contents of Mauritian endemic plant extracts on promoter activities of antioxidant enzymes. Free Radic Res 37:1215–1224
25. Naga OA, Seki M, Kobayashi H (1999) Inhibition of xanthine oxidase by flavonoids. Biosci Biotechnol Biochem 63:1787–1790
26. Yeh C-T, Yen G-C (2006) Induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance – associated protein 3 mRNA expression. J Nutr 136:11–15

27. Masella R, Di Benedetto R, Vari R, Filesi C, Giovanin C (2005) Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem 16:577–586
28. Haminiuk CWI, Plata-Oviedo MSV, Guedes AR, Stafussa AP, Bona E, Carpes ST (2011) Chemical, antioxidant and antibacterial study of Brazilian fruits. Food Sci Technol 46:1529–1537
29. Leite AV, Malta LG, Riccio MF, Eberlin MN, Pastore GM, Maróstica MR Jr (2011) Antioxidant potential of rat plasma by administration of freeze-dried jaboticaba peel (*Myrciaria jaboticab*a Vell Berg). J Agric Food Chem 59:2277–2283
30. Calloni C, Agnol RD, Martínez LS, Maron FS, Moura S, Salvador M (2015) Jaboticaba (*Plinia trunciflora* (O. Berg) Kausel) fruit reduces oxidative stress in human fibroblast cells (MRC-5). Food Res Int 70:15–22
31. Martins de Sá LZC, Castro PFS, Lino FMA, Bernardes MJC, Viegas JCJ, Dinis TCP, Santana MJ, Romao W, Vaz BGV, Lião LM, Ghedini PC, Rocha ML, Gil ES (2014) Antioxidant potential and vasodilatory activity of fermented beverages of jaboticaba berry (*Myrciaria jaboticaba*). J Funct Foods 8:169–179
32. Garcia-Diaz DF, Jimenez P (2013) Bioactive compounds and health benefits of exotic tropical red- black berries. J Funct Foods 5:539–549
33. Wang W-H, Tyan Y-C, Chen Z-S, Lin C-G, Yang M-H, Yuan S-S, Tsai W-C (2014) Evaluation of the antioxidant activity and antiproliferative effect of the jaboticaba (*Myrciaria cauliflora*) seed extracts in oral carcinoma cells. Biomed Res Int. https://doi.org/10.1155/2014/185946
34. Silva MC, de Sousa VB, Thomazini M, da Silva ER, Smaniotto T, de Carvalho RA, Genovese MI, Favaro-Trindade CS (2014) Use of the jaboticaba (*Myrciaria cauliflora*) depulping residue to produce a natural pigment powder with functional properties. LWT-Food Sci Technol 55:203–209
35. Havsteen BH (2002) The biochemistry and medical significance of the flavonoids. Pharmacol Therap 96:267–202
36. Tsuda T (2012) Dietary anthocyanin rich plants: biochemical basis and recent progress in health benefits studies. Mol Nutr Food Res 56:159–170
37. Kähkönen MP, Heinonen M (2003) Antioxidant activity of anthocyanins and their aglycons. J Agric Food Chem 51:628–633
38. Tsuda T, Horio F, Osawa T (2000) The role of anthocyanins as an antioxidant under oxidative stress in rats. Biofactors 13:133–139
39. Ramirez-Tortosa C, Andersen ØM, Gardner PT, Morrice PC, Wood SG, Duthie SJ, Collins AR, Duthie GG (2001) Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. Free Radic Biol Med 31:1033–1037
40. Priyadarsini KI, Khopde SM, Dumar SS, Mohan H (2002) Free radical studies of ellagic acid, a natural phenolic antioxidant. J Agric Food Chem 50:2200–2206
41. Han DH, Lee MJ, Kim JH (2006) Antioxidant and apoptosis-induct activity of ellagic acid. Anticancer Res 26:3601–3606
42. Aglitti FF, Duranti G, Rcordy R, Perticone P, Cozzi R (2001) Strong antioxidant activity of ellagic acid in mammalian cells in vitro revealed by the comet assay. Anticancer Res 21:3903–3908
43. Meyer AS, Heinonen M, Frankel EN (1998) Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. Food Chem 61:71–75
44. Turk G, Atessahin A, Sonmez M, Ceribasi AO, Yuce A (2008) Improvements of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. Fertil Steril 89:1474–1481
45. O'Byrne KJ, Dalgleish AG (2001) Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer 85:473–483
46. Lumeng CN, Bodzin JL, Saltiel A (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117:175–184
47. Wellen KE (2005) Inflammation, stress and diabetes. J Clin Invest 115:1111–1119

48. Lionett L, Mollica MP, Lombardi A, Cavaliere G, Gifuni G, Barletta A (2009) From chronic overnutrition to insulin resistance the role of fat storing capacity and inflammation. Nutr Metab Cardiovasc Dis 19:146–152
49. Chan BCL, Li LF, Hu SQ, Wat E, Wong ECW, Zhang VX, Lau CBS, Wong CK, Hon KLE, Hui PCL (2015) Gallic acid is the major active component of Cortex Moutan in inhibiting immune maturation of human monocyte-derived dendritic cells. Mol Ther 20:16388–16403
50. Dale BA (2002) Periodontal epithelium: a newly recognized role in health and disease. Periodontol 2000(30):70–78
51. Narayanan BA, Geoffroy O, Willingham MC, Re GG, Nixon DW (1999) p53/p21 (WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. Cancer Lett 136:215–221
52. Fakhry C, Marks MA, Gilman RH, Cabrerra L, Yori P, Kosek M, Gravitt PE (2013) Comparison of the immune microenvironment of the oral cavity and cervix in healthy women. Cytokine 64:597–604
53. Schröder J-M, Harder J (1999) Human beta-defensin-2. Int J Biochem Cell Biol 31:645–651
54. QuiÃones-Mateu ME, Lederman MM, Feng Z, Chakraborty B, Weber J, Rangel HR, Marotta ML, Mirza M, Jiang B, Kiser P (2003) Human epithelial Î²-defensins 2 and 3 inhibit HIV-1 replication. AIDS 17:F39–F48
55. Promsong A, Chung WO, Satthakarn S, Nittayananta W (2014) Ellagic acid modulates the expression of oral innate immune mediators: potential role in mucosal protection. J Oral Pathol Med 44:214–221
56. Modi MN, Goel T, Das T, Malik S, Suri S, Rawas AKS, Srivastava SK, Tuli R, Malhotra S, Gupta SK (2013) Ellagic acid and gallic acid from *Lagerstroemia speciosa L*. inhibit HIV-1 infection through the inhibition of HIV-1 protease and reverse transcriptase activity. Indian J Med Res 137:540–548
57. Jenning A, Welch AA, Spector T, Macgregar A, Cassidy A (2014) Intakes of anthocyanins and flavones are associated with biomarkers of insulin resistance and inflammation in women. J Nutr 144:202–208
58. Hassellund SS, Flaa A, Kjeldsen SE, Seljeflot I, Karlsen A, Erlund I, Rstrup M (2013) Effects of anthocyanins on cardiovascular risk factors and inflammation in pre-hypertensive men: a double-blind randomized placebo controlled crossover study. J Hum Hypertens 27:100–106
59. Mena P, Dominguez-Perles R, Girones-Vilaplan A, Baenas N, Garcia-Viguera C, Villano D (2014) Flavan-3-ols, anthocyanins, and inflammation. Lifestyles 66:745–758
60. Landete JM (2011) Ellagitannins, ellagic acid and their derived metabolites: a review about source, metabolism functions and health. Food Res Int 44:1150–1160
61. Siegel RL, Miller KD, Jemal A (2016) Cancer statistics. CA Cnacer J Clin 66:7–30
62. Adams J, Cory S (2007) The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26:1324–1337
63. Zee Y-K, O'connor JP, Parker GJ, Jackson A, Clamp AR, Taylor MB, Clarke NW, Jayson GC (2010) Imaging angiogenesis of genitourinary tumors. Nat Rev Urol 7:69–82
64. Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140:883–899
65. Palmer S (1985) Diet, nutrition and cancer. Prog Food Nutr Sci 9:283–341
66. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hipert DF, Griel AE, Etherton TD (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113:71–88
67. Losso JN, Bansode RR, Trappey A II, Bawadi HA, Truax R (2004) In vitro anti-proliferative activities of ellagic acid. J Nutr Biochem 15:672–678
68. Edderkaoui M, Odinokova I, Ohno I, Gukovshky I, Go VLW, Pandol SJ, Gukovskaya AS (2008) Ellagic acid induces apoptosis through inhibition of nuclear factor kB in pancreatic cancer cell lines. World J Gastroenterol 14:3672–3680
69. Stoner GD, Mukhtar H (1995) Polyphenols as cancer chemopreventive agents. J Cell Biochem 59:169–180

70. Maas JL, Galletta GJ, Stoner GD (1991) Ellagic acid, an anticarcinogen in fruits, especially strawberries: a review. HortSci 26:1–14
71. Ismail T, Calcabrini C, Diaz RA, Fimognari C, Turrini E, Catanzaro E, Akhtar S, Sestili P (2016) Ellagitannins in cancer chemoprevention and therapy. Toxins 8:1–22. https://doi.org/10.3390/toxins8050151
72. Kasimsetty SG, Bialonska D, Reddy MK, Ma G, Khan SI, Ferreira D (2010) Colon cancer chemopreventive activities of pomegranate ellagitannins and urolithins. J Agric Food Chem 58:2180–2187
73. Heber D (2008) Multitargeted therapy of cancer by ellagitannins. Cancer Lett 269:262–268
74. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D (2005) In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J Nutr Biochem 16:360–367
75. Lu Y, Jiang F, Jiang H, Wu K, Zheng W, Yizhong C, Katakowski M, Chopp M, To S-ST (2010) Gallic acid suppresses cell viability, proliferation, invasion and angiogenesis in human glioma cells. Eur J Pharmacol 641:102–117
76. Kaur M, Velmurugan B, Rajamankckam S, Agarwal R, Agarwal C (2009) Gallic acid, an active constituent of grape seed extract, exhibits anti-proliferative, pro-apoptotic and anti-tumorigenic effects against prostate carcinoma xenograft growth in nude cell. Pharm Res 25:213–2140
77. Verma S, Sing A, Mishra A (2013) Gallic acid: molecular rival of cancer. Enviorn Toxicol Pharmacol 35:473–485
78. Ho H-H, Chang C-S, Ho W-C, Liao S-Y, Wu C-H, Wang C-J (2010) Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF-κB activity and downregulation of PI3K/AKT/small GTPase signals. Food ChemToxicol 48:2508–2516
79. Toth M, Sohail A, Fridman R (2012) Assessment of gelatinases (MMP-2 and MMP-9) by gelatin zymography. Methos Mol Biol 878:121–135
80. Locatelli C, Filippin-Monteiro FB, Creczynski-Pasa TB (2013) Alkyl ester of gallic acid as anticancer agents: a review. Eur J Med Chem 60:233–239
81. Kong J-M, Chia L-S, Goh N-K, Chia T-F, Brouillard R (2003) Analysis and biological activities of anthocyanins. Phytochemistry 64:923–933
82. Zhang Y, Vareed SK, Nair MG (2005) Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. Life Sci 76:1465–1472
83. Feng R, Ni H-M, Wang SY, Tourkova IL, Shurin MR, Harada H, Yin X-M (2007) Cyanidin-3-rutinoside, a natural polyphenol antioxidant, selectively kills leukemic cells by induction of oxidative stress. J Biol Chem 282:13468–13476
84. Brandstetter H, Grams F, Glitz D, Lang A, Huber R, Bode W, Krell H-W, Engh RA (2001) The 1.8-Å crystal structure of a matrix metalloproteinase 8-barbiturate inhibitor complex reveals a previously unobserved mechanism for collagenase substrate recognition. J Biol Chem 276:17405–17412
85. Nagase H, Sasaki K, Kito H, Haga A, Sato T (1998) Inhibitory effect of delphinidin from *Solanum melongena* on human fibrosarcoma HT-1080 invasiveness in vitro. Planta Med 64:216–219
86. Bagchi D, Sen C, Bagchi M, Atalay M (2004) Anti-angiogenic, antioxidant, and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula. Biochemist 69:75–80
87. Wang L-S, Stoner GD (2008) Anthocyanins and their role in cancer prevention. Cancer Lett 269:281–290
88. Libby P, Sukhova G, Lee RT, Liao JK (1997) Molecular biology of atherosclerosis. Int J Cardiol 62:S23–S29
89. Libby P (2012) Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol 32: 2045–2051
90. Wang S, Wu D, Matthan NR, Lamon-Fava S, Lecker JL, Lichtenstein AH (2009) Reduction in dietary omega-6 polyunsaturated fatty acids: eicosapentaenoic acid plus docosahexaenoic acid

ratio minimizes atherosclerotic lesion formation and inflammatory response in the LDL receptor null mouse. Atherosclerosis 204:147–155
91. Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, Demer LL (1996) The Yin and Yang of oxidation in the development of the fatty streak. Arterioscler Thromb Vasc Biol 16:831–842
92. Aviram M (1993) Modified forms of low density lipoprotein and atherosclerosis. Atherosclerosis 98:1–9
93. Wang HX, Ng TB (1999) Natural products with hyperglycemic hypertensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. Life Sci 65(25):2663–2677
94. Batista ÂG, Lenquiste SA, Moldenhauer C, Gody JT, Pissini MR, Maróstica MR Jr (2013) Jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg) peel improved triglyceride excretion and hepatic lipid peroxidation in high fat fed rats. Rev Nutr 5:571–581
95. Alezandro MR, Granato D, Genovese MI (2013) Jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg), a Brazilian grape-like fruit, improves lipid profile in streptozotocin-mediated oxidative stress in diabetic rats. Food Res Int 54:650–659
96. German JB, Dillard CJ (2004) Saturated fats: what dietary intake?1,2,3. Am J Clin Nutr 80:550–559
97. Chang W-C, Yu Y-M, Chiang S-Y, Tseng C-Y (2008) Ellagic acid suppresses oxidised low-density lipoprotein-induced aortic smooth muscle cell proliferation: studies on the activation of extracellular signal-regulated kinase 1/2 and proliferating cell nuclear antigen expression. Br J Nutr 99:709–714
98. Papoutsi Z, Kassi E, Chinou I, Halabalaki M, Skaltsounis L, Moutsatsou P (2008) Walnut extract (*Juglans regia L.*) and its component ellagic acid exhibit anti-inflammatory activity in human aorta endothelial cells and osteoblastic activity in the cell line KS483. Br J Nutr 99:715–722
99. Yu Y-M, Wang Z-H, Liu C-H, Chen C-S (2007) Ellagic acid inhibits IL-1β-induced cell adhesion molecule expression in human umbilical vein endothelial cells. Br J Nutr 97:692–698
100. Chen XP, Zhang TT, Du GH (2007) Lectin-like oxidized low-density lipoprotein receptor-1, a new promising target for the therapy of atherosclerosis. Cardiovas Drug Rev 25:146–161
101. Lee W-J, Ou H-C, Hsu W-C, Chou M-M, Tseng J-J, Hsu S-L, Tsai K-L, Sheu WH-H (2010) Ellagic acid inhibits oxidized LDL-mediated LOX-1 expression, ROS generation and inflammation in human endothelial cells. J Vasc Surg 52:1290–1300
102. Zou M-H, Leist M, Ullrich V (1999) Selective nitration of prostacyclin synthase and defective vasorelaxation in atherosclerotic bovine coronary arteries. Am J Pathol 154:1359–1365
103. Larrosa M, García-Conesa MT, Espín JC, Tomás-Barberán FA (2010) Ellagitannins, ellagic acid and vascular health. Mol Asp Med 31:513–539
104. Xia X, Ling W, Ma J, Xia M, Hou M, Wang Q, Zhu H, Tang Z (2006) An anthocyanin-rich extract from black rice enhances atherosclerotic plaque stabilization in apolipoprotein E–deficient mice. J Nutr 136:2220–2225
105. de Pascual-Teresa S, Moreno DA, García-Viguera C (2010) Flavanols and anthocyanins in cardiovascular health: a review of current evidence. Int J Mol Sci 11:1679–1703
106. Wallace TC (2011) Anthocyanins in cardiovascular disease. Adv Cardiovasc Dis 2:1–7
107. Morton LW, Caccetta RAA, Puddy IB, Croft KD (2000) Chemistry and biological effect of dietary phenolic compounds: relevance to cardiovascular disease. Clin Exp Pharmacol Physiol 27:152–159
108. Kritchevsky D, Story JA (1978) Fiber hypercholesteremia and atherosclerosis. Lipids 13:366–369
109. Glore SR, Treeck DV, Knehans AW, Guild M (1994) Soluble fiber and serum lipids: a literature review. J Am Diet Assoc 94:425–436
110. Leveille GA, Sauberlich HE (1966) Mechanism of the cholesterol depressing effect of pectin in the cholesterol fed rat. J Nutr 88:209–214
111. Diamant M, Black EE, de Vos WM (2011) Do nutrient-gut-microbiota interactions play a role in human obesity, insulin resistance and type 2 diabetes? Obes Rev 12:272–281

112. Collins MD, Gibson GR (1999) Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am J Clin Nutr 69:1052S–1057S
113. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031
114. Roberfroid MB (2001) Prebiotics: preferential substrates for specific germs? Am J Clin Nutr 73:406S–409S
115. Gonzalez-Barrio R, Pilar Truchado P, Hideyuki I, Espín JC, Tomas-Barberan FA (2011) UV and MS identification of urolithins and nasutins, the bioavailable metabolites of ellagitannins and ellagic acid in different mammals. J Agric Food Chem 59:1152–1162
116. Faria A, Fernandes I, Norberto S, Mateus N, Calhau C (2014) Interplay between anthocyanins and gut microbiota. J Agric Food Chem 62:6969–6902
117. Okuda T, Yoshida T, Hatano T (1989) New methods of analyzing tannins. J Nat Prod 52:1–31
118. Mertens-Talcott SU, Jilma-Stohlawetz P, Rios J, Hingorani L, Derendorf H (2006) Absorption, metabolism, and antioxidant effects of pomegranate (*Punica granatum L.*) polyphenols after ingestion of a standardized extract in healthy human volunteers. J Agric Food Chem 54:8956–8961
119. Selma MV, Romo-Vaquero M, García-Villalba R, González-Sarrías A, Tomás-Barberán FA, Espín JC (2016) The human gut microbial ecology associated with overweight and obesity determines ellagic acid metabolism. Food Funct 7:1769–1774
120. Selma MV, Tomás-Barberán FA, Beltran D, García-Villalba R, Espín JC (2014) *Gordonibacter urolithinfaciens* sp. nov., a urolithin-producing bacterium isolated from the human gut. Int J Syst Evol Microbiol 64:2346–2352
121. Li Z, Henning SM, Lee RP, Lu Q-Y, Summanen PH, Thames G, Corbett K, Downes J, Tseng C-H, Finegold SM (2015) Pomegranate extract induces ellagitannin metabolite formation and changes stool microbiota in healthy volunteers. Food Funct 6:2487–2495
122. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP (2009) A core gut microbiome in obese and lean twins. Nature 457:480–484
123. Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444:1022–1023
124. Williamson G, Clifford MN (2010) Colonic metabolites of berry polyphenols: the missing link to biological activity? Br J Nutr 104:S48–S66
125. Hidalgo M, Oruna-Concha MJ, Kolida S, Walton GE, Kallithraka S, Spencer JP, de Pascual-Teresa S (2012) Metabolism of anthocyanins by human gut microflora and their influence. J Agric Food Chem 60:3882–3890
126. Gibson GR (1998) Dietary modulation of the human gut microflora using prebiotics. Br J Nutr 80:S209–S212
127. Jiang T, Gao X, Wu C, Tian F, Lei Q, Bi J, Xie B, Wang HY, Chen S, Wang X (2016) Apple-derived pectin modulates gut microbiota, improves gut barrier function, and attenuates metabolic endotoxemia in rats with diet induced obesity. Nutrients 8:126. https://doi.org/10.3390/nu8030126
128. Parkar SG, Redgat EL, Wibisono R, Luo X, Koh ETH, Roswitha Schroder R (2010) Gut health benefits of kiwifruit pectins: comparison with commercial functional polysaccharides. J Funct Foods 2:210–218
129. Nazzaro F, Fratianni F, Nicolaus B, Poli A, Orlando P (2010) The prebiotic source influences the growth, biochemical features and survival under simulate gastrointestinal conditions of the prebiotic *Lactobacillus acidophilus*. Anaerobe 18:280–285
130. Hopps E, Noto D, Caimi G, Averna MR (2010) A novel component of the metabolic syndrome: the oxidative stress. Nutr Metab Cardiovasc Dis 20:72–77
131. Eckel RH, Grandy SM, Zimmet PZ (2005) The metabolic syndrome. Lancet 365:1415–1428
132. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordan RMK, Savage PJ, Smith SC, Spertus JA, Costa F (2005) Diagnosis and management of the metabolic syndrome. Circulation 12:2735–2752

133. World Health Organization (2000) Obesity: preventing and managing the global epidemic. Defining the problem, who library cataloging in publication data. Technical report series, vol 894. WHO, Geneva, pp 1–13
134. Vincent HK, Innes KE, Vincent KR (2007) Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. Diabetes Obes Metab 9:813–839
135. Dragano NR, Cintra DE, Solon C, Morari J, Leite-Legatti AV, Velloso LA, Maróstica-Jr MR (2013) Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat- fed mice. Br J Nutr 110:447–455
136. Zimmet P, Alberti KG, Shaw J (2001) Global and societal implications of the diabetes epidemic. Nature 414:782–787
137. Lenquiste SA, Batista ÂG, da Silva MR, Dragano NRV, Maróstica MR (2012) Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. Food Res Int 49:153–160
138. Matsukawa T, Villareal MO, Isoda H (2016) The type 2 diabetes-preventive effect of cyanidin-3-glucoside on adipocytes. J Dev Sustain Agri 11:31–35
139. Garcia-Diaz DF, Johnson MH, de Mejia EG (2014) Anthocyanins from fermented berry beverages inhibit inflammation related adiposity response in vitro. J Med Med 18:489–496
140. Prior RL, Wu X, Gu L, Hager TJ, Hager A, Howard LR (2008) Whole berries versus berry anthocyanins: interactions with dietary fat levels in C5&7BL/6J mouse model of obesity. J Agric Food Chem 56:647–653
141. Tsuda T (2008) Regulation of adipocyte function by anthocyanins, possibility of preventing the metabolic syndrome. J Agric Food Chem 56:642–646
142. Sancho RAS, Pastore GM (2012) Evaluation of the effects of anthocyanins in type 2 diabetes. Food Res Int 46:378–386
143. Al-Muammar MN, Kahn F (2012) Obesity: the preventive role of the pomegranate (*Punica granatum*). Nutrition 28:595–604
144. Bigliardi B, Galati F (2013) Innovation trends in the food industry: the case of functional foods. Trends Food Sci Technol 31:118–129

Pomegranate Bioactive Molecules and Health Benefits

42

Saeed Akhtar, Tariq Ismail, and Anam Layla

Contents

Abstract

Fruits contribute an abundant supply of antioxidants to human diet and act as the first line of defense against the risks of chronic diseases occurrence. Pomegranate is one among the highly explored and appreciated fruits on account of its promising health-promoting and disease-preventing properties. Pomegranate fruit and its key components including rind, seed, and membranous network

S. Akhtar (✉) · T. Ismail · A. Layla
Institute of Food Science and Nutrition, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan
e-mail: saeedbzu@yahoo.com; ammarbintariq@yahoo.com; anamlayla1@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_78

have been evidently reported as carriers of a wide range of bioactive compounds including ellagitannins, hydroxycinnamic acids, hydroxybenzoic acids, flavons, flavonol-3-ols, anthocyanidins, anthocyanins, and conjugated and nonconjugated fatty acids, phytosterols, vitamins, and minerals. Traditional aspects of pomegranate exploitation as remedy against infections and gastrointestinal ailments have generated a basis for the modern-age research. Findings of the research carried out in the last two decades manifest fruit, flower, seeds, and peel of pomegranate as natural strategy to treat microbiological and parasitic pathogenesis and to act as a chemopreventive and therapeutic approach against inflammatory and infectious chronic ailments. Forthcoming sections of this chapter review fundamental biochemical composition of pomegranate and its anatomical fractions and provide recent updates on pomegranate perspective applications against the risks of various forms of cancers, cardiovascular diseases, diabetes, acute and chronic liver injury, renal disorders, impaired gut health, neurodegenerative disorders, microbiological pathogenesis, and parasitic infestation.

Keywords
Pomegranate · Ellagitannins · Inflammation · Cancer · Neurodegeneration

1 Introduction

Pomegranate (*Punica granatum* L; "seeded apple") was lauded in the Torah, Bible, and Al-Quran as a sacred fruit deliberating good luck, wealth, and power of fertility [1]. The mystical fruit traditionally implicated as folk or ethnic medicine to cure various health disorders [2]. The fruit-bearing plant is a predominant shrub of family Punicaceae that typically grows up to 12–16 feet and has several spiny branches with glossy lance-shaped leaves. The grenade-shaped fruiting body encloses clusters of delicious arils separated by pale white spongy mesocarp, with deep red leathery skin [1]. Since ancient times, the fruit is native and under cultivation from the northern Himalayas to Iran, Afghanistan, India, Pakistan, and China. The pomegranate cultivation was stretched from west of Persia (old name of Iran) over the entire Mediterranean region to Turkey and dried regions of American Southwest, Mexico, California, and Arizona [3].

The pomegranate plant, fruit, and their anatomical segments including flowers, fruit rind, leaves, bark, seeds, and roots encompasses a variety of biomolecules including phenolics, hydrolysable tannins, anthocyanins, flavonoids, and a wide range of essential micronutrients. The unique biochemical profile of the fruiting body and its major fractions attribute strong antioxidative, anti-inflammatory, apoptotic, and antimutagenic properties to curtail chronic maladies [4, 5]. Prophylactic properties of pomegranate are of broad spectrum and are anticipated to mitigate microbiological and parasitic pathogenesis [6, 7], gastric damage [8], cardiovascular disease [9], type 2 diabetes [10], several types of cancers [3], renal illnesses [11], liver complications [12], infertility [13], osteoarthritis [14], oral and dental health [15], and skin melanoma [16].

The consumption trend of fruit and associated structures is diversely alike fresh or dried arils, fresh or fermented juice, powdered extracts, rind powder tablets, capsules and soft gels, and extracts-based ointments and decoctions [5, 17, 18].

Pomegranate fruit waste including peel and seeds provides substantial amount of phytonutrients that can be exploited for their potent health benefits. The mechanistic properties of pomegranate fruit and peel extracts such as antioxidant, antimicrobial, flavoring, and colorant may also be implicated as a natural additive in the food industry for quality enhancement and food preservation [4, 19]. In addition, pomegranate seed oils and rind extracts deliberate photoprotective effect and can be utilized in cosmo care products [20]. The peel powders also have biosorption capacity for heavy metal from water and are capable of acting as phyto-remediating agent to ensure cost-effective waste water treatment and decontamination of chromium, nickel, and zinc contamination [21, 22].

A plethora of research has been conducted on health-promoting and food features of pomegranate and its anatomical fractions as in the last two decades. This chapter comprehensively covers the recent updates from research carried out to explore pomegranate fruit and its waste fractions' capability in delivering preventive and therapeutic aspects against certain human acute and chronic ailments of critical concern (Figs. 1, 2, and 3).

2 Biochemical Composition of Pomegranate

Globally, higher acceptability of pomegranate as a miraculous fruit is linked to its remarkable health benefits. Predominately, a unique biochemical profile ornamented with more than 124 phytochemicals is the differential factor responsible for a broad range of antioxidant, anti-inflammatory, and antimutagenic properties of

Fig. 1 Pomegranate flower

Fig. 2 Pomegranate ripened fruit

Fig. 3 Pomegranate edible fleshy sacs

pomegranate. Pomegranate fruit and its different anatomical fractions like flower, peel, juicy sacs, and seeds are referred as reservoirs of high molecular weight hydrolysable tannins, i.e., ellagitannins, alongside a wide range of anthocyanins, hydroxybenzoic acids, hydroxycinnamic acids, minerals, essential lipids, and complex polysaccharides [23–26]. Approximately 50% of the fruit weight is attributed to the rind portion that constitutes 30% of the fruit anthocyanidins contents.

Pomegranate peel, the waste fraction of the fruit, has been reported to carry 10% w/w crude polysaccharides that could further be exploited as a functional ingredient in attributing gelling and prebiotic properties to food formulations. A recent study from Tunisia reported 6.8–10.1% pectin contents in peel portion of four Tunisian pomegranate cultivars [27, 28]. Pomegranate seeds constitute 15–25% of the edible juicy arils and are considerably good sources of phytosterols, omega-5 fatty acids including punicic acids, tocopherols, and isomers of conjugated alpha-linolenic acid [29–31].

Variability in concentration of pomegranate bioactive compounds including phytochemicals of human health significance and their biological properties varies with the cultivar, anatomical part of the fruit, maturity period, type of extraction, and

mode of phenolics extraction. Low-cost and efficient recovery techniques of pomegranate phytochemicals, polyphenols, and seed oil have been introduced in the recent past including pulsed ultrasound-assisted extraction, green ultrasound-assisted extraction, ultrasound-assisted enzymatic extraction, enzyme-assisted supercritical fluid extraction, microwave-assisted extraction, microwave-treated supercritical CO_2, high-voltage electrical discharge, and hydroalcoholic extraction system [32–42].

Selection of a cost-effective, safer, and intelligent biomolecules extraction technique for pomegranate and its various fractions ensures higher rate of phenolics recovery with better ability of extracts/fractionated compounds in dispensing various biological promoting properties of human health concern (Table 1).

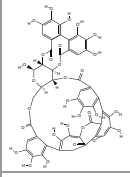
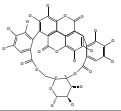
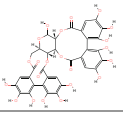
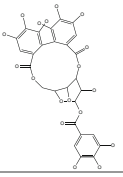
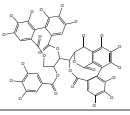
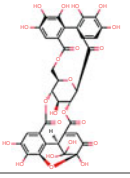
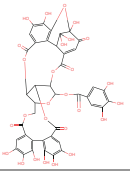
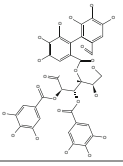
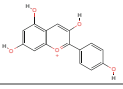
3 Health Benefits of Pomegranate and Its Fractions

3.1 Pomegranate Promotes Cardiac Health

Global estimates predict death toll due to cardiac complication to be 40% by the year 2020 [44]. Several epidemiological studies correlate inverse relation between adherence to relatively high intake of plant polyphenols and incidence of cardiovascular diseases (CVD). Experimental evidence suggested polyphenol-rich pomegranate fruits consumption to present modulatory effect in improving cardiac muscles tone, mitigating oxidative stress-mediated arterial hardening, and attenuating atherosclerosis. Healthy heart features are attributed predominately by the pomegranate ellagitannins especially punicalagin isomers α and β. Pomegranate hydrolyzable ellagitannins after being converted to ellagic acid are further metabolized into certain types of urolithins by intestinal microbiota. Studies on pomegranate consumption and association with improved cardiac health correlate urolithins as one among the known ellagitannins metabolites that stand responsible for attenuating various cardiac complications [9, 45]. Arterial lipid accumulation is a hallmark of atherosclerosis and is encouraged by low-density lipoprotein (LDL) cholesterol oxidation. A double-blind pilot scale study conducted in dyslipidemic obese patients receiving 500 mg pomegranate rind extract daily for a period of 8 weeks demonstrated a significant reduction in systolic blood pressure and improved lipid profile via reduced triglycerides and LDL levels and increased HDL level proclaiming pomegranates a potent antiatherogenic agent [46]. In a study conducted by Razani et al. [47], 100 hospitalized patients diagnosed with myocardial ischemia and unstable angina were randomly assigned to consume 220 ml pomegranate juice in addition to medical treatment; pomegranate diet therapy presented cardioprotective effect by significant reduction in serum level of malondialdehyde and troponin, intensity of angina pectoris, and attenuating reperfusion injury [47].

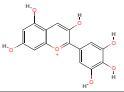
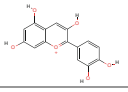
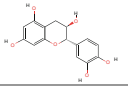
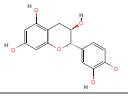
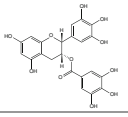
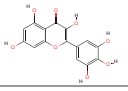
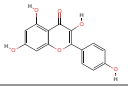
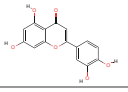
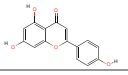
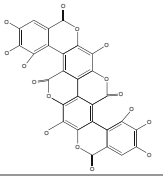
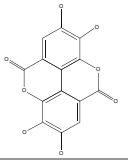
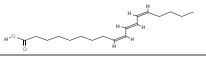
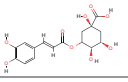
Pomegranate fruit and peel extracts have also been reported to exert an ameliorative role in vascular inflammation, oxidative stress, and elevated cardiac enzymes markers. Pomegranate extracts supplements (625 mg carrying 200 mg punicalagin) delivered to hyperlipidemic animal models have been found to prevent coronary

Table 1 Major biochemical constituents of pomegranate leaf, flower, fruit, peel, seeds, bark and root

Compound	Chemical class	Anatomical distribution	Structures
Punicalagin	Ellagitannins	Peel, leaf, bark, root	
Punicalin	Ellagitannins	Peel, leaf, bark, root	
Pedunculagin	Ellagitannins	Peel	
Corilagin	Ellagitannins	Peel, leaf	
Casuarinin	Ellagitannins	Peel	
Granatin A	Ellagitannins	Peel	
Granatin B	Ellagitannins	Peel	
Tellimagrandin I	Ellagitannins	Peel	
Pelargonidin	Anthocyanins	Juice, peel	

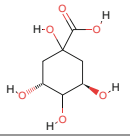
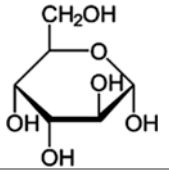
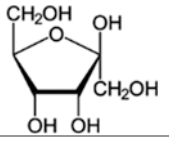
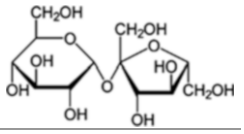
(*continued*)

Table 1 (continued)

Compound	Chemical class	Anatomical distribution	Structures
Delphinidin	Anthocyanins	Juice, peel	
Cyanidin	Anthocyanins	Juice, peel	
Catechin	Flavanoids	Juice, peel	
Epicatechin	Flavanoids	Juice, peel	
Epigallocatechin 3-gallate	Flavanoids	Juice, peel	
Quercetin	Flavanoids	Juice, peel	
Kaempferol	Flavanoids	Peel	
Luteolin	Flavanoids	Peel	
Apigenin	Flavanoids	Leaf	
Gallic acid	Organic acid	Juice, peel, flower	
Ellagic acid	Organic acid	Juice, peel, seed	
Punicic acid	Organic acid	Seed	
Chlorogenic acid	Organic acid	Juice, peel	

(*continued*)

Table 1 (continued)

Compound	Chemical class	Anatomical distribution	Structures
Quinic acid	Organic acid	Juice, peel	
Glucose	Simple sugars	Juice	
Fructose	Simple sugars	Juice	
Sucrose	Simple sugars	Juice	

Source: Lansky and Newman [3]; Ambigaipalan et al. [43]

endothelial dysfunction. The study further delineates inhibition of vascular inflammation and stress-mediated coronary DNA damage and stimulating Akt/endothelial nitric oxide-synthase pathway to improve vasodilatory values as the fundamental mechanism of pomegranate extracts in preventing coronary endothelial dysfunction [9]. Oxidative stress-mediated myocardial infarction results in coronary muscle atrophy and necrosis. Pomegranate fruit extracts have been found to present an ameliorative effect in isoproterenol-toxicated myocardial infarction by attenuating oxidative stress and level of cardiac enzymes markers, i.e., CK-MB and troponin [48]. Clinical findings are therefore suggestive of pomegranate fruit juice, seeds, rind extracts, and other derived products as potent source of antioxidants and nutrients offering cardioprotective and therapeutic properties.

3.2 Pomegranate and Its Role in Cancer Chemoprevention

Cancer is one of the leading causes of death in global perspective, and it has been reported that new cancer cases will increase by 70% at the end of 2030. Healthier eating practices and dietary preferences toward more fruits and vegetable consumption have been reported to reduce risks of cancer insurgencies and associated mortalities [49, 50]. Pomegranate ranks among the fruits that carry highest concentration of cancer chemopreventive and therapeutic biomolecules including

ellagitannins, flavonoids, and anthocyanins. Pomegranate polyphenols dispensed either in the form of crude, purified fruit extracts or as fruit juice furnish anticancer activities by inducing apoptosis, cell cycle arrest, antiangiogenesis, and anti-mutagenesis activities [49].

Pomegranate anticancerous properties alike other chemopreventive agents have been reported to be associated with antioxidant activities of biomolecules. Pomegranate juice prospectively contains 2 g/L ellagitannins of which punicalagin accounts 1500–1900 mg/L [51]. It's among the reasons that pomegranate juice adds three times higher antioxidant capacity in terms of inhibition of the production of DNA oxidation products, reactive nitrogen species, lipid peroxidation, and in scavenging reactive oxygen species [51–54].

Clinical findings are indicative of pomegranate biomolecules metabolites accumulation in prostate tissues of prostate cancer patients administrated with 250 ml pomegranate juice consecutively for a period of 4 weeks [55]. However, statistically nonsignificant impact of pomegranate juice administration on cancer development and progression markers was indicated probably due to shorter treatment duration. Another 18-month study wherein patients were provided with 1 or 3 g pomegranate extracts was found to extend prostate-specific antigen doubling time from 12–19 months and 12–17.5 months, respectively for low-dose and high-dose treated groups [56]. Inflammation – one among the cancer initiation factors activates cancer cells and induces DNA damages alongside epigenetic changes. Pomegranate as juice, fruit extracts, and individually fractionated biomolecules exerts significant impact on expression of inflammatory cell signaling protein in cancer cells [57]. A study carried out in human colon cancer cell line indicated pomegranate juice, pomegranate tannins, and punicalagin to significantly reduce expression of cyclo-oxygenase – 2 (COX-2) expression. COX-2 proteins stand responsible for production of prostanoids that induce inflammation [58]. Ellagic acid – another metabolite of pomegranate ellagitannins has been reported to inhibit intestinal inflammation by downregulating certain inflammation-mediating compounds such as COX-2 and iNOS and blocking cell signaling pathways including NF-kB, p38 MAPK, IL6, and STAT3 in tissues of the colon [59].

A study carried out by Mehta and Lansky [60] highlighted fermented pomegranate juice carrying 10 μg/ml polyphenols to exert chemopreventive properties by 90% suppression of mammary cancerous lesion formation [60]. Pomegranate extracts possessing up to 200 μg/ml bioactive phenolic compounds have been reported to reduce breast cancer cells viability and block mammary cancer cell cycle progression [61]. Pomegranate as fruit and peel extracts exhibits skin protection properties against UVA- and UVB-mediated reactions. Pretreatment with pomegranate fruit extracts protects skin fibroblast from UV-mediated cell death. A significant inhibition in production of UV-induced reactive oxygen species and increment in intracellular antioxidant levels however can only be achieved with too higher pomegranate polyphenols concentration, i.e., 500 to 10,000 mg/L [62, 63].

Albeit, a plethora of research have been undertaken to explore chemopreventive and therapeutic properties of pomegranate against various types of cancers including

breast, colon, liver, bladder, skin, lung, and cerebral cancer, yet the bioavailability of pomegranate bioactive compounds including ellagitannins, flavonoids, and anthocyanins in the target cells need to be determined. Furthermore, maximum tolerable doses of combined extracts and individual compounds capable of delivering chemopreventive and therapeutic role and exact underlying anticancer mechanism are yet to be explored.

3.3 Antidiabetic Properties of Pomegranate

Traditionally pomegranate fruit and associated structures alike flowers and leaves were recommended in conventional therapy of diabetes [64]. Since last decade, scientific work has consolidated effects of fruit in curing type 2 diabetes and its complications. Oxidative stress-mediated damage of pancreatic β-cell is one of the proposed mechanism involved in the onset of type 2 diabetes. Pomegranate being the healing fruit owing to its free radical scavenging properties has the ability to safeguard pancreatic β-cells from injury through neutralizing the effect of free radicals [65, 66]. In human trials, fruit juice and derived products presented a diabetes-preventive role that may be related with enhanced activity of antioxidative enzymes such as paraoxonase 1 (PON1), reduction of lipid peroxidation, and inhibition of inflammation mediator transcription factor NF-kB activation [67, 68]. Oral supplementation of pomegranate peel powder (200 mg/kg B.W) for 20 days substantially improved serum level of ALT and AST, glutathione and SOD contents, hepatic glucose-6 phosphate dehydrogenase activity, and regenerated pancreatic β-cells in streptozotocin-induced diabetic animal model [69, 70]. In vivo and in vitro application of fruit extracts and their individual components like punicalagin, punicalin, ellagic acid, and gallic acid exhibited anti-glycation properties and reduced levels of glycation products alike serum AGEs, hemoglobin A1c, and glycoalbumin [71]. Ellagic acid and its glycosides have a profound effect on insulin-sensitizing activity through increasing gene expression of PPAR-γ and GLUT4 that activate insulin signaling pathways for the glucose uptake in L6 myotubes [72]. Phenolic-rich pomegranate peel and flower extracts have been reported to offer α-glucosidase inhibitory activity with IC50 value of 5.56 μg/ml and 29.77 μg/ml, respectively. The studies further suggest ellagitannins to bind the active site of α-glucosidase surface rendering it inactive and delaying carbohydrate digestion that ultimately facilitate in lower glucose absorption [10, 73]. The fruit also exhibits ameliorative effect in treating diabetic nephropathy via improving blood glucose level, kidney hypertrophy index, strengthening glomeruli tubules, increasing basement membrane thickness, and reducing renal disease markers [74, 75]. On account of abovementioned exploratory features, pomegranate, fruit, and peel-derived extracts and fractionated compounds may be suggested as functional food and nutraceutical ingredient in diabetes care.

3.4 Hepatoprotective Role of Pomegranate

The liver is principally the metabolic powerhouse of the human body that stands responsible for the metabolization of nutrients and detoxification. Oxidative stress is among the mechanisms involved in liver pathogenesis and progression of chronic liver disease. Pomegranate fruit on account of its polyphenols including hydrolysable tannins, anthocyanins, flavonoids, and compounds of alike structure prevent progression of liver illnesses. Oral feeding of pomegranate (800 mg/kg B.W) for 2 weeks have shown to protect sepsis-mediated acute liver injury. The study further demonstrated free radical scavenging properties and anti-inflammatory activities of the fruit phenolics as the fundamental protection mechanism of pomegranate phenolics to protect and allay the risks of acute liver injury. Pomegranate phenolics consumed in the form of juice or extracts activate SOD and glutathione activity and reduce serum malondialdehyde contents that further help in mediating TLR4 and NF-*k*B inflammatory pathways [76].

Administration of pomegranate peel extracts rich in ellagitannins to animal models with clinical signs of steatosis (fatty liver) showed considerable protective effect on hepatic morphology, inhibited lipogenesis in cytoplasm of hepatocytes, and improved liver enzymes [12]. Ellagic acid, one of the key pomegranate phenolic attenuates alcohol-induced hepatotoxicity by reducing expressions of pro-fibrogenic and pro-inflammatory cytokines like interleukins, TNF-α, and TGF-β that are reported to increase in alcohol-induced liver fibrosis and inflammation [77, 78]. Pomegranate seed extracts alike peel extracts have been recorded to offer remarkable protective effect against xenobiotics (e.g., CCl_4)-mediated liver fibrosis. The mechanism might be correlated with secondary metabolites of ellagitannins like ellagic acid, urolithins, increased free radical scavenging activity, and inhibition of TGF-β1 level and collagen synthesis [77, 79].

3.5 Pomegranate and Brain Health

Brain aging is generally recognized by irreversible loss in gross motor and sensory neurons and associated physiological functions including cognition. Alzheimer's disease is a suboptimal neurodegenerative disorder symptomized with dementia and cognitive impairment. Senile plaque formation through accretion of amyloid β peptides and generation of neurofibrillary tangles are the hallmark lesions associated with Alzheimer's disease. Research findings have shown redox impairment to be involved in the amyloid β-associated neurotoxicity [80, 81]. Fruits like pomegranate bearing substantial free radical quenching properties could serve as a natural neuroprotective agent. A study conducted in transgenic amyloid precursor protein (APP) mutated mice models-associated diet consumption enriched with 4% pomegranate juice to generate significant impact on boosting cognitive performance of the animal models [82]. The study further witnessed pomegranate juice consumption to

anticipate improved memory, learning ability, and motor coordination and reduced anxiety level in treated mice model as compared to standard diet-fed mice. In another study wherein pomegranate peel extracts in combination with *Gmelina arborea* bark extracts were found to present gamma secretase modulatory and acetylcholinesterase inhibitory effect in reducing pathogenesis of Alzheimer's diseases, it concluded that the extracts derived from both plants offer neuroprotective effect in cognitive dysfunction [83]. Human trials further endorsed consumption of pomegranate juice (8 ounce/day) for a duration of 4 weeks to boost memory and brain functionality via strengthening brain antioxidants pool [84].

3.6 Anti-Inflammatory and Bone Health Promotion Properties

Inflammation is an undefined immune system hyperactivity that progressively triggers transcription factors, i.e., TNF-α and NF-*k*B, that elevate level of pro-inflammatory cytokines like prostaglandin E2 (PGE-2) and interleukin (IL-1, IL-6, IL-8) and overexcite production of reactive oxygen and nitrogen species [85, 86]. A recent study reported that punicalagin A & B, ellagic acid and gallic acid possess anti-inflammatory effect by exerting potent inhibitory effect on lipopolysaccharides stimulated macrophages that downregulate COX-2 protein release from RAW264.7 cells. Consequently, COX-2 protein expression inhibition reduces the level of pro-inflammatory mediators alike inducible NO (*i*NO), PGE-2, interleukins, and ROS [87]. Inflammation negatively affects bone health and compromises quality of life through pannus formation and bone destruction that might consequently lead toward disability. Long-term use of pharmacological preparations for bone rehabilitation alike cyclooxygenase inhibitor or nonsteroidal anti-inflammatory drugs (NSAIDs) contributes in cardiac complications or gastrointestinal discomfort [88]. Botanical extracts like those derived from pomegranate are referred as potent carrier of antioxidants and anti-inflammatory compounds that could further be exploited in bone health restoration. Rheumatoid arthritis affecting about 0.5–1% world population is a systematic autoimmune bone disorder of synovial joints. Higher infiltration rate of inflammatory cells in synovial fluid of patients with rheumatoid arthritis progresses toward irreversible cartilage and bone damage. Ellagic acid inhibits foot paw edematous inflammation [89]. The study conferred ellagic acid to attenuate etiology of adjuvant-induced arthritis (AIA)-linked pathogenesis by downregulation of pro-inflammatory cytokines IL -1β, TNF-α, and IL −17 and upregulation serum level of anti-inflammatory cytokines IL-10 and interferon γ (IFN-γ). Pomegranate fruit extracts offer protection against osteoarthritis by improving cartilage stiffness and physical fitness, decreasing level of cartilage catabolizing enzymes, and strengthening antioxidative defense system [14]. Osteoporosis is the structural deterioration of the microarchitecture array of the bone during remodeling phase and progressively leads toward bone fragility and bone loss. Pomegranate peel extract-enriched diet significantly improves bone mineralization by increasing

osteogenic transcription factors and stimulating activity of bone maker protein, i.e., alkaline phosphatase (ALP) [90]. Pomegranate seed oil (PSO) on account of its punicic acid (conjugated linolenic acid)-rich profile offers osteblastogenesis development and suppression of osteoclastogenesis in animal models fed with 5% PSO-supplemented diet [91]. Consequent upon pomegranate antioxidants and anti-inflammatory compounds bearing features, the fruit and its biomolecules can be suggested as functional food to prevent bone loss and associated disorders.

3.7 Microbial Pathogenesis Inhibitory Role of Pomegranate

Multidrug-resistant bacteria, pandemic viruses, and opportunistic fungi are well-known illnesses and life-threatening infections causing microorganism. Microbiological pathogenesis and infections urge to find an alternative therapy like botanical extracts for contending with widespread multidrug resistance among microorganisms of human health concern. Phytonutrients including polyphenols, flavonoids, tannins (condensed and hydrolysable), terpenoids, and phytosterols are extensively explored and exploited for their potential antimicrobial activity [92, 93]. Aqueous and methanolic extracts of pomegranate pericarp and rind holding up appreciable concentration of anthocyanins, punicalagin, ellagic acid, gallic acid, and other polyphenols have been widely explored for antimicrobial efficacy and in treating a wide range of contagions [6, 94]. Pomegranate peel methanolic extract (80%) presents inhibitory effect against hemorrhagic *Escherichia coli*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *and Listeria monocytogenes* with minimum inhibitory concentration (MICs) ranging between 6 and 12 mg/ml [95, 96]. The toxicity of these phytonutrients against bacterial cell is due to complex formation with enzymes cofactors and sulfhydryl group of proteins that alter permeability of membrane and disturb respiratory chain [97, 98]. In vitro and in situ application of 80% methanolic extracts of pomegranate whole fruit, seed, and rind presented antifungal activity against *Penicillium citrinum*, *Aspergillus niger, Trichoderma reesei*, *Rhizopus oryzae*, and *Mucor indicus* [99]. The crude extract of pomegranate rind shows cytotoxicity at conidial and hyphal stage against dermatophytes, e.g., *Microsporum canis* and *Trichophyton mentagrophytes*, with MICs values of 250 μg/ml and 125 μg/ml, respectively. Polyphenols including punicalagin, catechin, and epigallocatechin being potential antifungal agents induce precipitation of microbial cell membranous proteins and intercellular leakage and alter composition of cytoplasm and outer cell membranes that inhibit fungal growth [100, 101]. Pomegranate hydroalcoholic extracts have also been reported to offer antiviral activity against human immunodeficiency virus type 1, herpes simplex virus 1, influenza A, adenovirus, and hepatitis C virus. The possible mechanism behind viral cell damage is a complex formation between condensed tannins and viral glycoprotein that affect viral attachment with host cell. This further suppresses viral transcription/translation and prevents viral replication [102–104].

3.8 Anthelmintic Activities of Pomegranate

Parasites (ecto- and endoparasites) depend on host for food and shelter rendering a compromised immune system to host, increased disease burden in human and domestic animals, and huge economic loss. Crude pomegranate rind, bark, and root extracts gained a lot of attention in curing parasitic infections since ages [105–107]. Pomegranate fruit extracts have been explored as anti-cestoda, vermifugal, and antiprotozoan mainly due to its tannins, triterpenes, sterols, glycosides, and alkaloids [108, 109]. Oral administration of 40 mg/ml of pomegranate rind crude extract to animals infested with tapeworms (*Raillietina spiralis)* and roundworms (*Ascaridia galli)* induced parasites paralysis and reduced death time in comparison with anthelmintics piperazine and albendazole. Ethanolic and aqueous extracts of pomegranate exhibit ovicidal and larvicidal properties against *Gastrothylax indicus* and *Hymenolepis nana* manifesting it a novel source of anthelmintic agent [7, 110, 111]. Pomegranate peel extracts show protective role against *Plasmodium*-induced parasitosis (malaria). Alongside its hepatoprotective effects, pomegranate extracts reverse signs of anemia provoked by plasmodium infestation through the hemoglobin, and erythrocytes count back to the normal level [112, 113].

3.9 Gut Health Promotion Properties of Pomegranate

Gut microbiota represent complex and mutualistic interplay of the human's largest microbial biome that considerably influence homeostasis and healthy conditions of the host. Dietary and other nutritional interventions have been shown to exert a modulatory effect on the composition of gut microbiota. Polyphenols-rich pomegranate juice and fruit extracts demonstrate beneficial effects on gut health and microbiome. The gut microbes transform intact phenolic compounds, e.g., ellagitannins and anthocyanins, into bioactive metabolites such as ellagic acid and urolithins. Intact ellagitannins and anthocyanins act as prebiotic and have a synergistic effect in promoting probiotics properties of *Lactobacilli* and *Bifidobacteria.* It further inhibits growth of pathogenic microbes and preserves gut microbiome balance [114, 115]. Incorporation of pomegranate peel at 10% of the animal diet exhibit modulatory effect on sodium oxide dismutase 1 (SOD1) and glutathione activity and reduced level of malondialdehyde (MDA) in the small intestine of the ruminant as compared to control diet. Pomegranate polyphenols particularly ellagitannins, punicalagin, and ellagic acid attenuate small intestine lipid peroxidation by strengthening free radicals ($\bullet O_2^-$ and H_2O_2) scavenging system and regulating enzymatic antioxidative pathways as the first-line defense system of the small intestine [116]. Pomegranate peel extracts and seed oil prevent chronic inflammation and necrotizing enterocolitis-mediated intestinal damage. Pomegranate polyphenols especially ellagitannins protect from ulcerative colitis through reducing ERK1/2 activity by downregulation of mTOR downstream pathway and inhibit production of pro-inflammatory cytokines at molecular level [117, 118]. Pomegranate seeds have been reported to be exploited as traditional remedy for the management of

diarrhea. Antidiarrheal activity is also supported by the findings of Souli et al. [2] suggesting methanolic extracts of pomegranate juice to significantly reduce gastrointestinal transit time and number of defecation against castor oil-induced diarrhea in dose-dependent manner. Pomegranate seed oil is a rich source of minerals that might help in regulating mineral balance in diarrheal episodes. Hydrolysable tannins offer protein denaturation properties and form a protein-tannate complex that encourages more resistance to intestinal mucosa, inhibits gastrointestinal transit, and reduces excretion favoring its antidiarrheal activity [119, 120].

3.10 Role of Pomegranate in Modulating Renal Disorders

The importance of kidney function in clearing blood wastes is very evident from the fact that they receive 20% of total blood supplies with each cardia beat to clear the waste. Kidneys eliminate xenobiotics including endogenous metabolites, exogenous chemicals, and drugs through urine and prevent loss of nutrients such as glucose, inorganic ions, and oligopeptides. While in elimination and detoxification, kidneys are utmost exposed to xenobiotics that might cause oxidative stress, nephrotoxicity, and lipid peroxidation [121, 122]. Pomegranate aril and peel extracts show protective effect against nitrosamine and CCl_4-induced apoptosis and kidney injury via inhibiting generation of reactive oxygen species, lowering lipid peroxidation, and enhancing host antioxidative defense mechanisms [123]. Cisplatin – a platinum based inorganic drug – is widely used in the treatment of carcinoma. The drug is reported to cause renal functions impairment through ROS generation, inhibition of protein synthesis, and mitochondrial dysfunction. Pomegranate seed oil being rich in polyunsaturated fatty acids and antioxidants has been suggested as potent dietary approach to avert the risks of cisplatin-induced kidneys dysfunction. Pomegranate seed oil also reduces serum urea, renal nitric oxide, and malondialdehyde levels and upregulate thiol contents [124]. Use of pomegranate accessions like flower extracts (25 mg/kg b.w.) also improves gentamicin-induced weight loss and renal toxicity [125]. Pomegranate juice consumption by the heavy smokers has also shown remarkable ameliorative effects against nicotine-induced hepatorenal toxicity [126]. Pomegranate juice and peel extracts orally administrated to animal models at the rate of 3 ml/kg b.w. and 200 mg/kg b.w., respectively, for a period of 8 weeks substantially reduced cytokines markers and significantly improved steroid-induced kidney inflammation [11]. The clinical trials on exploring renal protective role of pomegranate demonstrate bioactive constituents of the fruit and waste fraction as precursors to activate immune system and enhance cellular antioxidative enzymes production that protect kidneys from inflammation-induced injury and dysfunction [4].

3.11 Pomegranate Perspective Potential in Improving Fertility

Pomegranates have potent beneficial effects in strengthening reproduction through improving spermatogenesis and semen production, maturation of epididymal

spermatozoa, and increasing blood testosterone level [13]. Consumption of phytosterols, punicalagin, quercetin, ellagic acid, and vitamin A-rich natural beverages alike pomegranate fruit juice improves sperm cell abnormalities induced by oxidative stress, bisphenol A, CCl_4, and lead acetate toxicity [127, 128]. Pomegranate juice significantly improves changes in female sex hormone levels and reduces symptoms of polycystic ovarian syndrome [129]. Oral administration of isoflavonoid-rich extracts of pomegranate rind to the animal models at 0.5 g/kg b.w. increased fertility through improved sperm count, sperm motility, and sperm quality. Cautious intake of pomegranate whole fruit extracts up to 5 mg/mL avoiding higher doses has potential to promote fertility [130, 131].

3.12 Wound Healing Properties of Pomegranate Accessions

Wound healing is a distinct process of biochemical and cellular events that involves immediate inflammation after injury to achieve homeostasis, tissue granulation, and collagen formation that imparts tensile strength in the remodeling phase of skin regeneration [132, 133]. Pomegranate pericarp and epicarp are historically used in folk medicines for their ameliorative effects in various ailments including excised and incised wound healing. Biochemical and histological examination revealed that pomegranate comprises tremendous antimicrobial and antioxidant features that aid in epithelialization and production of hydroxyproline to regenerate wounds [134, 135]. Pomegranate pericarp and epicarp extract-based ointment and gels have been extensively explored in the recent past to calculate their wound healing potential. The findings of the studies suggest that topical application of pomegranate polyphenols and lipophilic fractions-based ointments on cutaneous wounds (sores and lesions) result in significant wound recovery notably in diabetic patients [136, 137]. Topical administration of 100 mg/kg pomegranate extracts for a period of 15 days to incised wounds in experimental animals reduces 95% wound area as compared to 85% in control petroleum jelly-based ointment [138]. Pomegranate extracts (10–20%) when topically applied to burn wounds have also significantly reduced period of epithelialization analog to standard drug [139]. Pomegranate pericarp and epicarp extracts contain effective bioactive ingredients that aid in skin epithelialization and regeneration and may be recommended as a natural remedy in wound healing.

3.13 Cosmo Care Properties of Pomegranate

The skin is the most targeted and exposed site for pathogenesis from exogenous factors especially ultraviolet radiations (UVA and UVB) and microbiological infections. Exogenous factors like UV radiations induce genotoxicity and oxidative stress consequently creating skin disorders including immunosuppression, skin carcinoma, sunburn, and photoaging [140]. Pomegranate a mystical fruit famed as *a pharmacy unto itself* owing to its polyphenolic compounds, hydrolysable tannins, and organic acid fractions has a tonic effect in skin care [141, 142]. Fluorescence microscopy and

anisotropic measurements present skin-protective effect of pomegranate seed oil and peel extracts nanoemulsions entrapped with punicic acid and polyphenol-rich ethyl acetate fraction against reactive oxygen species and UVB-mediated DNA and lipid bilayer biomembrane damage [143, 144]. Acne – a plaguing skin disorder – is recognized by sebaceous glands hyperactivity and inflammation leaving scars due to loss in equilibrium of symbiotic skin microbiota. Pomegranate fruit rind and bark rich in hydrolysable tannins (especially punicalagin) exhibit multiple anti-acne abilities including blood purification, antibacterial, anti-inflammatory, and anti-keratinocyte proliferation activity [145, 146]. Owing to photoprotective and eco-friendly effects of pomegranate, seed oils and rind extracts of the fruit can be utilized in skin beautification, combination cosmo care products, and as skin protection factor sunscreen [20, 147].

3.14 Pomegranate Role in Maintaining Oral Hygiene

Orodental health is mostly affected by plaque-forming biofilms and cariogenic bacteria that seek habitat in oral cavity. Several chemotherapeutic agents including chlorhexidine, bisbiguanides, and fluorides containing mechanical floss and gels have been formulated to ensure orodental hygiene [148]. However, long-term use of such chemotherapeutic agents develops resistance in bacterial strains and promotes tooth staining. Such a situation calls for the development of herbal products as an alternative therapy to orodental chemotherapeutic agents [149]. In vivo and in vitro studies have confirmed flavonoids, tannins, vitamins, and minerals-rich fractions of pomegranate as anticariogenic and anti-plaque natural formulations. Pomegranate polyphenols have strong tendency toward inhibiting growth of plaque-forming bacteria including *Streptococcus sanguis* and *Pseudomonas aeruginosa* [150, 151]. Use of pomegranate mouthwash effectively reduced gingivitis (gum bleeding) and periodontitis (gum infection) in comparison with 0.2% chlorhexidine standard mouth rinse [15, 152]. Topical application of oral gel containing 10% pomegranate extracts relieves pain and reduces time for wound healing in aphthous stomatitis (oral ulcer) disorder [153]. Pomegranate fruit extracts efficiently reduce production of bacterial secondary metabolites, sucrose catabolizing enzyme α-glucosidase activity, aspartate aminotransferase activity, and upregulation of ceruloplasmin that inhibits oral oxidative stress [93, 154]. Promising features of pomegranate extracts against several orodental distresses encourage development of pomegranate and its extracts-based natural oral care formulations as alternate to synthetic and chemo-therapeutic products.

4 Conclusions

Clinical trials in human subjects and animal models have demonstrated significant effect of pomegranate consumption in improving the body's inherited defense against various forms of infections, inflammatory, and non-inflammatory disorders.

In comparison with the fruits of the same and other classes, pomegranate has shown broader applicability against serious maladies. Unlike synthetic drugs, heterogeneous biochemical composition of pomegranate and its extracts has ability to manipulate multiple biochemical pathways that can further be exploited for the treatment of various complex disorders. Controlled and clinical trials on pomegranate and its hydroalcoholic extracts have been regarded as safe for human health at orally administrated doses supplying up to 2000 mg polyphenols per day. However, exploitation of pomegranate and derived bioactive compounds as therapeutic agent either alone or in combination calls for careful experimentation to rule out genotoxic response of the plant material.

References

1. Jurenka J (2008) Therapeutic applications of pomegranate (Punica granatum L.): a review. Altern Med Rev 13:128
2. Souli A, Sebai H, Rtibi K, Chehimi L, Sakly M, Amri M, El-Benna J, Marzouki L (2015) Inhibitory effects of two varieties of Tunisian pomegranate (Punica granatum L.) extracts on gastrointestinal transit in rat. J Med Food 18:1007–1012. https://doi.org/10.1089/jmf.2014.0110
3. Lansky EP, Newman RA (2007) Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 109:177–206. https://doi.org/10.1016/j.jep.2006.09.006
4. Ismail T, Sestili P, Akhtar S (2012) Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects. J Ethnopharmacol 143:397–405. https://doi.org/10.1016/j.jep.2012.07.004
5. Akhtar S, Ismail T, Fraternale D, Sestili P (2015) Pomegranate peel and peel extracts: chemistry and food features. Food Chem 174:417–425. https://doi.org/10.1016/j.foodchem.2014.11.035
6. Gullon B, Pintado ME, Pérez-Álvarez JA, Viuda-Martos M (2016) Assessment of polyphenolic profile and antibacterial activity of pomegranate peel (Punica granatum) flour obtained from co-product of juice extraction. Food Control 59:94–98. https://doi.org/10.1016/j.foodcont.2015.05.025
7. Al-Megrin WA (2016) Efficacy of pomegranate (Punica granatum) peel extract against Hymenolepis nana in infections mice. Biosci Biotechnol Res Asia 13:103–108. https://doi.org/10.13005/bbra/2010
8. Moghaddam G, Sharifzadeh M, Hassanzadeh G, Khanavi M, Dolatshahi F, Sadeghi N, Hajimahmoodi M et al (2014) Anti-ulcerative potential of Punica granatum L. hydroalcoholic peel extract. Trop J Pharm Res 13:1093–1097. https://doi.org/10.4314/tjpr.v13i7.12
9. Vilahur G, Padró T, Casaní L, Mendieta G, López JA, Streitenberger S, Badimon L (2015) Polyphenol-enriched diet prevents coronary endothelial dysfunction by activating the Akt/eNOS pathway. Rev Esp Cardiol 68:216–225. https://doi.org/10.1016/j.rec.2014.04.021
10. Bekir J, Cazaux S, Mars M, Bouajila J (2016) In vitro anti-cholinesterase and anti-hyperglycemic activities of flowers extracts from seven pomegranate varieties. Ind Crop Prod 81:176–179. https://doi.org/10.1016/j.indcrop.2015.11.066
11. Ali H, Naheed K, Khalil A, Qamar K, Saga Z (2017) Effects of pomegranate juice and peel extract on steroid induced inflammation in mice kidneys. JSZMC 8:1218–1221
12. Al-Shaaibi SN, Waly MI, Al-Subhi L, Tageldin MH, Al-Balushi NM, Rahman MS (2016) Ameliorative effects of pomegranate peel extract against dietary-induced nonalcoholic fatty liver in rats. Prev Nutr Food Sci 21:14–23. https://doi.org/10.3746/pnf.2016.21.1.14

13. Rao F, Tian H, Li W, Hung H, Sun F (2016) Potential role of punicalagin against oxidative stress induced testicular damage. Asian J Androl 18:627. https://doi.org/10.4103/1008-682X.168792
14. Ghoochani N, Karandish M, Mowla K, Haghighizadeh MH, Jalali MT (2016) The effect of pomegranate juice on clinical signs, matrix metalloproteinases and antioxidant status in patients with knee osteoarthritis. J Sci Food Agric 96:4377–4381. https://doi.org/10.1002/jsfa.7647
15. de Medeiros Nóbrega DR, Santos RL, Soares RDSC, Alves PM, Medeiros ACD, Pereira JV (2015) A randomized, controlled clinical trial on the clinical and microbiological efficacy of Punica granatum Linn mouthwash. BRPDIC 15:301–308. https://doi.org/10.4034/PBOCI.2015.151.32
16. Kang SJ, Choi BR, Lee EK, Kim SH, Yi HY, Park HR, Song CH, Lee YJ, Ku SK (2015) Inhibitory effect of dried pomegranate concentration powder on melanogenesis in B16F10 melanoma cells; involvement of p38 and PKA signaling pathways. Int J Mol Sci 16:24219–24242. https://doi.org/10.3390/ijms161024219
17. Kumari A, Dora J, Kumar A, Kumar A (2012) Pomegranate (Punica granatum) overview. Int J Pharm Chem Biol Sci 1:1218–1222
18. Zhao X, Yuan Z, Fang Y, Yin Y, Feng L (2013) Characterization and evaluation of major anthocyanins in pomegranate (Punica granatum L.) peel of different cultivars and their development phases. Eur Food Res Technol 236:109–117. https://doi.org/10.1007/s00217-012-1869-6
19. Qu W, Breksa AP III, Pan Z, Ma H, Mchugh TH (2012) Storage stability of sterilized liquid extracts from pomegranate peel. J Food Sci 77:765–772. https://doi.org/10.1111/j.1750-3841.2012.02779.x
20. Emanuele E, Bertona M, Biagi M (2017) Comparative effects of a fixed Polypodium leucotomos/pomegranate combination versus Polypodium leucotomos alone on skin biophysical parameters. Neuroendocrinol Lett 38:101–105
21. Revathi M, Saravanan M, Chiya AB, Velan M (2012) Removal of copper, nickel, and zinc ions from electroplating rinse water. CLEAN 40:66–79. https://doi.org/10.1002/clen.201000477
22. Dhir B (2014) Potential of biological materials for removing heavy metals from wastewater. Environ Sci Pollut Res 21:1614–1627. https://link.springer.com/article/10.1007/s11356-013-2230-8. Accessed online 3 Dec 2013
23. Heber D (2011) Pomegranate ellagitannins. In: Benzie IFF, Wachtel-Galor S (eds) Herbal medicine: biomolecular and clinical aspects, 2nd edn. CRC Press, Boca Raton
24. Neyrinck AM, Van Hée VF, Bindels LB, De Backer F, Cani PD, Delzenne NM (2012) Polyphenol-rich extract of pomegranate peel alleviates tissue inflammation and hypercholesterolaemia in high-fat diet-induced obese mice: potential implication of the gut microbiota. Br J Nutr 7:1–8. https://doi.org/10.1017/S0007114512002206
25. Syed DN, Chamcheu JC, Adham MV, Mukhtar H (2013) Pomegranate extracts and cancer prevention: molecular and cellular activities. Anticancer Agents Med Chem (Formerly Curr Med Chem Anticancer Agents) 13:1149–1161
26. Orgil O, Schwartz E, Baruch L, Matityahu I, Mahajna J, Amir R (2014) The antioxidative and anti-proliferative potential of non-edible organs of the pomegranate fruit and tree. LWT-Food Sci Technol 58:571–577. https://doi.org/10.1016/j.lwt.2014.03.030
27. Abid M, Cheikhrouhou S, Renard CM, Bureau S, Cuvelier G, Attia H, Ayadi MA (2017) Characterization of pectins extracted from pomegranate peel and their gelling properties. Food Chem 215:318–325. https://doi.org/10.1016/j.foodchem.2016.07.181
28. Khatib M, Giuliani C, Rossi F, Adessi A, Al-Tamimi A, Mazzola G, Di Gioia D, Innocenti M, Mulinacci N (2017) Polysaccharides from by-products of the wonderful and Laffan pomegranate varieties: new insight into extraction and characterization. Food Chem 235:58–66. https://doi.org/10.1016/j.foodchem.2017.05.041
29. Kýralan M, Gölükcü M, Tokgöz H (2009) Oil and conjugated linolenic acid contents of seeds from important pomegranate cultivars (Punica granatum L.) grown in Turkey. J Am Oil Chem Soc 86:985–990. https://link.springer.com/article/10.1007/s11746-009-1436-x. Accessed Online 12 July 2009

30. Modaresi J, Nasri MF, Rashidi L, Dayani O, Kebreab E (2011) Effects of supplementation with pomegranate seed pulp on concentrations of conjugated linoleic acid and punicic acid in goat milk. J Dairy Sci 94:4075–4080. https://doi.org/10.3168/jds.2010-4069
31. Melo IL (2012) Evaluation of the effects of pomegranate seed oil (Punica granatum L.) on tissue lipid profile and its influence on biochemical parameters in oxidative processes of rats [thesis]. Pharmaceutical Science Faculty of Sao Paulo University, Sao Paulo
32. Amyrgialaki E, Makris DP, Mauromoustakos A, Kefalas P (2014) Optimisation of the extraction of pomegranate (Punica granatum) husk phenolics using water/ethanol solvent systems and response surface methodology. Ind Crop Prod 59:216–222. https://doi.org/10.1016/j.indcrop.2014.05.011
33. Moorthy IG, Maran JP, Muneeswari S, Naganyashree S, Shivamathi CS (2014) Response surface optimization of ultrasound assisted extraction of pectin from pomegranate peel. Int J Biol Macromol 72:1323–1328. https://doi.org/10.1016/j.ijbiomac.2014.10.037
34. Mushtaq M, Sultana B, Anwar F, Adnan A, Rizvi SS (2015) Enzyme-assisted supercritical fluid extraction of phenolic antioxidants from pomegranate peel. J Supercrit Fluids 104:122–131
35. Lantzouraki DZ, Sinanoglou VJ, Zoumpoulakis P, Proestos C (2016) Comparison of the antioxidant and antiradical activity of pomegranate (Punica granatum L.) by ultrasound-assisted and classical extraction. Anal Lett 49:969–978. https://doi.org/10.1080/00032719.2015.1038550
36. Kazemi M, Karim R, Mirhosseini H, Hamid AA (2016) Optimization of pulsed ultrasound-assisted technique for extraction of phenolics from pomegranate peel of Malas variety: Punicalagin and hydroxybenzoic acids. Food Chem 206:156–166. https://doi.org/10.1016/j.foodchem.2016.03.017
37. Çavdar HK, Yanık DK, Gök U, Göğüş F (2017) Optimisation of microwave-assisted extraction of pomegranate (Punica granatum L.) seed oil and evaluation of its physicochemical and bioactive properties. Food Sci Biotechnol 55:86. https://doi.org/10.17113/ftb.55.01.17.4638
38. Đurđević S, Milovanović S, Šavikin K, Ristić M, Menković N, Pljevljakušić D, Petrović S, Bogdanović A (2017) Improvement of supercritical CO_2 and n-hexane extraction of wild growing pomegranate seed oil by microwave pretreatment. Ind Crop Prod 104:21–27. https://doi.org/10.1016/j.indcrop.2017.04.024
39. Goula AM, Papatheodorou A, Karasavva S, Kaderides K (2018) Ultrasound-Assisted Aqueous Enzymatic Extraction of Oil from Pomegranate Seeds. Waste Biomass Valoriz 9:1–11. https://link.springer.com/article/10.1007/s12649-016-9740-9. Accessed online 18 Oct 2016
40. Huang J, He W, Yan C, Du X, Shi X (2017) Microwave assisted extraction of flavonoids from pomegranate peel and its antioxidant activity. Bio Web Conf 8:03008. EDP Sciences
41. Xi J, He L, Yan LG (2017) Continuous extraction of phenolic compounds from pomegranate peel using high voltage electrical discharge. Food Chem 230:354–361. https://doi.org/10.1016/j.foodchem.2017.03.072
42. Zhai X, Zhu C, Li Y, Zhang Y, Duan Z, Yang X (2018) Optimization for pectinase-assisted extraction of polysaccharides from pomegranate peel with chemical composition and antioxidant activity. Int J Biol Macromol 109:244–253
43. Ambigaipalan P, de Camargo AC, Shahidi F (2016) Phenolic compounds of pomegranate byproducts (outer skin, mesocarp, divider membrane) and their antioxidant activities. J Agric Food Chem 64:6584–6604. https://doi.org/10.1021/acs.jafc.6b02950
44. Taghadosi M, Gilasy HR (2008) The general and specific quality of life in patients with ischemia in Kashan. Iran J Nurs Res 3:39–46
45. Espín JC, Larrosa M, García-Conesa MT, Tomás-Barberán F (2013) Biological significance of urolithins, the gut microbial ellagic acid-derived metabolites: the evidence so far. Evid Based Complement Alternat Med 2013:270418. https://doi.org/10.1155/2013/270418
46. Haghighian MK, Rafraf M, Moghaddam A, Hemmati S, Jafarabadi MA, Gargari BP (2016) Pomegranate (Punica granatum L.) peel hydro alcoholic extract ameliorates cardiovascular

risk factors in obese women with dyslipidemia: a double blind, randomized, placebo controlled pilot study. EuJIM 8:676–682. https://doi.org/10.1016/j.eujim.2016.06.010
47. Razani Z, Dastani M, Kazerani HR (2017) Cardioprotective effects of pomegranate (Punica granatum) juice in patients with ischemic heart disease. Phytother Res 31:1731–1738. https://doi.org/10.1002/ptr.5901
48. Aloutaibi GT, Gashlan H, Moselhy SS, Al-Malki AL, Khan JA (2017) Possible cardioprotective action of pomegranate juice punica granatum and propolis against myocardial infarction induced in rats. AJTCAM 14:138–146. https://doi.org/10.21010/ajtcam.v14i5.17
49. Turrini E, Ferruzzi L, Fimognari C (2015) Potential effects of pomegranate polyphenols in cancer prevention and therapy. Oxidative Med Cell Longev 2015:938475. https://doi.org/10.1155/2015/938475
50. Siegel R, Ward E, Brawley O, Jemal A (2011) The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin 61:212–236
51. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA (2000) Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 48:4581–4589. https://doi.org/10.1021/jf000404a
52. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D (2005) In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J Nutr Biochem 16:360–367. https://doi.org/10.1016/j.jnutbio.2005.01.006
53. Kulkarni AP, Mahal HS, Kapoor S, Aradhya SM (2007) In vitro studies on the binding, antioxidant, and cytotoxic actions of punicalagin. J Agric Food Chem 55:1491–1500. https://doi.org/10.1021/jf0626720
54. Aqil F, Munagala R, Vadhanam MV, Kausar H, Jeyabalan J, Schultz DJ, Gupta RC (2012) Anti-proliferative activity and protection against oxidative DNA damage by punicalagin isolated from pomegranate husk. Food Res Int 49:345–353. https://doi.org/10.1016/j.foodres.2012.07.059
55. Freedland SJ, Carducci M, Kroeger N, Partin A, Rao JY, Jin Y, Kerkoutian S, Wu H, Li Y, Creel P, Mundy K (2013) A double-blind, randomized, neoadjuvant study of the tissue effects of POMx pills in men with prostate cancer before radical prostatectomy. Cancer Prev Res 6(10):1120–1127. https://doi.org/10.1158/1940-6207
56. Paller CJ, Ye X, Wozniak PJ, Gillespie BK, Sieber PR, Greengold RH, Stockton BR, Hertzman BL, Efros MD, Roper RP, Liker HR (2013) A randomized phase II study of pomegranate extract for men with rising PSA following initial therapy for localized prostate cancer. Prostate Cancer Prostatic Dis 16:50–55. https://doi.org/10.1038/pcan.2012.20
57. Pohl C, Hombach A, Kruis W (2000) Chronic inflammatory bowel disease and cancer. Hepatogastroenterology 47:57–70
58. Adams LS, Seeram NP, Aggarwal BB, Takada Y, Sand D, Heber D (2006) Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. J Agric Food Chem 54:980–985. https://doi.org/10.1021/jf052005r
59. Marín M, Giner RM, Ríos JL, Recio MC (2013) Intestinal anti-inflammatory activity of ellagic acid in the acute and chronic dextrane sulfate sodium models of mice colitis. J Ethnopharmacol 150:925–934. https://doi.org/10.1016/j.jep.2013.09.030
60. Mehta R, Lansky EP (2004) Breast cancer chemopreventive properties of pomegranate (Punica granatum) fruit extracts in a mouse mammary organ culture. Eur J Cancer Prev 13:345–348. https://doi.org/10.1097/01.cej.0000136571.70998.5a
61. Dai Z, Nair V, Khan M, Ciolino HP (2010) Pomegranate extract inhibits the proliferation and viability of MMTV-Wnt-1 mouse mammary cancer stem cells in vitro. Oncol Rep 24:1087–1091. https://doi.org/10.3892/or_00000959
62. Syed DN, Malik A, Hadi N, Sarfaraz S, Afaq F, Mukhtar H (2006) Photochemopreventive effect of pomegranate fruit extract on UVA-mediated activation of cellular pathways in normal

human epidermal keratinocytes. J Photochem Photobiol 82:398–405. https://doi.org/10.1562/2005-06-23-RA-589
63. Pacheco-Palencia LA, Noratto G, Hingorani L, Talcott ST, Mertens-Talcott SU (2008) Protective effects of standardized pomegranate (Punica granatum L.) polyphenolic extract in ultraviolet-irradiated human skin fibroblasts. J Agric Food Chem 56:8434–8441. https://doi.org/10.1021/jf8005307
64. Xu KZY, Zhu C, Kim MS, Yamahara J, Li Y (2009) Pomegranate flower ameliorates fatty liver in an animal model of type 2 diabetes and obesity. J Ethnopharmacol 123:280–287. https://doi.org/10.1016/j.jep.2009.03.035
65. Hunt JV, Smith CC, Wolff SP (1990) Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes 39:1420–1424. https://doi.org/10.2337/diab.39.11.1420
66. Tzulker R, Glazer I, Bar-Ilan I, Holl D, Aviram M, Amir R (2007) Antioxidant activity, polyphenol content and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. J Agric Food Chem 55:9559–9570. https://doi.org/10.1021/jf071413n
67. Rock W, Rosenblat M, Miller-Lotan R, Levy AP, Elias M, Aviram M (2008) Consumption of wonderful variety pomegranate juice and extract by diabetic patients increases paraoxonase 1 association with high-density lipoprotein and stimulates its catalytic activities. J Agric Food Chem 56:8704–8713. https://doi.org/10.1021/jf801756x
68. Rosenblat M, Hayek T, Aviram M (2006) Anti-oxidative effects of pomegranate juice (PJ) consumption by diabetic patients on serum and on macrophages. Atherosclerosis 187:363–371. https://doi.org/10.1016/j.atherosclerosis.2005.09.006
69. Saad EA, Hassanien MM, El-Hagrasy MA, Radwan KH (2015) Antidiabetic, hypolipidemic and antioxidant activities and protective effects of Punica granatum peels powder against pancreatic and hepatic tissues injuries in streptozotocin induced IDDM in rats. Int J Pharm Pharm Sci 7:397–402
70. Hasona NASA, Qumani MA, Alghassab TA, Alghassab MA, Alghabban AA (2017) Ameliorative properties of Iranian Trigonella foenum-graecum L. seeds and Punica granatum L. peel extracts in streptozotocin-induced experimental diabetic guinea pigs. Asian Pac J Trop Biomed 7:234–239. https://doi.org/10.1016/j.apjtb.2016.12.004
71. Kumagai Y, Nakatani S, Onodera H, Nagatomo A, Nishida N, Matsuura Y, Wada M (2015) Anti-glycation effects of pomegranate (Punica granatum L.) fruit extract and its components in vivo and in vitro. J Agric Food Chem 63:7760–7764. https://doi.org/10.1021/acs.jafc.5b02766
72. Nankar RP, Doble M (2015) Ellagic acid potentiates insulin sensitising activity of pioglitazone in L6 myotubes. J Funct Foods 15:1–10. https://doi.org/10.1016/j.jff.2015.03.010
73. Çam M, İçyer NC (2015) Phenolics of pomegranate peels: extraction optimization by central composite design and alpha glucosidase inhibition potentials. J Food Sci Technol 52:1489–1497. https://doi.org/10.1007/s13197-013-1148-y
74. Ankita P, Deepti B, Nilam M (2015) Flavonoid rich fraction of Punica granatum improves early diabetic nephropathy by ameliorating proteinuria and disturbed glucose homeostasis in experimental animals. Pharm Biol 53:61–71. https://doi.org/10.3109/13880209.2014.910533
75. Mestry SN, Dhodi JB, Kumbhar SB, Juvekar AR (2017) Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract. JTCM 7:273–280. https://doi.org/10.1016/j.jtcme.2016.06.008
76. Makled MN, El-Awady MS, Abdelaziz RR, Atwan N, Guns ET, Gameil NM et al (2016) Pomegranate protects liver against cecal ligation and puncture-induced oxidative stress and inflammation in rats through TLR 4/NF-κB pathway inhibition. Environ Toxicol Pharmacol 43:182–192. https://doi.org/10.1016/j.etap.2016.03.011
77. García-Nino WR, Zazueta C (2015) Ellagic acid: pharmacological activities and molecular mechanisms involved in liver protection. Pharmacol Res 97:84–103. https://doi.org/10.1016/j.phrs.2015.04.008

78. Mandrekar P, Szabo G (2009) Signalling pathways in alcohol-induced liver inflammation. J Hepatol 50:1258–1266. https://doi.org/10.1016/j.jhep.2009.03.007
79. Wei XL, Fang RT, Yang YH, Bi XY, Ren GX, Luo AL, Zhao M, Zang WJ (2015) Protective effects of extracts from pomegranate peels and seeds on liver fibrosis induced by carbon tetrachloride in rats. BMC Complement Altern Med 15:389. https://doi.org/10.1186/s12906-015-0916-9
80. Asseburg H, Hagl S, Eckert GP (2014) Nutritional approaches for healthy aging of the brain and the prevention of neurodegenerative diseases. Pharma-Nutrition, pp 457–479. Springer. https://doi.org/10.1007/978-3-319-06151-1_23
81. E Abdel Moneim A (2015) Oxidant/antioxidant imbalance and the risk of Alzheimer's disease. Curr Alzheimer Res 12:335–349
82. Subash S, Braidy N, Essa MM, Zayana AB, Ragini V, Al-Adawi S, Guillemin GJ (2015) Long-term (15 mo) dietary supplementation with pomegranates from Oman attenuates cognitive and behavioral deficits in a transgenic mice model of Alzheimer's disease. Nutrition 31:223–229. https://doi.org/10.1016/j.nut.2014.06.004
83. Vasudev P, Shreedhara CS, Chandrashekar KS, Yamini D (2015) Neuroprotective action of Gmelina arborea (bark) and Punica granatum (peel) extracts. Asian J Pharm. https://doi.org/10.1016/j.ajps.2015.11.004
84. Bookheimer SY, Renner BA, Ekstrom A, Li Z, Henning SM, Brown JA, Small GW (2013) Pomegranate juice augments memory and FMRI activity in middle-aged and older adults with mild memory complaints. Evid Based Complement Alternat Med 2013:946298. https://doi.org/10.1155/2013/946298
85. Conner EM, Grisham MB (1996) Inflammation, free radicals, and antioxidants. Nutrition 12:274–277. https://doi.org/10.1016/S0899-9007(96)00000-8
86. Nathan C (2006) Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol 6(3):173–182. https://doi.org/10.1038/nri1785
87. BenSaad LA, Kim KH, Quah CC, Kim WR, Shahimi M (2017) Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A&B isolated from Punica granatum. BMC Complement Altern Med 17:47. https://doi.org/10.1186/s12906-017-1555-0
88. Akhtar N, Haqqi TM (2012) Current nutraceuticals in the management of osteoarthritis: a review. Ther Adv Musculoskelet Dis 4:181–207. https://doi.org/10.1177/1759720X11436238
89. Allam G, Mahdi EA, Alzahrani AM, Abuelsaad AS (2016) Ellagic acid alleviates adjuvant induced arthritis by modulation of pro-and anti-inflammatory cytokines. Cent Eur J Immunol 41:339. https://doi.org/10.5114/ceji.2016.65132
90. Spilmont M, Léotoing L, Davicco MJ, Lebecque P, Miot-Noirault E, Pilet P et al (2015) Pomegranate peel extract prevents bone loss in a preclinical model of osteoporosis and stimulates osteoblastic differentiation in vitro. Nutrients 7:9265–9284. https://doi.org/10.3390/nu7115465
91. Spilmont M, Léotoing L, Davicco MJ, Lebecque P, Mercier S, Miot-Noirault E, Pilet P, Rios L, Wittrant Y, Coxam V (2013) Pomegranate seed oil prevents bone loss in a mice model of osteoporosis, through osteoblastic stimulation, osteoclastic inhibition and decreased inflammatory status. J Nutr Biochem 24:1840–1848. https://doi.org/10.1016/j.jnutbio.2013.04.005
92. Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA (2007) Antibacterial activity directed isolation of compounds from Punica granatum. J Food Sci 72:M341–M345. https://doi.org/10.1111/j.1750-3841.2007.00533.x
93. Howell AB, D'Souza DH (2013) The pomegranate: effects on bacteria and viruses that influence human health. Evid Based Complement Alternat Med 2013:606212. https://doi.org/10.1155/2013/606212
94. Tanveer A, Farooq U, Akram K, Hayat Z, Shafi A, Nazar H, Ahmad Z (2015) Pomegranate extracts: a natural preventive measure against spoilage and pathogenic microorganisms. Food Rev Int 31:29–51. https://doi.org/10.1080/87559129.2014.961074
95. Al-Zoreky NS (2009) Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. J Food Microbiol 134:244–248. https://doi.org/10.1016/j.ijfoodmicro.2009.07.002

96. Prashanth D, Asha MK, Amit A (2001) Antibacterial activity of Punica granatum. Fitoterapia 72:171–173. https://doi.org/10.1016/S0367-326X(00)00270-7
97. Cristani M, D'Arrigo M, Mandalari G, Castelli F, Sarpietro MG, Micieli D, Venuti V, Bisignano G, Saija A, Trombetta D (2007) Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. J Agric Food Chem 55:6300–6308. https://doi.org/10.1021/jf070094x
98. Goel G, Puniya AK, Aguilar CN, Singh K (2005) Interaction of gut microflora with tannins in feeds. Naturwissenschaften 92:497–503
99. Miguel MG, Neves MA, Antunes MD (2010) Pomegranate (Punica granatum L.): a medicinal plant with myriad biological properties – a short review. J Med Plant Res 4:2836–2847
100. Foss SR, Nakamura CV, Ueda-Nakamura T, Cortez DA, Endo EH, Dias Filho BP (2014) Antifungal activity of pomegranate peel extract and isolated compound punicalagin against dermatophytes. Ann Clin Microbiol Antimicrob 13:32. https://doi.org/10.1186/s12941-014-0032-6
101. Dahham SS, Ali MN, Tabassum H, Khan M (2010) Studies on antibacterial and antifungal activity of pomegranate (Punica granatum L.). Am Eurasian J Agric Environ Sci 9:273–281
102. Cheng HY, Lin TC, Yang CM, Wang KC, Lin CC (2004) Mechanism of action of the suppression of herpes simplex virus type 2 replication by pterocarnin A. Microbes Infect 6:738–744. https://doi.org/10.1016/j.micinf.2004.03.009
103. Reddy BU, Mullick R, Kumar A, Sudha G, Srinivasan N, Das S (2014) Small molecule inhibitors of HCV replication from pomegranate. Sci Rep 4:5411. https://doi.org/10.1038/srep0541
104. Moradi MT, Karimi A, Alidadi S, Saedi-Marghmaleki M (2015) In vitro anti-adenovirus activity of pomegranate (Punica granatum L.) peel extract. Adv Herb Med 1:1–8
105. Akhtar MS, Aslam M (1988) Anthelmintic efficacies of total alkaloids and glycosides isolated from Punica granatum fruit-rind (anar). Pak J Agric Sci 25:161–168
106. Brouwer N, Liu Q, Harrington D, Kohen J, Vemulpad S, Jamie J, Randall M, Randall D (2005) An ethnopharmacological study of medicinal plants in New South Wales. Molecules 10:1252–1262. https://doi.org/10.3390/10101252
107. Subhedar S, Goswami P, Rana N, Gupta A, Shukla P (2011) Herbal alternatives: anthelmintic activity of Punica granatum (pomegranate). Int J Drug Discov Herb Res 1:150–152
108. Dell'Agli M, Galli GV, Corbett Y, Taramelli D, Lucantoni L, Habluetzel A et al (2009) Antiplasmodial activity of Punica granatum L. fruit rind. J Ethnopharmacol 125:279–285. https://doi.org/10.1016/j.jep.2009.06.025
109. Tantray MA, Akbar S, Khan R, Tariq KA, Shawl AS (2009) Humarain: a new dimeric gallic acid glycoside from Punica granatum L. bark. Fitoterapia 80:223–225. https://doi.org/10.1016/j.fitote.2009.01.013
110. Aggarwal R, Kaur K, Suri M, Bagai U (2016) Anthelmintic potential of Calotropis procera, Azadirachta indica and Punica granatum against Gastrothylax indicus. J Parasit Dis 40:1230–1238. https://doi.org/10.1007/s12639-015-0658-0
111. Ali N, Jamil A, Shah S, Shah I, Ahmed G, Junaid M, Ahmed Z (2015) Parasiticidal and brine shrimp cytotoxicity potential of crude methanolic extract of rind of Punica granatum Linn against round worms and tape worms. Pak J Pharm Sci 28:959–962
112. Singh K, Hasan A, Ahmad A, Mir SS (2016) Anti-malarial treatment: herbal medicine a ray of hope. Int J Herb Med 4:119–125
113. Hafiz TA, Mubaraki MA, Al-Quraishy S, Dkhil MA (2016) The potential role of Punica granatum treatment on murine malaria-induced hepatic injury and oxidative stress. Parasitol Res 115:1427–1433. https://doi.org/10.1007/s00436-015-4876-2
114. Li Z, Summanen PH, Komoriya T, Henning SM, Lee RP, Carlson E et al (2015) Pomegranate ellagitannins stimulate growth of gut bacteria in vitro: implications for prebiotic and metabolic effects. Anaerobe 34:164–168. https://doi.org/10.1016/j.anaerobe.2015.05.012
115. Mosele JI, Gosalbes MJ, Macià A, Rubió L, Vázquez-Castellanos JF, Jiménez Hernández N et al (2015) Effect of daily intake of pomegranate juice on fecal microbiota and feces

metabolites from healthy volunteers. Mol Nutr Food Res 59:1942–1953. https://doi.org/10.1002/mnfr.201500227
116. Al-Gubory KH, Blachier F, Faure P, Garrel C (2016) Pomegranate peel extract decreases small intestine lipid peroxidation by enhancing activities of major antioxidant enzymes. J Sci Food Agric 96:3462–3468. https://doi.org/10.1002/jsfa.7529
117. Hollebeeck S, Winand J, Hérent MF, During A, Leclercq J, Larondelle Y, Schneider YJ (2012) Anti-inflammatory effects of pomegranate (Punica granatum L.) husk ellagitannins in Caco-2 cells, an in vitro model of human intestine. Food Funct 3:875–885. https://doi.org/10.1039/C2FO10258G
118. Kim H, Banerjee N, Ivanov I, Pfent CM, Prudhomme KR, Bisson WH et al (2016) Comparison of anti-inflammatory mechanisms of mango (Mangifera Indica L.) and pomegranate (Punica Granatum L.) in a preclinical model of colitis. Mol Nutr Food Res 60:1912–1923. https://doi.org/10.1002/mnfr.201501008
119. El Kar C, Ferchichi A, Attia F, Bouajila J (2011) Pomegranate (Punica granatum) juices: chemical composition, micronutrient cations, and antioxidant capacity. J Food Sci 76: C795–C800. https://doi.org/10.1111/j.1750-3841.2011.02211.x
120. Kouitcheu MLB, Penlap BV, Kouam J, Ngadjui BT, Fomum ZT, Etoa FX (2006) Evaluation of antidiarrhoeal activity of the stem bark of Cylicodiscus gabunensis (mimosaceae). Afr J Biotechnol 5:1062–1066
121. Cheng X, Klaassen CD (2009) Tissue distribution, ontogeny, and hormonal regulation of xenobiotic transporters in mouse kidneys. Drug Metab Dispos 37:2178–2185. https://doi.org/10.1124/dmd.109.027177
122. Viladomiu M, Hontecillas R, Lu P, Bassaganya-Riera J (2013) Preventive and prophylactic mechanisms of action of pomegranate bioactive constituents. Evid Based Complement Alternat Med 2013:789764. https://doi.org/10.1155/2013/789764
123. Hamouda AF, Shaban NZ, Talaat IM (2015) Effects of some pyrimidine derivatives and pomegranate juice on male rat kidney injuries induced by diethylnitrosamine and carbon tetrachloride. Biol Chem Res 201:215–229
124. Boroushaki MT, Rajabian A, Farzadnia M, Hoseini A, Poorlashkari M, Taghavi A, Dolati K, Bazmandegan G (2015) Protective effect of pomegranate seed oil against cisplatin-induced nephrotoxicity in rat. Ren Fail 37:1338–1343. https://doi.org/10.3109/0886022X.2015.1073496
125. Sadeghi F, Nematbakhsh M, Noori-Diziche A, Eshraghi-Jazi F, Talebi A, Nasri H et al (2015) Protective effect of pomegranate flower extract against gentamicin-induced renal toxicity in male rats. JRIP 4:45–50. https://doi.org/10.12861/jrip.2015.10
126. Albasha MO, Azab AE (2016) Hepatorenal protective effects of pomegranate (Punica granatum) Juice against nicotine induced toxicity in Guinea pigs. J Adv Biol Biotechnol 5:1–13. https://doi.org/10.9734/JABB/2016/21996
127. Türk G, Çeribaşı S, Sönmez M, Çiftçi M, Yüce A, Güvenç M et al (2016) Ameliorating effect of pomegranate juice consumption on carbon tetrachloride-induced sperm damages, lipid peroxidation, and testicular apoptosis. Toxicol Ind Health 32:126–137. https://doi.org/10.1177/0748233713499600
128. Leiva KP, Rubio J, Peralta F, Gonzales GF (2011) Effect of Punica granatum (pomegranate) on sperm production in male rats treated with lead acetate. Toxicol Mech Methods 21:495–502. https://doi.org/10.3109/15376516.2011.555789
129. Hossein KJ, Leila KJ, koukhdan Ebrahim T, Nazanin SJ, Farzad P, Elham R (2015) The effect of pomegranate juice extract on hormonal changes of female Wistar rats caused by polycystic ovarian syndrome. BPJ 8:971–977. https://doi.org/10.13005/bpj/849
130. AL-Saeed MH, Hadi NS (2015) Study the effect of Isoflovonoid extract of Punica granatum rinds on fertility efficiency and semen fluid characteristic in male rabbits. Bas J Vet Res 14:17–30
131. Kılıçgün H, Arda N, Uçar EÖ (2015) Identification of longevity, fertility and growth-promoting properties of pomegranate in Caenorhabditis elegans. Pharmacogn Mag 11:356–359. https://doi.org/10.4103/0973-1296.153089

132. Beanes SR, Dang C, Soo C, Ting K (2003) The phases of cutaneous wound healing. Exp Rev Mol Med 5:1–22
133. Falanga V (2005) Wound healing and its impairment in the diabetic foot. Lancet 366:1736–1743. https://doi.org/10.1016/S0140-6736(05)67700-8
134. Hayouni EA, Miled K, Boubaker S, Bellasfar Z, Abedrabba M, Iwaski H et al (2011) Hydroalcoholic extract based-ointment from Punica granatum L. peels with enhanced in vivo healing potential on dermal wounds. Phytomedicine 18:976–984. https://doi.org/10.1016/j.phymed.2011.02.011
135. Adiga S, Tomar P, Rajput RR (2010) Effect of Punica granatum peel aqueous extract on normal and dexamethasone suppressed wound healing in wistar rats. Int J Pharm Sci Rev Res 5:34–37. Available online at www.globalresearchonline.net
136. Yan H, Peng KJ, Wang QL, Gu ZY, Lu YQ, Zhao J et al (2013) Effect of pomegranate peel polyphenol gel on cutaneous wound healing in alloxan-induced diabetic rats. Chin Med J 126:1700–1706
137. Klein M (2008) Pomegranate-derived products for the treatment of skin sores & lesions. US Patent Appl 12:676–957
138. Nayak SB, Rodrigues V, Maharaj S, Bhogadi VS (2013) Wound healing activity of the fruit skin of Punica granatum. J Med Food 16:857–861. https://doi.org/10.1089/jmf.2012.0229
139. Rajput R, Sagar VS, Adiga S (2011) Effect of punica granatum peel extract on burn wound healing in albino wistar rats. Int J Appl Biol Pharm 2:353–357
140. Nichols JA, Katiyar SK (2010) Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. Arch Dermatol Res 302:71–83. https://link.springer.com/article/10.1007/s00403-009-1001-3. Accessed online 7 Nov 2009
141. Viuda-Martos M, Fernández-López J, Pérez-Álvarez JA (2010) Pomegranate and its many functional components as related to human health: a review. Compr Rev Food Sci Food Saf 9:635–654. https://doi.org/10.1111/j.1541-4337.2010.00131.x
142. Melo ILP, Carvalho EBT, Mancini-Filho J (2014) Pomegranate seed oil (Punica Granatum L.): a source of punicic acid (conjugated α-linolenic acid). J Hum Nutr Food Sci 2:1024
143. Baccarin T, Mitjans M, Ramos D, Lemos-Senna E, Vinardell MP (2015a) Photoprotection by Punica granatum seed oil nanoemulsion entrapping polyphenol-rich ethyl acetate fraction against UVB-induced DNA damage in human keratinocyte (HaCaT) cell line. J Photochem Photobiol 153:127–136. https://doi.org/10.1016/j.jphotobiol.2015.09.005
144. Baccarin T, Mitjans M, Lemos-Senna E, Vinardell MP (2015b) Protection against oxidative damage in human erythrocytes and preliminary photosafety assessment of Punica granatum seed oil nanoemulsions entrapping polyphenol-rich ethyl acetate fraction. Toxicol In Vitro 30:421–428. https://doi.org/10.1016/j.tiv.2015.09.020
145. Lee CJ, Chen LG, Liang WL, Wang CC (2017) Multiple activities of punica granatum linne against acne vulgaris. Int J Mol Sci 18:141. https://doi.org/10.3390/ijms18010141
146. Karodi RS, Mahendrakumar CB, Bhise K (2013) Evaluation of anti-acne activity of hydroalcoholic extract of Punica granatum Linn. J Pharmacogn Phytother 5:160–163. https://doi.org/10.5897/JPP12.044
147. Badea G, Lăcătușu I, Badea N, Ott C, Meghea A (2015) Use of various vegetable oils in designing photoprotective nanostructured formulations for UV protection and antioxidant activity. Ind Crop Prod 67:18–24. https://doi.org/10.1016/j.indcrop.2014.12.049
148. Marsh PD (2010) Controlling the oral biofilm with antimicrobials. J Dent 38:S11–S15. https://doi.org/10.1016/S0300-5712(10)70005-1
149. Van Leeuwen MPC, Slot DE, Van der Weijden GA (2011) Essential oils compared to chlorhexidine with respect to plaque and parameters of gingival inflammation: a systematic review. J Periodontol 82:174–194. https://doi.org/10.1902/jop.2010.100266
150. Menezes SM, Cordeiro LN, Viana GS (2006) Punica granatum (pomegranate) extract is active against dental plaque. J Herb Pharmacother 6:79–92. https://doi.org/10.1080/J157v06n02_07

151. Umar D, Dilshad B, Farhan M, Ali A, Baroudi K (2016) The effect of pomegranate mouthrinse on Streptococcus mutans count and salivary pH: an in vivo study. J Adv Pharma Tech Res 7:13–16. https://doi.org/10.4103/2231-4040.173266
152. Sedigh-Rahimabadi M, Fani M, Rostami-chijan M, Zarshenas MM, Shams M (2017) A traditional mouthwash (Punica granatum var pleniflora) for controlling gingivitis of diabetic patients: a double-blind randomized controlled clinical trial. J Evid Based Complementary Altern Med 22:59–67. https://doi.org/10.1177/2156587216633370
153. Ghalayani P, Zolfaghary B, Farhad AR, Tavangar A, Soleymani B (2013) The efficacy of Punica granatum extract in the management of recurrent aphthous stomatitis. JRPP 2:88–92
154. DiSilvestro RA, DiSilvestro DJ, DiSilvestro DJ (2009) Pomegranate extract mouth rinsing effects on saliva measures relevant to gingivitis risk. Phytother Res 23:1123–1127. https://doi.org/10.1002/ptr.2759

Part VII

Functional Foods

Antidiabetic Functional Foods with Antiglycation Properties

43

Mutiu Idowu Kazeem, Habeeb Adebodun Bankole, Azeez Ayomide Fatai, Abiola Fatimah Adenowo, and Theophilus Clavell Davies

Contents

M. I. Kazeem (✉) · H. A. Bankole · A. A. Fatai
Department of Biochemistry, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria
e-mail: mikazeem@gmail.com; habeeb.bankole@lasu.edu.ng; azeez.fatai@lasu.edu.ng

A. F. Adenowo
Department of Biochemistry and Microbiology, University of Zululand, Kwadlangezwa, South Africa
e-mail: afaatimah@yahoo.com

T. C. Davies
Department of Geology, University of Nigeria, Nsukka, Nigeria
e-mail: theo.clavellpr3@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_16

Abstract

Diabetes mellitus is a disease that requires long-term management and sometimes last throughout the lifetime of the patient. Persistent hyperglycemia experienced in diabetes over long period results in diabetic complications, such as nephropathy, neuropathy, retinopathy and cardiovascular diseases. One of the mechanisms involved in the development of diabetic complications is glycation which caused the production of advanced glycation end products (AGEs). Therefore, any agent that prevents the formation of AGEs may be suitable for the management of diabetes and its complications. There is a plethora of studies on the antiglycation properties of many foods and their products, but there is no repository of their information. This is an attempt to review the available information on the role of antiglycation agents from functional foods in the management of diabetic complications. We hope this information will assist diabetic patients in the choice of their diets and stimulate further research on these foods.

Keywords

Glycation · Advanced glycation end products · Functional foods · Diabetes · Diabetic complications

List of Abbreviations

AGE	Advanced glycation end products
BSA	Bovine serum albumin
GOLD	Glyoxal-lysine dimer
MOLD	Methyglyoxal-lysine dimer
RAGE	Receptor for advanced glycation endproduct

1 Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to defects in insulin secretion and/or action [1]. It causes alteration in the carbohydrate, lipid, and protein metabolism of its sufferers leading to a variety of micro- and macrovascular complications, which include nephropathy, neuropathy, and retinopathy [2]. Diabetes is a global problem affecting 382 million people in 2013, and this number is projected to increase to 572 million in 2035 [3]. The disease caused 5.1 million deaths and more than 21 million live births were affected by it during pregnancy, making it the third "killer" of mankind after cancer and cardiovascular diseases [3].

Persistent hyperglycemia experienced by diabetic patients promotes an unhealthy interaction between the excess blood glucose and some proteins like collagen, hemoglobin, and lens crystallins [4], and this is called glycation. Glycation is a spontaneous nonenzymatic reaction between the carbonyl group of reducing sugars and amino group of amino acids, peptides, or proteins to form a freely reversible Schiff base that rearranges to a more stable ketoamine called Amadori products [4]. This is then followed by complex series of reactions such as condensation and oxidative modification to produce several heterogenous products known as advanced glycation end products (AGEs) [5] (Fig. 1). Glycation is also referred to as nonenzymatic glycosylation or Maillard reaction because the process was first described by Louis Camille Maillard in 1912 [6].

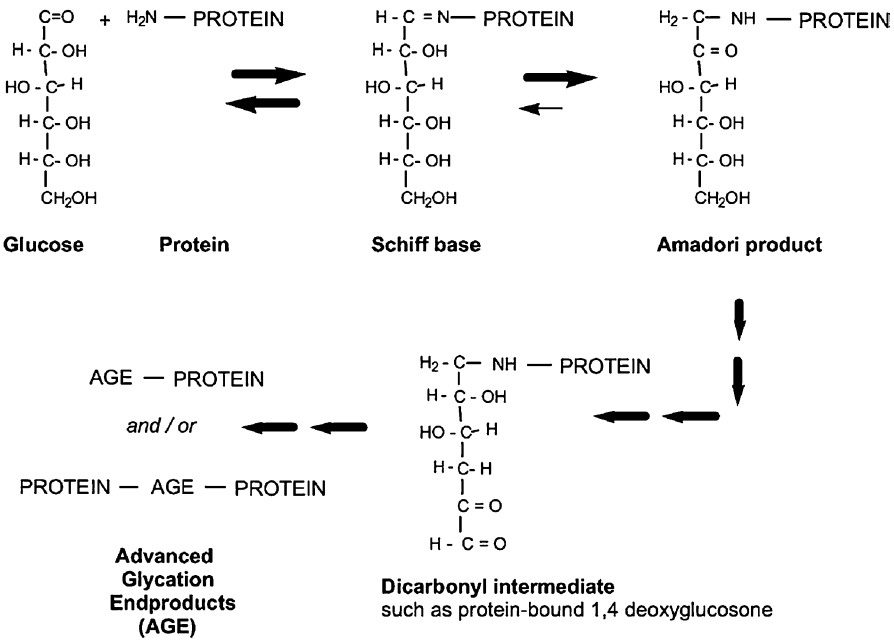


Fig. 1 Chemistry of glycation reaction to form advanced glycation end products [6]

The formation of AGEs may alter the physicochemical properties of the proteins, and in turn adversely affect their functional properties [7]. Excessive generation of AGEs is implicated in several pathological conditions, such as cardiovascular diseases, neurodegenerative diseases, inflammation, aging, and diabetes mellitus [4]. Previous studies have also established the role of AGEs in the development of diabetic complications [8–10]. Therefore the mitigation of protein glycation will be an effective medium of preventing or ameliorating these complications. There is a continuous demand for agents with antiglycation properties, as they may be useful in preventing diabetic complications [11, 12]. Though some synthetic antiglycation agents like aminoguanidine and pyridoxamine have been discovered, they have serious toxicity issues [8]. Therefore, food-derived antiglycation agents will be more appropriate in the management of diabetic complications due to their safety and availability to the general populace.

This review attempts to compile a repository of plant foods, which have been validated to display antiglycation potential (Table 1). This will serve as a guide to future references for researchers in institutions and pharmaceutical industries, for further studies aimed at isolation of their active components and large-scale production. It will also assist diabetics and their families in the choice of foods that will be consumed, in order to ameliorate the debilitating effects of diabetes and its complications.

2 Formation of Advanced Glycation End Products

AGEs are complex, heterogeneous molecules that cross-link proteins, exhibit browning, and generate fluorescence. The generation of reactive intermediate products, such as α-dicarbonyls, α,β-unsaturated aldehydes, dialdehydes, and keto-aldehydes, is very important in the formation of AGEs [6]. α-Dicarbonyls include 3-deoxyglucosone, glyoxal, and methylglyoxal. 3-Deoxyglucosone is formed by nonoxidative rearrangement as well as hydrolysis of Amadori product, and by fructose-3-phosphate. It rapidly reacts with protein amino groups to form AGE such as imidazolone, pyrraline, and carboxymethylysine [4, 13]. Methyglyoxal can be produced nonenzymatically from the spontaneous decomposition of triose phosphates, autoxidation of carbohydrates, glucose degradation, and also by several minor metabolic pathways, including the Maillard reaction [10]. In addition to reaction with arginine residues to form imidazolone adducts, methyglyoxal reacts with lysine residues in protein to form carboxyethylysine and the imidazolium crosslink, methylglyoxal-lysine dimer (MOLD).

Due to the variety of AGEs, they are broadly classified on the bases of their formation into three:

(i) Fluorescent cross-linking AGEs, e.g., pentosidine and crossline
(ii) Nonfluorescent cross-linking AGEs, e.g., Glyoxal-lysine dimer (GOLD) and methyglyoxal-lysine dimer (MOLD)
(iii) Noncross-linking AGEs, e.g., carboxymethylysine and pyralline

Table 1 List of functional foods with antiglycation potential

Name	Part	Extract	Test	Method(s)	Ref(s)
Bael	Fruits	Methanol	In vitro	BSA-glucose	[14]
Apple	Fruits	Water	In vitro	BSA-fructose	[15]
Banana	Inflorescence	Methanol	In vitro	BSA-glucose	[16]
	Inflorescence	Ethanol	In vitro	BSA-fructose	[17]
	Flower	Diet	In vivo	STZ-diabetic rats	[18]
Black tea	Leaves	Water	In vitro	BSA-glucose	[19]
Buckwheat	Hull	Water	In vitro	BSA-glucose	[20]
	Seed	Ethanol	In vitro	BSA, carbonyl level	[21]
Shaddock	Fruit pulp	Methanol	In vitro	BSA-fructose	[22]
Cornelian cherry	Fruit	Water	In vitro	BSA-fructose	[23]
	Fruits	Water	In vivo	STZ-diabetic rats	[24]
	Fruits	Glycosides	In vivo	STZ-diabetic rats	[25]
	Fruits	Morroniside	In vivo	STZ-diabetic rats	[26]
Cumin	Seeds	Water	In vitro/ vivo	BSA/STZ-DM rat	[27]
	Seeds	Methanol	In vitro/ vivo	BSA/STZ-DM rat	[12]
	Seeds	Cymene	In vitro/ vivo	BSA/STZ-DM rat	[28]
Dendrobii	Whole plant	Polysaccharides	In vitro	BSA-glucose	[29]
Black nightshade	Leaf	Ethanol, water	In vitro	BSA, carbonyl level	[30]
Ginger	Rhizome	Ethylacetate	In vitro	BSA-glucose	[31]
	Rhizome	Polyphenol	In vitro	BSA-glucose	[32]
	Rhizome	Polyphenol	In vivo	STZ-diabetic rats	[33]
Guava	Leaf	Water	In vitro	BSA, fructosamine	[34]
	Leaf	Water	In vitro	Plasma glycation	[35]
	Leaf	Methanol	In vivo	STZ-diabetic rats	[36]
	Leaf	Methanol	In vivo	STZ-diabetic rats	[37]
Yerba mate	Leaf	Water	In vitro	BSA-methglyoxal	[38]
Common juniper	Fruits	Essential oil	In vitro	Insulin glycation	[39, 40]
Longan	Fruits	Polysaccharides	In vitro	BSA-glucose	[41]
Lotus	All parts	Methanol	In vitro	BSA glucose	[42]
Saucer berry	Whole plant	Compounds	In vitro	BSA glucose	[43]
Mung bean	Seed	Ethanol	In vitro	BSA glucose	[44]
Passion fruit	Leaf	Water	In vitro	BSA glucose	[45]
Pomegranate	Fruit	Polysaccharides	In vitro	BSA glucose	[46]
Grape vine	Fruit	Water	In vitro	BSA, carbonyl level	[47]
Water apple	Leaf	Ethanol	In vitro	BSA glucose	[48]
Turmeric	Rhizome	Ethylacetate	In vitro	BSA glucose	[49]
Lingonberry	Fruit	Ethanol	In vitro	BSA glucose	[50]
Bitter melon	Fruits	Capsule	In vivo	Diabetic subjects	[51]
Green tea	Leaf	Ethanol	In vivo	STZ-diabetic rats	[52]

(*continued*)

Table 1 (continued)

Name	Part	Extract	Test	Method(s)	Ref(s)
Rambutan	Fruit	Ethanol	In vitro	BSA glucose	[53]
	Fruit	Geraniin	In vitro	BSA glucose	[54]
Sickle senna	Seeds	Glucosides	In vitro	BSA glucose	[55]
	Seeds	Anthraquinones	In vitro	BSA glucose	[56]
Kokum	Fruits	Garcinol	In vitro	BSA fructose	[57]
Luobuma tea	Leaves	Flavonoids	In vitro	BSA glucose	[58]
Sangwhang	Fruiting body	Isolates	In vitro	HbA1c formation	[59]
Clove	Buds	Water	In vitro	BSA-fructose	[60]

However, AGEs can also be classified on the basis of their lifespan into long-lived and short-lived molecules. In addition, most AGEs are formed endogenously but some can also be derived from exogenous sources such as foods and tobacco [9].

3 Antiglycation Agents from Fruits

3.1 Banana

Banana (*Musa paradisiaca*) is an herbaceous plant used as foods by man worldwide. The flower and pseudostem are used for the culinary purpose in some countries [61], while the fruit is consumed raw, cooked, or fried in all parts of the world. The leaves are also used in the wrapping of foods which adds characteristic aroma to the food and prevents burning. The flower and inflorescence have been reported with antidiabetic activity [62, 63]. Methanolic extract and different solvent fractions of banana inflorescence were evaluated for their antiglycation activities using the BSA-glucose antiglycation model [16]. Methanol extract as well as ethanol, water, and butanol fractions displayed good antiglycation activities with IC_{50} 44, 31, 55, and 68 μg/mL respectively, and are comparable to standard antiglycation agent, aminoguanidine (IC_{50}: 61 μg/mL).

Also, the antiglycation effect of ethanol extract of banana flower and two compounds isolated from it was investigated [17]. Ethanol extract of banana flower, lupeol (Fig. 2a), and umbelliferone (Fig. 2b) inhibited the formation of early-stage glycation product (fructosamine) by 85–90% and protein carbonyl compounds by 80–86%. However, fluorescence study on the late stage glycation moieties (AGEs) showed that the extract and compounds inhibited it by 71–82%. The rate of inhibition by this extract and compounds were significantly higher than the standard, aminoguanidine.

To ascertain the antiglycation effect of banana in animal model, the effect of banana flower and pseudostem on serum fructosamine and advanced glycation end

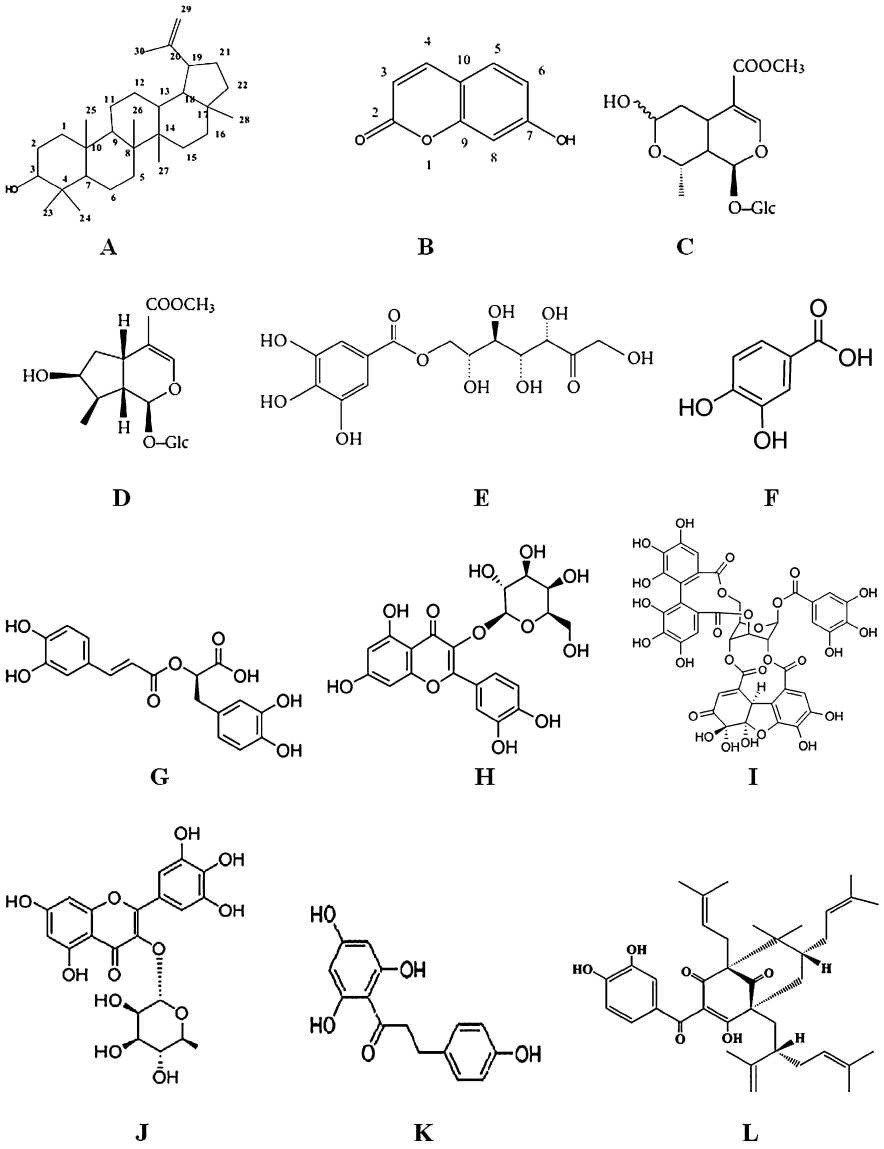


Fig. 2 Structures of some antiglycation compounds from fruits. (**a**) Lupeol; (**b**) Umbelliferone; (**c**) Morroniside; (**d**) Loganin; (**e**) 7-*O*-galloyl-D-sedoheptulose; (**f**) Protocatechuic acid; (**g**) Rosmarinic acid; (**h**) Quercetin-3-*O*-galactoside; (**i**) Geraniin; (**j**) Myricetin-3-O-rhamnoside; (**k**) Phloretin; (**l**) Garcinol

products (AGEs) in streptozotocin-induced diabetic rats was reported [18]. Supplementation of Wistar rat's diet with banana flower and pseudostem significantly decreased the serum fructosamine levels (2.15 and 2.37 moles/mg protein) compared with diabetic untreated rats (3.19 moles/mg protein). The concentration of serum

AGEs was also reduced by 61.5% and 51.2% in the banana flower and pseudostem fed rats compared with the diabetic control. This showed that banana exhibited antiglycation property in both in vitro as well as in vivo model, and this property may be due to the presence of umbelliferone and lupeol.

3.2 Cornelian Cherry

Cornelian cherry (*Corni fructus* or *Cornus officinalis*) is a common plant in China, Korea, and Japan, where it found usage as a functional food and as a component of traditional medicine [25]. Several studies have been carried out on the in vivo antiglycation potential of Cornelian cherry in rats. The beneficial effect of Cornelian cherry on AGE-mediated renal injury in streptozotocin-induced diabetic rats was reported [24]. Administration of 100 and 200 mg/kg of Cornelian cherry extract to diabetic rats for 10 days significantly reduced the renal AGE level to 5.32 and 4.92 IU compared with 6.19 IU in the diabetic control group. The renal AGE level of the diabetic rats administered 200 mg/kg cornelian cherry was similar to the group that received standard antiglycation agent, aminoguanidine.

Administration of 20 mg/kg iridoid glycoside and low molecular weight polyphenol fractions of Cornelian cherry to streptozotocin-induced diabetic rats for 10 days also showed that the iridoid glycosides fraction significantly reduced serum glycosylated protein (19.7 nmol/mg protein) and renal AGE (3.90 IU) compared with diabetic control (21.5 nmol/mg protein and 4.45 IU respectively), while the polyphenol fractions caused downregulation of receptor for advanced glycation end products (RAGE) to a value of 1.58 and 1.21-fold lower compared with the normal rats [24]. The active components of the iridoid glycoside fraction were identified to be morroniside (Fig. 2c), loganin (Fig. 2d), mevaloside, 5-hydroxymethyl-2-furfural, and loganic acid while the only component of the polyphenol fraction was 7-*O*-galloyl sedoheptulose (Fig. 2e).

In order to identify the compounds responsible for the antiglycation property of this plant, aqueous extract of Cornelian cherry and some of its active compounds (loganin, morroniside and 7-*O*-galloyl-D-sedoheptulose) were tested for their antiglycation activities using BSA-fructose model [23]. Out of this compounds, only 7-*O*-galloyl-D-sedoheptulose displayed good antiglycation activity (61.90%) at 25 μg/mL. Elsewhere, another component of the cornelian cherry fraction, morroniside, was also evaluated for its protective effect on renal damage in streptozotocin-induced diabetic rats [26]. Administration of 100 mg/kg of morroniside to diabetic rats for 20 days caused a significant reduction in the level of serum glycosylated protein (HbA1c) and thiobarbituric acid reactive substances, compared with the diabetic control. It also reduced the expression of AGE-related proteins such N-carboxylmethylysine and RAGE of the diabetic rats. It, therefore, implies that cornelian cherry exhibited antiglycation potential, which may due to the presence of its phytochemicals, 7-*O*-galloyl-D-sedoheptulose and morroniside.

3.3 Guava

Guava (*Psidium guajava* L.) is a widely cultivated plant in tropical and subtropical countries. The fresh fruit is eaten raw while the combination of its dried leaves and fruits are used in the preparation of drink known as guava tea [36]. Several compounds have been isolated from different parts of this plant, which include guavanoic acid, guavacoumaric acid, β-sitosterol-3-*O*-β-D-glucopyranoside, and jacoumaric acid [64]. The antiglycation potential of ethylacetate fraction of guava leaf extract in streptozotocin-induced diabetic rats was investigated [36]. Oral administration of 100 mg/kg of ethylacetate fraction of guava leaf extract to diabetic rats for 30 days caused about a two-fold reduction in the plasma glycated hemoglobin (HbA1c) (3.94%) compared to the diabetic control group (7.62%). Significant decrease was also witnessed in the serum fructosamine level of the guava-extract fed diabetic rats (227.97 μmol/L) compared with diabetic untreated group (258.30 μmol/L). This report is also corroborated by a similar study [37].

In order to evaluate the in vitro antiglycation effect of guava, guava leaf extracts and some of its active compounds were tested on α-dicarbonyl compounds-induced blood coagulation [35]. Guava leaf extract (0.001 mg/mL) significantly inhibited hypercoagulation induced by methylglyoxal but does not have an effect on the prothrombin clotting time. Guava leaf extract and its active compounds (gallic acid, ferulic acid, and quercetin) also protected antithrombin III from methylglyoxal-induced loss of activity. Guava leaf extract at the concentration of 50 μg/mL also exhibited strong inhibition (over 90%) of α-dicarbonyl compounds formation [34]. Catechin, gallic acid, and quercetin (from the guava extract) also displayed over 80% inhibitory effect on α-dicarbonyl compounds formation while ferulic acid did not. Both the extract and active compounds of guava leaf inhibited the formation of Amadori products (fructosamine) and AGEs from albumin in the presence of glucose. This α-dicarbonyl compounds formation was confirmed when the ethyacetate extract of guava leaf exhibited an IC_{50} value of 38.95 μg/mL for the inhibition of protein glycation, which is similar to the activity of standard antiglycation agent, aminoguanidine (IC_{50}: 33.82 μg/mL) [36]. Therefore, guava is a potent source of antiglycation agent, which may be due to the presence of phytochemicals such as gallic acid, quercetin, and ferulic acid.

3.4 Apples

Apples (*Malus domestica*) are one of the most important fruits in the world that are rich in phytochemicals, which include catechins, epicatechin, chlorogenic acid, *p*-coumaric acid, and protocatechuic acid [65]. Though apple is eaten raw as food, it is also processed into different products such as juice, nectar, and puree due to its health-promoting potentials. In a BSA-fructose antiglycation assay, aqueous extract of apple and green-tea fortified apple displayed anti-AGE formation activities of 15 and 48 mg/kg dry weight, respectively [15]. This implies that aqueous extract of apple displayed good antiglycation potential in-vitro, however fortification of apple with green tea increased its ability to inhibit AGEs formation.

3.5 Bael

Bael (*Aegle marmelos* L. Corr.), also known as golden apple, bael apple, wood apple, and stone apple, is native to India. It is a commonly used food with varieties of biological properties [66]. Several compounds isolated from the bael fruits, include aegelin, eugenol, marmelosin, lupeol, and fagarin [67]. It also contains tartaric acid, flavon-3-ols, anthocyannins, and flavonoid glycosides [68]. The fresh and dried fruits are used in the preparation of lemonade-like "soft drink" [14] while the leaves are eaten as salads. Methanolic extract of bael apple exhibited strong antiglycation potential with an EC_{50} value of 60.14 μg/mL in a BSA-glucose antiglycation experiment [14]. This suggests that consumption of bael fruits could help in the prevention and or amelioration debilitating effect of diabetes and its complications.

3.6 Bitter Melon

Bitter melon (*Momordica charantia* L.) is a plant which is consumed as food and used as an ingredient in some Asian curries. The stem and fruits could be a source of teas while the fruits are eaten raw mostly in Asia [69]. It is one of the popular plants used in the management of diabetes mellitus and its complications in different parts of the globe [70]. Several compounds have been isolated from its fruit, which includes charantin, momordicins, cucurbitacin, gentisic acid, and diosgenin [69]. A pilot study on the hypoglycemic and antiglycation potential of bitter melon was investigated in type 2 diabetic patients for 16 weeks [51]. The study involved the consumption of 6.0 g dried fruit powder of bitter melon daily by the diabetic patients throughout the duration of the experiment, which resulted in significant reduction in the levels of blood glucose, glycated hemoglobin (HbA1c), and serum AGEs of the patients by 9.49%, 6.69%, and 6.65%, respectively. It implies that consumption of bitter melon fruit can ameliorate diabetes mellitus and prevent the onset of its complications. However, there is a need for in vitro evaluation of the antiglycation activity of bitter melon and subsequent bioassay-guided fractionation of its active components.

3.7 Common Juniper

Common juniper (*Juniperus communis* and *oblonga*) is an evergreen shrub which is endemic to Europe, Asia, and North America. Common juniper is used as spice for food such as meat and as flavor in the preparation of alcoholic beverages [71]. Inhibition of protein glycation by essential oils of branchlets and fruits of common juniper was investigated [39]. Essential oils from the branchlet of male tree, female tree, and fruits inhibited the glycation of hemoglobin at all concentrations (200, 400, and 600 μg/mL) tested. However, the rate of inhibition of glycation is inversely proportional to the concentration of the oils which accounted for the highest inhibitory activities occurring at the lowest concentration (89.9%, 74.7%, and 62.8% for male branchlet, female branchlet, and fruit oils, respectively). Essential oil from the

fruit also displayed its highest inhibitory activity on insulin glycation at 200 μg/mL (78.4%), while that of the male (64%) and female branchlet (81%) inhibited it most at 600 μg/mL [40]. The antiglycation activity of common juniper may be due to the variety of components present in its essential oil. These include α-pinene, limonene, α-thujene, terpinen-4-ol, and isoterpinolene [39].

3.8 Grapevine

Grapevine (*Vitis vinifera* L.) is one of the largest food crops in the world due to its widespread consumption among people [72]. Its fruit is a berry which can be green, red, or purple in color, and can be taken fresh, processed to make juice and wine, or dried to produce raisins. It is a rich source of many phenolic compounds ranging from catechins, epicatechin, epicatechin-3-*O*-gallate, and procyanidins [72]. The inhibition of AGEs by red grape skin extract was investigated [47]. After 4 weeks of incubation, the grape extract (0.031–0.5 mg/mL) inhibited the formation of AGE by 55.23–63.52%. The extract (0.5 mg/mL) also reduced the level of fructosamine and carbonyl content generated by 10.5% and 41.7%, respectively. Incubation with 0.5 mg/mL extract also caused significant improvement (50.4%) in the level of protein thiol group and concomitant decrease (58.1%) in the carboxylmethylysine content. The study suggests that red grape extract inhibited all the stages (early, intermediate, and late stage) involved in the formation of AGEs, which makes it a veritable source of antiglycation agents.

3.9 Saucer Berry

Grey-leaved saucer berry (*Cordia sinensis* Lam.) is found in Eastern African and Middle Eastern countries of the World. The fruits and gum are consumed as food, while the fruits are also taken in many cuisines. Several chemical compounds have been isolated from this plant, which include transcaffeic acid, methylrosmarinate, protocatechuic acid (Fig. 2f), kaempferide-3-*O*-β-D-glucopyranoside, kaempferol-3-*O*-β-D-glucopyranoside, rosmarinic acid (Fig. 2g), and quercetin-3-*O*-β-D-glucopyranoside [43]. The evaluation of isolated compounds from grey-leaved saucer berry for their in vitro antiglycation potential was also reported [43]. All the compounds tested showed good antiglycation activities by exhibiting higher percentage inhibition of glycation which ranges between 68.0% (for protocatechuic acid) and 88.4% (methylrosmarinate). Though these compounds displayed good antiglycation activities, there is need for more tests to determine their IC_{50} values. This will enable comparison with similar plants and with standard antiglycation agents.

3.10 Lingonberry

Lingonberry (*Vaccinium vitis-idaea* L.) is a low shrub bearing bright red fruits which is most common in Russia and the Nordic countries. The fruits are eaten raw or

processed into jam, juice or syrup, and as an ingredient in dishes [73]. It is also used by the Cree population of Canada in the treatment of diabetic symptoms like frequent urination, abscesses, and snow blindness [74]. The inhibitory effect of lingonberry and its active compounds on the formation of AGEs was investigated [50]. Ethanol extract of lingonberry exhibited concentration-dependent inhibition of BSA-glycation which culminated in IC_{50} of 13.50 μg/mL. Four compounds isolated from this extract were also tested for their antiglycation activities. Quercetin-3-*O*-galactoside (Fig. 2h), cyanidin-3-*O*-galactoside, and catechin exhibited good anti-glycation potential with IC_{50} 2.86, 3.10, and 8.35 μg/mL respectively, while *p*-coumaric was not active. This study demonstrated lingonberry as an inhibitor of AGEs formation, however there is a need for validation of this property in animal or human model. The antiglycation activity may be due to the presence of phytochemicals like quercetin-3-*O*-galactoside, cyanidin-3-*O*-galactoside, and catechin.

3.11 Longan

Longan (*Dimocarpus longan* Lour.) is a tropical tree that produces exotic, attractive, and edible fruits. The fruit is a thin and indehiscent pericarp with succulent edible mesocarp and dark brown seed [75]. The fruits and seeds of this plant are rich in phenolic compounds, such as ellagic acid, corilagin, kaempferol, grevifolin, and gallic acid [76]. The fruit is also eaten by consumers globally due to its sweet juicy mouth feel and aromatic flavor [77]. The antiglycation activity of polysaccharides of longan fruit was investigated [41]. The study showed that polysaccharides obtained after ultrasonic treatment under different conditions exhibited a potent antiglycation effect in BSA-glucose glycation system. However, detailed characterization of the possible antiglycation property of this fruits is imperative.

3.12 Passion Fruit

Passion fruit (*Passiflora manicata*) is a tropical plant which is characterized with brightly colored flower and edible fruit. Its fruit is also used in the preparation of beverages, while the leaves are used for medicinal purpose as sedative and tranquilizer. The presence of chemical compounds such as vitexin, isovitexin (Fig. 4a), and orientin in the leaf extract of passion fruit was reported [78]. Coincubation of aqueous extract of passion fruit (1–100 μg/mL) with BSA-glucose medium caused significant reduction in the total glycated protein, and these results were similar to that of the standard phenolic compound, tannic acid [45]. HPLC analysis of the extract also showed the presence of active compounds such as isoorientin, vitexin, and isovitexin, which might be responsible for the antiglycation property of the plant.

3.13 Pomegranate

Pomegranate (*Punica granatum* L.) is a shrub or small tree which grows between 5 and 8 m tall, and possesses large berry fruits. The plant originated from the Middle

East and extends throughout the Mediterranean to Southern America [79]. All parts of the plant are useful, especially the fruit which is used in the preparation of juicy drink while the seeds are used as spice for food. Several chemical compounds are reportedly present in this plant, which include gallic acids, ellagic acid, caffeic acid, luteolin, apigenin, dephinidin, and pelargonidin [79]. The antiglycation property of polysaccharide fraction isolated from the fruit rind of pomegranate was investigated [46]. The polysaccharide fraction (10–20 μg/mL) was able to inhibit BSA glycation by only 28% as against 41% displayed by vitamin C. It also reduced the formation of fructosamine by 50% after 7 day incubation, which suggested mild antiglycation potential. The presence of polyphenols, such as gallic acid, ellagic acid, and caffeic acid, may be responsible for the antiglycation activity of this plant.

3.14 Shaddock

Shaddock (*Citrus grandis* L. Osbeck), also referred to as pomelo or pummelo, is one of the most cultivated crops in South-East Asia due to its consumption as food and usage in traditional medicine [80]. Previous study has showed this fruit is rich in bioactive compounds such hesperidin, naringenin, and naringin [81]. The effect of shaddock fruit on the concentration of AGEs, fructosamine, carbonyl carbonyl, and carboxymethylysine was reported [22]. The shaddock extract (0.25–2.00 mg/mL) significantly inhibited the formation of AGEs in concentration-dependent manner to a maximum of 90.68% on the 28th day. The extract also reduced fructosamine concentration by 3.7, 9.9, 17.5, and 30% at 0.25, 0.50, 1.00, and 2.0 mg/mL, respectively. Concomitant reduction was also witnessed in the formation of carbonyl and thiol compounds, following incubation with shaddock extract. The antiglycation activities of shaddock may due to the presence of phytochemicals like neohesperidin, naringin, hesperidin, and naringenin [22].

3.15 Rambutan

Rambutan (*Nephelium lappaceum* L.) is a tropical fruit which is native to South East Asia. It is mostly found in Malaysia, Indonesia, Thailand as well as Philippines, and spread to other parts of the world. Its fruit has a refreshing flavor and exotic appearance and can be consumed fresh or in processed form [82]. Some chemical compounds have been isolated and identified from this fruit, such as ellagic acid, corilagin, and geraniin [83]. The potency of rambutan in the management of hyperglycemia was investigated [53]. Ethanolic extract of rambutan inhibited the formation of AGEs by 43% in a BSA-glucose glycation model. This result was better than that of green tea (38%), which was earlier reported as better antiglycation agent than the standard, aminoguanidine [34]. Isolation and purification procedures on rambutan fruits yielded a pure compound, geraniin (Fig. 2i), which exhibited a very high (98%) inhibition of AGE formation [54]. It can therefore be concluded that rambutan displayed antiglycation activity, which is due to the presence of geraniin in it.

3.16 Water Apple

Water apple (*Syzygium aqueum* Alston) is a tropical plant which originated from Malaysia and Indonesia. It is cultivated for its sweet tasty, bell-like fruits called apples that are consumed fresh, and its leaves which are used as vegetables and in wrapping other foods [84]. The inhibitory effect of ethanol leaf extract of water apple on the formation of AGEs and the isolation of its active components was investigated [48]. The extract displayed strong inhibitory potential against the formation of AGEs (89%), which was higher than green tea (45%) which served as positive control. Six pure compounds were also isolated from the extract which included 4-hydroxylbenzadehyde, myricetin-3-*O*-rhamnoside (Fig. 2j), europetin-3-*O*-rhamnoside, phloretin (Fig. 2k), myrigalone-G, and myrigalone-B. Therefore, water apple exhibited good antiglycation potential, which may be due to the presence of the chemical compounds present in it.

3.17 Kokum

Kokum (*Garcinia indica* Choicy) is an underexploited tree distributed throughout the tropical Asian and African countries. Its fruit is characterized by pleasant flavor and sour taste, and is used as an acidulant in dishes as well as in the production of beverages and butter [85]. Phytochemical study showed that the fruits contain cyanidin-3-glucoside, cyanidin-3-sambubioside, garcinol (Fig. 2l), isogarcinol, and hydroxycitric acid [68]. The leaves and fruits are traditionally used in the treatment of different ailments like inflammation, diarrhea, dysentery, ulcers, and indigestion. The antiglycation activity of garcinol isolated from the fruit rind of kokum was investigated in BSA fructose model [57]. They showed that garcinol suppressed fluorescence and protein cross-link formation in the reaction system, better than aminoguanidine. Therefore, kokum leaves inhibited the formation of AGEs in vitro, which may be attributed to its phytochemical compounds especially garcinol.

4 Antiglycation Agents from Beverages

4.1 Black Tea

Black tea (*Camellia sinensis* L.) is one of the most popular beverages consumed worldwide, and is made by fermentation and drying of the leaves of the plant, *Camellia sinensis*. The fermentation of this tea converts catechins to theaflavins and thearubigins, thereby decreasing catechin content [86]. The main components of black tea include catechins (e.g., epigallocatechin gallate), quercetin, rutin, caffeic acid, theaflavins, and thearubigins [87]. The in vitro antiglycation and cross-link breaking potential of black tea from Sri Lanka was reported [19]. Black tea brew exhibited strong antiglycation (IC_{50}: 19.04 μg/mL) and AGEs cross-link breaking (IC_{50}: 82.89 μg/mL) activities, and these activities were similar to that of popular antiglycation agent, rutin.

4.2 Green Tea

The steaming of freshly harvested leaves of *Camellia sinensis* yields dry and stable product referred to as green tea [86]. Though it is obtained from the same plant as black tea, its chemical composition is quite different because it has higher quantity of catechins (epicatechin, epicatechin-3-gallate, epigallocatechin-3-gallate, and epigallocatechin) and do not possess theaflavins and thearubigins [86]. The reduction of protein glycation by ethanol extract of green tea in the cardiac tissue of streptozotocin-induced diabetic rats was reported [52]. Oral administration of 300 mg/kg green tea extract to diabetic rats for 4 weeks significantly reduced the extent of cardiac protein glycation from 235.70 mg/mg protein in diabetic control rats to 185.30 mg/mg protein in the green tea–treated diabetic rats. The study also revealed the extract contained 33.5%, 28.5%, 13.5%, and 3.8% of epigallocatechin, epigallocatechin-3-gallate, epicatechin-3-gallate, and epicatechin, respectively. This antiglycation property may be connected to the presence of catechins in the tea.

4.3 Luobuma Tea

Another tea plant with antiglycation potential is luobuma (*Apocynum venetum* L.). This plant is native to China but widely distributed in the temperate zones of Eurasia and North America. Its leaves are used in the preparation of beverage called luobuma tea in China, Japan, and the USA. It is also used in Chinese traditional medicine in the treatment of inflammation, hypertension, hepatitis, and diuresis [88]. Several phytochemicals have been isolated from this plant, which includes apocyanins A, B, C, and D, as well as apocynosides I and II [88]. Aqueous extract of Luobuma leaves exhibited better antiglycation activity than the standard, aminoguanidine with IC_{50} 37.2 and 59.2 μg/mL, respectively [58]. Furthermore, all the seven isolated compounds (catechin (Fig. 3a), epicatechin (Fig. 3b), gallocatechin (Fig. 3c), epigallocatechin, epicatechin-(4β-8)-gallocatechin, epigallocatechin-(4β-8)-epicatechin and procyanidin B-2) from this extract also displayed excellent antiglycation activities, with IC_{50} values ranging between 9.1 and 19.8 μg/mL. Therefore, antiglycation compounds from luobuma tea could be explored further as possible candidates for the development of antiglycation drugs in the treatment of diabetic complications.

4.4 Yerba Mate

Yerba mate (*Ilex paraguariensis* St. Hil.) is a popular tree in the South American countries of Paraguay, Uruguay as well as parts of Argentina and Brazil. It is used in the preparation of tea called Mate tea by the infusion of dried leaves of the plant. It is commercially packaged in tea bags or concentrates, for usage in the food and dietary supplement industries [89]. Due to its nutraceutical properties, mate is used as an ingredient in energy drinks, candy, and beer [90]. Coincubation of bovine serum albumin (BSA) and methylglyoxal with yerba mate extract caused a dose-dependent

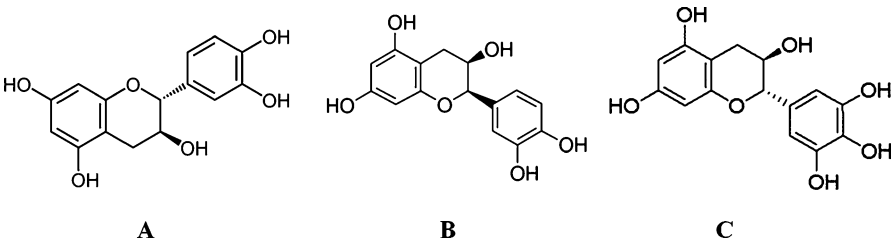


Fig. 3 Structures of some antiglycation compounds from beverages. (**a**) Catechin; (**b**) Epicatechin; (**c**) Gallocatechin

inhibition of glycation reaching up to 40% at 20 μL/mL of the extract, which implies good antiglycation effect [38]. This may be due to the presence of some phytochemicals, such as methylxanthines, matesaponnins, chlorogenic acids, and 4,5-dicaffeoylquinic acid [89], in the plant.

4.5 Dendrobii

Dendrobii (*Dendrobium huoshanense*) is another antiglycation tea plant which is endemic to Huoshan town, Anhui Province of China. It is used as spice in the preparation of foods such as soups and as herbal tea or functional beverage for the protection of the eye, liver, and stomach [91]. Phytochemical analysis revealed the presence of many compounds like polysaccharides, 6,8-di-*C*-glycosyl flavonoids, shikimic acid, and salicylic acid [92]. The antiglycation activity of polysaccharides extracted from dendrobii has been reported [29]. Polysaccharides from dendrobii inhibited the formation of amadori product in a dose-dependent manner to maximum of 72.54%, which was about 1.95 fold greater than that of aminoguanidine. The formation of intermediate dicarbonyl compound and AGEs was also decreased to a minimum of 51.9%, which was lower than that of aminoguanidine. The inhibition of glycation by these polysaccharides of dendrobii may be due to their antioxidant properties.

5 Antiglycation Agents from Legumes

5.1 Mung Bean

Mung bean (*Phaseolus radiata* L. Wilczek), also referred to as green gram or moong bean, is a legume which is native to the Indian subcontinent but widely cultivated in the Southeast Asian countries. The inhibitory effect of mung bean extract and some of its active constituents on the formation of AGEs was investigated [44]. Hydroethanol extract of mung bean displayed the highest inhibitory activity (80.4%) against AGEs formation compared to other variety of beans such as black bean (72.1%), soybean (70.1%), and cowpea (67.3%). Two active components isolated from mung bean, vitexin (Fig. 4a)

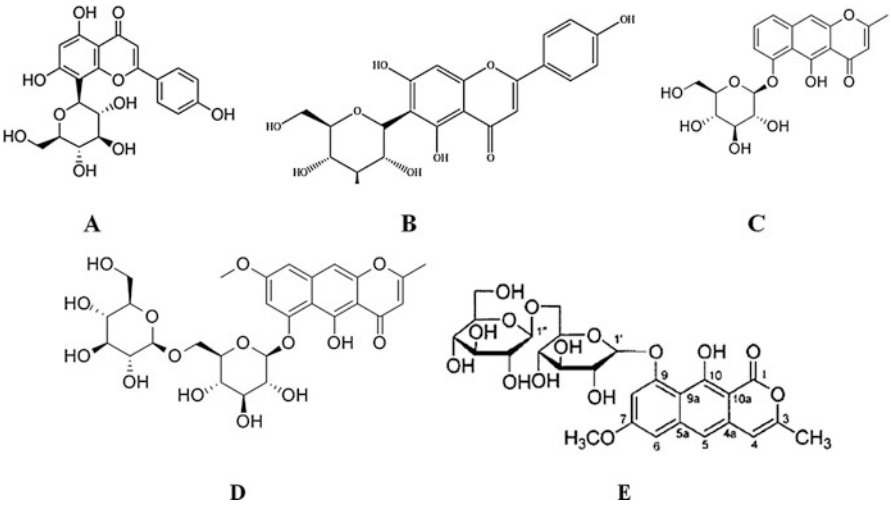


Fig. 4 Structures of some antiglycation compounds from legumes. (**a**) Vitexin; (**b**) Isovitexin; (**c**) Cassiaside; (**d**) Rubrofusarin-6-O-β-D-gentiobioside; (**e**) Toralactone-9-*O*-β-gentiobioside

and isovitexin (Fig. 4b) at 100 μM, also exhibited a strong inhibitory effect on fluorescent AGEs formation induced by methyglyoxal similar to the activity of 1 mM aminoguanidine [44]. However, both compounds have weaker direct methylglyoxal-trapping activities than aminoguanidine. Therefore, mung bean displayed antiglycation effect which may be due to the presence of phytochemicals like vitexin and isovitexin.

5.2 Buckwheat

Buckwheat (*Fagopyrum sp.*) is a plant that is cultivated for its seeds, which is used as food. There are two major varieties of buckwheat: common buckwheat (*Fagopyrum esculentum*) which is sweet, and tartary buckwheat (*Fagopyrum tataricum*) which has a bitter taste [93]. However, the chemical composition of both is similar which include chlorogenic acid, orientin, isoorientin, vitexin, isovitexin, and rutin [94]. Buckwheat is also a rich source of starch, protein, minerals, vitamins, dietary fiber, and antioxidants. The in vitro antiglycation activity of common buckwheat hull tea infusion was investigated [20]. The ready-to-drink buckwheat hull tea displayed lower inhibition (34.90%) of AGEs in BSA-glucose system compared to green tea.

Ethanol extract (200 μg/mL) of tartary buckwheat exhibited strong inhibition of AGEs (83%), fructosamine (50%), and α-dicarbonyl compounds (65%) formation [21]. It, therefore, reduced the generation of AGEs through the suppression of fructosamine and α-dicarbonyl compounds formation. This implies that tartary buckwheat possessed higher antiglycation activity than the common buckwheat, and this may be due to variation in the quantities of phytochemicals present in them.

5.3 Sickle Senna

Sickle senna (*Cassia tora* L.) is a leguminous plant found in the South East Asia and South West Pacific. The young leaves of this plant are cooked as vegetable while the roasted seeds are consumed as tea. It is used in the treatment of several diseases, such as bronchitis, leprosy, dyspepsia, hypertension, and cardiac disorders [95]. Three naphthopyrone glucosides, namely cassiaside (Fig. 4c), rubrofusarin-6-*O*-β-D-gentiobioside (Fig. 4d), and toralactone-9-*O*-β-D-gentiobioside (Fig. 4e), isolated from sickle senna seeds displayed antiglycation property and showed better inhibition of AGE formation (IC_{50}: 6.4–32.2 μg/mL) than the standard antiglycation agent, aminoguanidine (IC_{50}: 34.6 μg/mL) [55]. Nine anthraquinones isolated from the seeds of sickle senna were also evaluated for their antiglycation activity, out of which only obtusifolin (IC_{50}: 28.9 μM) and emodin (IC_{50}: 118.0 μM) displayed significant inhibition of AGE formation, which were also more potent than aminoguanidine (IC_{50}: 961.0 μM) [56]. Therefore, the antiglycation activities of sickle senna may be due to the presence of naphthopyrone glucosides, as well as obtusifolin and emodin.

6 Antiglycation Agents from Vegetables and Spices

6.1 Black Nightshade

Black nightshade (*Solanum nigrum* L.), also called hounds berry or stubble berry, is a common food plant in Africa, Asia, and Europe. Its leaves and fruits are used in the preparation of foods such as soup, jam, salad, and fruit juices [96]. This plant is reported to contain steroidal glycosides (e.g., solarmargine, desgalactotigonin) and steroidal saponins (e.g., solanigrosides as well as nigrumnins I and II) [97]. The antiglycation property of ethanolic extract of the leaves of black nightshade and two of its isolated compounds, solamargine (Fig. 5a) and solasonine (Fig. 5b), were investigated [30]. The result revealed that 95% ethanolic extract of black nightshade inhibited the generation of AGEs in a dose-dependent manner, via the lowering of fructosamine and α-dicarbonyl compounds formation. Solasonine also exerted stronger antiglycation activity for attenuating AGEs, fructosamine, and α-dicarbonyl compounds formation than solarmargine. This implies that black nightshade leaves possess antiglycation effects which may be due to the presence of solasonine.

6.2 Sangwhang

Sangwhang (*Phellinus linteus*), also called black hoof mushroom, is one of the mushrooms that are considered functional foods in Japan, China, and South Korea, though it is also found in tropical America and Africa. It is used in Asian herbal medicine for the management of several diseases such as liver disorder, oral ulcer, allergies, and diabetes mellitus [98]. Nine compounds (protocatechuic acid, protocatechualdehyde, caffeic acid, ellagic acid, hispidin, davallialactone (Fig. 5c),

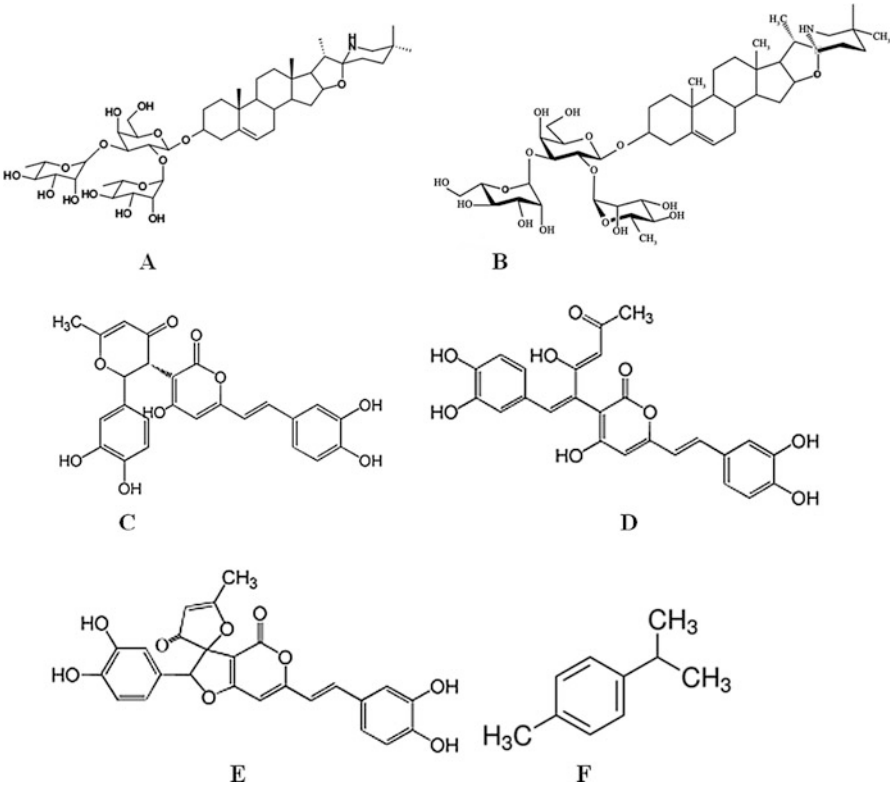


Fig. 5 Structures of some antiglycation compounds from vegetables and spices. (**a**) Solamargine; (**b**) Solasonine; (**c**) Davallialactone; (**d**) Interfungins A; (**e**) Inoscavin A; **(f)** p-Cymene

hypolomin B, interfungins A (Fig. 5d), and inoscavin A (Fig. 5e)) isolated from the ethylacetate fraction of sangwhang were evaluated for antiglycation activities [59]. Davallialactone (50.2%), interfungins A (63.1%), and inoscavin A (45.7%) exhibited inhibitory effect on HbA1c formation, while protocatechualdehyde (IC_{50}: 144.28 μM), davallialactone (IC_{50}: 158.66 μM), and inoscavin A (IC_{50}: 213.15 μM) prevented methylglyoxal-mediated protein modification. However, only interfungin A (IC_{50}: 1.15 mM) inhibited protein cross-link formation even better than aminoguanidine (IC_{50}: 11.93 mM). Therefore, sangwhang displayed good inhibitory potential against AGEs formation which is due mainly to the presence of interfungin A.

6.3 Turmeric

Turmeric (*Curcuma longa* L.) is a rhizomatous plant which is distributed in the Asian continent especially India. It is used in the preparation of foods as spice, preservatives, and coloring agent [99]. It is also used in folk medicine for the

treatment of various ailments like anorexia, cough, rheumatism, hepatic disorder, and diabetic wounds [100]. Several compounds have been isolated from this plant, which includes curcumin, demethoxycurcumin, bisdemethoxycurcumin, and ar-turmerone [101]. The antiglycation potential of hexane, ethylacetate, methanol, and aqueous extracts of turmeric rhizome was reported [49]. Ethylacetate (IC_{50}: 0.09 μg/mL) displayed highest inhibitory effect, followed by methanol (1.48 μg/mL), and aqueous (10.42 μg/mL) extract, while the hexane extract had no effect. In fact, the antiglycation potential of the ethylacetate extract was about 800 times higher than that of ascorbic acid, which is the standard. Therefore, turmeric is an excellent inhibitor of glycation and AGEs formation.

6.4 Lotus

Lotus (*Nelumbo nucifera* Gaertn.) is an aquatic plant cultivated mostly in China and India but distributed throughout Asia. The young leaves, seeds, and rhizome of the plant are used as vegetables while the matured leaves are used as functional foods [102]. The leaf is also used as ingredients in the preparation of healthy beverages and tea bags in China [103]. It is rich in several phytochemicals, which include dauricine, lotusine, nelumboside, pronuciferine, anonaine, and arbutin [104]. The inhibitory effect of lotus leaves, stamens, seeds, embryos, and rhizomes on the formation of advanced glycated end products was investigated [42]. Only the methanol extract of the leaves and stamens demonstrated good antiglycation activities with IC_{50} values of 110.5 μg/mL and 125.5 μg/mL, respectively, compared to the standard, aminoguanidine. However, only the ethylacetate fraction of the leaf effectively inhibited AGE formation (IC_{50} 28.18 μg/mL). Though lotus exhibited good antiglycation effects, further study is required to isolate the bioactive compound(s) from its ethylacetate fraction.

6.5 Ginger

Ginger (*Zingiber officinale* Roscoe) is one of the commonly used plants as spice or condiments in food preparation. It is a functional food which provides medical benefit to its consumers, in addition nutritional function [105]. This is why its rhizome is widely used in food processing and pharmaceutical industries. Major chemical constituents of this plant include 6-gingerol, 6-shogaol, 6-paradol, α-zingiberene, and cineole [106]. The inhibition of protein glycation by ginger extract in an in vitro model was investigated [31]. Ethylacetate extract of ginger at varying concentrations (100–500 μg/mL) displayed dose-dependent inhibition of albumin glycation, which culminated in its IC_{50} value 290.84 μg/mL for the antiglycation activity. This is in conformity with our study on the antiglycation potential of polyphenol extract of ginger, where the IC_{50} value for the antiglycation effect was 285 μg/mL [32]. We also investigated the antiglycation effects of polyphenols extracted from ginger in streptozotocin-induced diabetic rats [33]. Oral administration of 500 mg/kg of free and bound polyphenols of ginger to diabetic rats for 42 days significantly reduced the glycated hemoglobin (HbA1c) level (7.10%)

compared to the diabetic control (10.33%). The formation of AGEs in the serum was also drastically decreased from 6.80 μg/mL in the diabetic control rats to 1.98 μg/mL in the ginger-treated diabetic animals. These reports suggest that ginger possessed antiglycation property both in vitro and in vivo, which may be due to the presence of polyphenols such as gingerol and zingiberene.

6.6 Cumin

Cumin (*Cuminum cyminum* L.), commonly called jeera, is a widely used spice condiment in vegetarian and nonvegetarian foods mostly in Asian countries [107]. It is also used in folklore medicine as an analgesic, antihypertensive, carminative, purgative, and antidiabetic [108]. Its essential oil components include α- and β-pinene, *p*-cymene (Fig. 2f), limonene, safranal, as well as α- and γ-terpinene. The antiglycation potential of aqueous extract of cumin was tested on fructose-induced glycation [27]. Coincubation of fructose with cumin extract resulted in dose-dependent reduction of AGE fluorescence and prevented the formation of predominant AGE, carboxymethylysine. The presence of the extract also prevented the formation of glycation mediated protein cross-links, thereby displaying potent antiglycation activity. Methanolic extract of cumin seed was also tested for its inhibitory effect on glucose-induced BSA glycation [12]. Cumin extract exhibited concentration-dependent inhibition of BSA glycation and subsequent formation of fluorescent glycation products, culminating in low IC_{50} of 1.17 mg/mL.

The in vivo antiglycation activity of cumin in streptozotocin-induced diabetic rats was reported [27]. Supplementation of diabetic rats' diet with 0.5% cumin powder for 8 weeks significantly decreased glycated hemoglobin (HbA1c) level of the rats (5.1%) compared to the diabetic control rats. It also caused protein carbonyl concentration to reduce from 8.1 to 6.3 nmol/mg protein in the cumin-fed rats. Feeding diabetic rats with cumin-supplemented diet also prevented the hyperglycemia-mediated formation of protein cross-links and improved the total and soluble protein levels. Administration of methanolic extract of cumin seed to diabetic rats for 28 days also caused significant reduction in their serum glycated hemoglobin (8.39%) and renal AGE (2.25 IU/mg protein), compared to the glycated hemoglobin (15.39%) and renal AGE (10.09 IU/mg protein) of the diabetic control respectively [12]. Treatment with the extract also reduced glycated and rat tail tendon collagen of the diabetic rats.

In order to identify the antiglycation agents present in cumin, one of its active components, cymene, was investigated for its effect on glycation [28]. The coincubation of 100 μM cymene in BSA-glucose system caused drastic reduction in fructosamine formation and its effect was similar to that of 2 mM aminoguanidine. This implies that cymene is 20 times more potent as an antiglycation agent than aminoguanidine. Varying concentrations (25–100 μM) of cymene also elicited a significant decrease in the protein-bound carbonyl compound formation. Cymene (100 μM) also caused 56.6% and 49.16% reduction in the formation of total AGEs and pentosidine in the BSA-glucose system. Administration of 20 mg/kg cymene to streptozotocin-induced diabetic rats for 60 days also caused significant reduction in

their glycated hemoglobin (HbA1c) level (6.1%) compared to the diabetic control (11.3%). The concentration of serum fructosamine was also decreased significantly from 3.98 mM/L to 2.32 mM/L, which means 58.29% reduction was obtained. It can be concluded that cumin possesses antiglycation property in vitro and in vivo, which may be due to the presence of cymene.

6.7 Clove

Clove (*Syzygium aromaticum* (L.) Merr. & Perry) is a plant which is native to tropical America and Australia. It is popularly used as spice in food preparation and in the treatment of diseases, including inflammation, infections, toothache, and respiratory and gastrointestinal disorders [109]. Chemical compounds present in this plant include eugenol, limonene, β-pinene, ferulic acid, and kaempferol [110]. The inhibition of AGEs formation by clove extract was investigated [60]. Clove extract (0.25–1.00 mg/mL) inhibited the formation of AGEs to a maximum of 95.2% as against the standard, aminoguanidine (91.5%). It also caused 72.8% reduction in the formation of N-carboxymethylysine [60]. Coincubation of the clove extract with the glycation reaction system also decreased the formation of protein carbonyl content by 73.7%, as compared to aminoguanidine (60.0%). However, it significantly elevated the level of protein thiol group better than the standard. This implies that clove extract inhibited all the stages involved in the formation of AGEs, and therefore can be consumed to prevent diabetic complications.

7 Possible Mechanisms of Antiglycation by Foods

The inhibition of glycation and subsequent formation of AGEs by food plants may occur through one or more mechanisms. This includes cleavage of the AGE-crosslink or blocking of the AGE receptor, trapping of reactive dicarbonyl species, as well as through antioxidant activities [9, 11]. Other possible mechanisms of antiglycation are inhibition of aldose reductase and reduction of blood glucose level using hypoglycemic agents, such as acarbose and metformin [4, 6]. Antiglycation agents either function as AGE inhibitors, AGE breakers, or receptor for AGE (RAGE) blockers. AGE inhibitors include aminoguanidine, benfotiamine, and pyridoxamine. N-phenacyl thiazolium compound and alagebrium are AGE breakers while cerivastatin is an example of RAGE blocker [6]. Some antioxidant compounds have also been validated as antiglycation agents, which include vitamin C and E [8].

8 Conclusion

The incidence of diabetic complications ranging from diabetic foot, kidney dysfunction, and cardiovascular disease, which may be due to nonenzymatic glycation, is on the increase. Synthetic drugs manufactured to ameliorate these conditions are

bedevilled with serious side effects and nonavailability, which necessitated the search for antiglycation agents from food plants. Beverages, fruits, legumes, vegetables, and spices, with antiglycation potential, are presented in this review. However, further study will be required to isolate the bioactive component(s) of these foods for possible drug development.

Acknowledgments The support of Lagos State University, Ojo, Lagos, Nigeria, is gracefully acknowledged.

References

1. Harris M (2004) Definition and classification of diabetes mellitus and the criteria for diagnosis. Diabetes mellitus: a fundamental and clinical. Text 3:457–467
2. Altan V (2003) The pharmacology of diabetic complications. Curr Med Chem 10(15): 1317–1327
3. Federation ID (2013) IDF Diabetes Atlas. International Diabetes Federation, Brussells
4. Harding J, Ganea E (2006) Protection against glycation and similar post-translational modifications of proteins. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics 1764(9): 1436–1446
5. Rojas A, Morales M (2004) Advanced glycation and endothelial functions: a link towards vascular complications in diabetes. Life Sci 76(7):715–730
6. Peyroux J, Sternberg M (2006) Advanced glycation endproducts (AGEs): pharmacological inhibition in diabetes. Pathol Biol 54(7):405–419
7. Monnier V, Mustata G, Biemel K, Reihl O, Lederer M, Zhenyu D et al (2005) Cross-linking of the extracellular matrix by the maillard reaction in aging and diabetes: an update on "a puzzle nearing resolution". Ann N Y Acad Sci 1043(1):533–544
8. Singh R, Barden A, Mori T, Beilin L (2001) Advanced glycation end-products: a review. Diabetologia 44(2):129–146
9. Ahmed N (2005) Advanced glycation endproducts—role in pathology of diabetic complications. Diabetes Res Clin Pract 67(1):3–21
10. Daroux M, Prevost G, Maillard-Lefebvre H, Gaxatte C, D'agati V, Schmidt A et al (2010) Advanced glycation end-products: implications for diabetic and non-diabetic nephropathies. Diabetes Metab 36(1):1–10
11. Rahbar S, Figarola J (2003) Novel inhibitors of advanced glycation endproducts. Arch Biochem Biophys 419(1):63–79
12. Jagtap A, Patil P (2010) Antihyperglycemic activity and inhibition of advanced glycation end product formation by *Cuminum cyminum* in streptozotocin induced diabetic rats. Food Chem Toxicol 48(8):2030–2036
13. Nishikawa T, Edelstein D, Brownlee M (2000) The missing link: a single unifying mechanism for diabetic complications. Kidney Int 58:S26–S30
14. Prathapan A, Krishna M, Nisha V, Sundaresan A, Raghu K (2012) Polyphenol rich fruit pulp of *Aegle marmelos* (L.) Correa exhibits nutraceutical properties to down regulate diabetic complications – an in vitro study. Food Res Int 48(2):690–695
15. Lavelli V, Corey M, Kerr W, Vantaggi C (2011) Stability and anti-glycation properties of intermediate moisture apple products fortified with green tea. Food Chem 127(2):589–595
16. Nisha P, Mini S (2014) Vitro antioxidant and Antiglycation properties of methanol extract and its different solvent fractions of *Musa paradisiaca* L.(Cv. Nendran) inflorescence. Int J Food Prop 17(2):399–409
17. Ramu R, Shirahatti P, Zameer F, Ranganatha L, Prasad M (2014) Inhibitory effect of banana (*Musa* sp. var. Nanjangud rasa bale) flower extract and its constituents Umbelliferone and

Lupeol on α-glucosidase, aldose reductase and glycation at multiple stages. S Afr J Bot 95:54–63
18. Bhaskar J, Shobha M, Sambaiah K, Salimath P (2011) Beneficial effects of banana (*Musa* sp. var. elakki bale) flower and pseudostem on hyperglycemia and advanced glycation end-products (AGEs) in streptozotocin-induced diabetic rats. J Physiol Biochem 67(3):415–425
19. Ratnasooriya W, Abeysekera W, Muthunayake T, Ratnasooriya C (2014) Vitro Antiglycation and cross-link breaking activities of Sri Lankan low-grown orthodox Orange pekoe grade black tea (*Camellia sinensis* L.) Trop J Pharm Res 13(4):567–571
20. Zielinska D, Szawara-Nowak D, Zielinski H (2013) Antioxidative and anti-glycation activity of buckwheat hull tea infusion. Int J Food Prop 16(1):228–239
21. Lee C-C, Lee B-H, Lai Y-J (2015) Antioxidation and antiglycation of *Fagopyrum tataricum* ethanol extract. J Food Sci Technol 52(2):1110–1116
22. Caengprasath N, Ngamukote S, Mäkynen K, Adisakwattana S (2013) The protective effects of pomelo extract (*Citrus Grandis* L. Osbeck) against fructose-mediated protein oxidation and glycation. EXCLI J 12:491
23. Park C, Tanaka T, Kim H, Park J, Yokozawa T (2012) Protective effects of *Corni fructus* against advanced glycation endproducts and radical scavenging. Evid Based Complement Alternat Med 2012:1–7
24. Yamabe N, Kang K, Goto E, Tanaka T, Yokozawa T (2007) Beneficial effect of *Corni fructus*, a constituent of Hachimi-jio-gan, on advanced glycation end-product-mediated renal injury in streptozotocin-treated diabetic rats. Biol Pharm Bull 30(3):520–526
25. Yamabe N, Kang K, Matsuo Y, Tanaka T, Yokozawa T (2007) Identification of antidiabetic effect of iridoid glycosides and low molecular weight polyphenol fractions of *Corni fructus*, a constituent of Hachimi-jio-gan, in streptozotocin-induced diabetic rats. Biol Pharm Bull 30(7): 1289–1296
26. Yokozawa T, Yamabe N, Kim H, Kang K, Hur J, Park C et al (2008) Protective effects of morroniside isolated from *Corni fructus* against renal damage in streptozotocin-induced diabetic rats. Biol Pharm Bull 31(7):1422–1428
27. Kumar P, Reddy P, Srinivas P, Reddy G (2009) Delay of diabetic cataract in rats by the antiglycating potential of cumin through modulation of α-crystallin chaperone activity. J Nutr Biochem 20(7):553–562
28. Joglekar M, Panaskar S, Arvindekar A (2014) Inhibition of advanced glycation end product formation by cymene–A common food constituent. J Funct Foods 6:107–115
29. Li X-L, Xiao J-J, Zha X-Q, Pan L-H, Asghar M-N, Luo J-P (2014) Structural identification and sulfated modification of an antiglycation *Dendrobium huoshanense* polysaccharide. Carbohydr Polym 106:247–254
30. Hou T-H, Chung J-P, Chen S-S, Chang T-L (2013) Antioxidation and antiglycation of 95% ethanolic extracts prepared from the leaves of black nightshade (*Solanum nigrum*). Food Sci Biotechnol 22(3):839–844
31. Rani M, Krishna M, Padmakumari K, Raghu K, Sundaresan A (2012) *Zingiber officinale* extract exhibits antidiabetic potential via modulating glucose uptake, protein glycation and inhibiting adipocyte differentiation: an in vitro study. J Sci Food Agric 92(9):1948–1955
32. Kazeem M, Akanji M, Hafizur R, Choudhary M (2012) Antiglycation, antioxidant and toxicological potential of polyphenol extracts of alligator pepper, ginger and nutmeg from Nigeria. Asian Pacific J Trop Biomed 2(9):727–732
33. Kazeem M, Akanji M, Yakubu M, Ashafa A (2015) Antiglycation and hypolipidemic effects of polyphenols from *Zingiber officinale* Roscoe (Zingiberaceae) in streptozotocin-induced diabetic rats. Trop J Pharm Res 14(1):55–61
34. Wu J-W, Hsieh C-L, Wang H-Y, Chen H-Y (2009) Inhibitory effects of guava (*Psidium guajava* L.) leaf extracts and its active compounds on the glycation process of protein. Food Chem 113(1):78–84
35. Hsieh C-L, Lin Y-C, Yen G-C, Chen H-Y (2007) Preventive effects of guava (*Psidium guajava* L.) leaves and its active compounds against α-dicarbonyl compounds-induced blood coagulation. Food Chem 103(2):528–535

36. Soman S, Rauf A, Indira M, Rajamanickam C (2010) Antioxidant and antiglycative potential of ethyl acetate fraction of *Psidium guajava* leaf extract in streptozotocin-induced diabetic rats. Plant Foods Hum Nutr 65(4):386–391
37. Soman S, Rajamanickam C, Rauf A, Indira M (2013) Beneficial effects of *Psidium guajava* leaf extract on diabetic myocardium. Exp Toxicol Pathol 65(1):91–95
38. Lunceford N, Gugliucci A (2005) *Ilex paraguariensis* extracts inhibit AGE formation more efficiently than green tea. Fitoterapia 76(5):419–427
39. Emami S, Asgary S, Naderi G, Ardekani M, Aslani S, Airin A et al (2012) Investigation of antioxidant and anti-glycation properties of essential oils from fruits and branchlets of *Juniperus oblonga*. Rev Bras 22(5):985–993
40. Asgary S, Naderi G, Ardekani M, Sahebkar A, Airin A, Aslani S et al (2014) Inhibition of protein glycation by essential oils of branchlets and fruits of *Juniperus Communis* subsp. hemisphaerica. Res Pharmaceut Sci 9(3):179
41. Yang B, Zhao M, Jiang Y (2009) Anti-glycated activity of polysaccharides of longan (*Dimocarpus longan* Lour.) fruit pericarp treated by ultrasonic wave. Food Chem 114(2): 629–633
42. Jung H, Jung Y, Yoon N, Jeong D, Bae H, Kim D-W et al (2008) Inhibitory effects of *Nelumbo nucifera* leaves on rat lens aldose reductase, advanced glycation endproducts formation, and oxidative stress. Food Chem Toxicol 46(12):3818–3826
43. Al-Musayeib N, Perveen S, Fatima I, Nasir M, Hussain A (2011) Antioxidant, anti-glycation and anti-inflammatory activities of phenolic constituents from *Cordia sinensis*. Molecules 16(12):10214–10226
44. Peng X, Zheng Z, Cheng K-W, Shan F, Ren G-X, Chen F et al (2008) Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation endproducts. Food Chem 106(2):475–481
45. da Silva Morrone M, de Assis A, da Rocha R, Gasparotto J, Gazola A, Costa G et al (2013) *Passiflora manicata* (Juss.) aqueous leaf extract protects against reactive oxygen species and protein glycation in vitro and ex vivo models. Food Chem Toxicol 60:45–51
46. Rout S, Banerjee R (2007) Free radical scavenging, anti-glycation and tyrosinase inhibition properties of a polysaccharide fraction isolated from the rind from *Punica granatum*. Bioresour Technol 98(16):3159–3163
47. Jariyapamornkoon N, Yibchok-anun S, Adisakwattana S (2013) Inhibition of advanced glycation end products by red grape skin extract and its antioxidant activity. BMC Complement Altern Med 13(171):1–9
48. Manaharan T, Appleton D, Cheng H, Palanisamy U (2012) Flavonoids isolated from *Syzygium aqueum* leaf extract as potential antihyperglycaemic agents. Food Chem 132(4):1802–1807
49. Lekshmi P, Arimboor R, Nisha V, Menon A, Raghu K (2014) In vitro antidiabetic and inhibitory potential of turmeric (*Curcuma longa* L) rhizome against cellular and LDL oxidation and angiotensin converting enzyme. J Food Sci Technol 51(12):3910–3917
50. Beaulieu LP, Harris C, Saleem A, Cuerrier A, Haddad P, Martineau L et al (2010) Inhibitory effect of the Cree traditional medicine wiishichimanaanh (*Vaccinium vitis-idaea*) on advanced glycation endproduct formation: identification of active principles. Phytother Res 24(5): 741–747
51. Trakoon-osot W, Sotanaphun U, Phanachet P, Porasuphatana S, Udomsubpayakul U, Komindr S (2013) Pilot study: hypoglycemic and antiglycation activities of bitter melon (*Momordica charantia* L.) in type 2 diabetic patients. J Pharm Res 6(8):859–864
52. Babu P, Sabitha K, Shyamaladevi C (2006) Green tea impedes dyslipidemia, lipid peroxidation, protein glycation and ameliorates Ca 2+−ATPase and Na+/K+−ATPase activity in the heart of streptozotocin-diabetic rats. Chem Biol Interact 162(2):157–164
53. Palanisamy U, Manaharan T, Teng L, Radhakrishnan A, Subramaniam T, Masilamani T (2011) Rambutan rind in the management of hyperglycemia. Food Res Int 44(7):2278–2282
54. Palanisamy U, Ling L, Manaharan T, Appleton D (2011) Rapid isolation of geraniin from *Nephelium lappaceum* rind waste and its anti-hyperglycemic activity. Food Chem 127(1): 21–27

55. Lee G, Jang D, Lee Y, Kim J, Kim J (2006) Naphthopyrone glucosides from the seeds of *Cassia tora* with inhibitory activity on advanced glycation end products (AGEs) formation. Arch Pharm Res 29(7):587–590
56. Jang D, Lee G, Kim Y, Lee Y, Kim C-S, Yoo J et al (2007) Anthraquinones from the seeds of *Cassia tora* with inhibitory activity on protein glycation and aldose reductase. Biol Pharm Bull 30(11):2207–2210
57. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H (2000) Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. J Agric Food Chem 48(2):180–185
58. Yokozawa T, Nakagawa T (2004) Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. Food Chem Toxicol 42(6):975–981
59. Lee Y, Kang Y-H, Jung J-Y, Lee S, Ohuchi K, Shin K et al (2008) Protein glycation inhibitors from the fruiting body of *Phellinus linteus*. Biol Pharm Bull 31(10):1968–1972
60. Suantawee T, Wesarachanon K, Anantsuphasak K, Daenphetploy T, Thien-Ngern S, Thilavech T et al (2015) Protein glycation inhibitory activity and antioxidant capacity of clove extract. J Food Sci Technol 52(6):3843–3850
61. Joshi S (2000) *Eugenia Jambolana* L, *Musa paradisiaca* L. In: Medicinal Plants, Joshi, S. (Ed.). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, pp 286–294
62. Pari L, Umamaheswari J (2000) Antihyperglycaemic activity of *Musa sapientum* flowers: effect on lipid peroxidation in alloxan diabetic rats. Phytother Res 14(2):136–138
63. Bhaskar J, Salimath P, Nandini C (2011) Stimulation of glucose uptake by *Musa* sp.(cv. elakki bale) flower and pseudostem extracts in Ehrlich ascites tumor cells. J Sci Food Agric 91(8): 1482–1487
64. Begum S, Hassan S, Siddiqui B (2002) Two new triterpenoids from the fresh leaves of *Psidium guajava*. Planta Med 68(12):1149–1152
65. Vrhovsek U, Rigo A, Tonon D, Mattivi F (2004) Quantitation of polyphenols in different apple varieties. J Agric Food Chem 52(21):6532–6538
66. Lambole VB, Krishna M, Upendra K, Bhatt S, Vipul G (2010) Phytopharmacological properties of *Aegle marmelos* as a potential medicinal tree: an overview. Int J Pharmaceut Rev Res 5(2):67–71
67. Maity P, Hansda D, Bandyopadhyay U, Mishra D (2009) Biological activities of crude extracts and chemical constituents of Bael, *Aegle marmelos* (L.) Corr. Indian J Exp Biol 47:849–861
68. Baliga M, Bhat H, Joseph N, Fazal F (2011) Phytochemistry and medicinal uses of the bael fruit (*Aegle marmelos* Correa): a concise review. Food Res Int 44(7):1768–1775
69. Grover J, Yadav S (2004) Pharmacological actions and potential uses of *Momordica charantia*: A review. J Ethnopharmacol 93(1):123–132
70. Leung L, Birtwhistle R, Kotecha J, Hannah S, Cuthbertson S (2009) Anti-diabetic and hypoglycaemic effects of *Momordica charantia* (bitter melon): a mini review. Br J Nutr 102(12):1703–1708
71. Miceli N, Trovato A, Dugo P, Cacciola F, Donato P, Marino A et al (2009) Comparative analysis of flavonoid profile, antioxidant and antimicrobial activity of the berries of *Juniperus communis* L. var. communis and *Juniperus communis* L. var. saxatilis Pall. from Turkey. J Agric Food Chem 57(15):6570–6577
72. Jayaprakasha G, Singh R, Sakariah K (2001) Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. Food Chem 73(3):285–290
73. Ek S, Kartimo H, Mattila S, Tolonen A (2006) Characterization of phenolic compounds from lingonberry (*Vaccinium vitis-idaea*). J Agric Food Chem 54(26):9834–9842
74. Leduc C, Coonishish J, Haddad P, Cuerrier A (2006) Plants used by the Cree Nation of Eeyou Istchee (Quebec, Canada) for the treatment of diabetes: a novel approach in quantitative ethnobotany. J Ethnopharmacol 105(1):55–63
75. Jiang Y, Zhang Z, Joyce D, Ketsa S (2002) Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.) Postharvest Biol Technol 26(3):241–252
76. Zheng G, Xu L, Wu P, Xie H, Jiang Y, Chen F et al (2009) Polyphenols from longan seeds and their radical-scavenging activity. Food Chem 116(2):433–436

77. Rangkadilok N, Sitthimonchai S, Worasuttayangkurn L, Mahidol C, Ruchirawat M, Satayavivad J (2007) Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. Food Chem Toxicol 45(2):328–336
78. Zucolotto S, Fagundes C, Reginatto F, Ramos A, Castellanos L, Duque C et al (2012) Analysis of C-glycosyl flavonoids from south American Passiflora species by HPLC-DAD and HPLC-MS. Phytochem Anal 23(3):232–239
79. Lansky E, Newman R (2007) *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 109(2):177–206
80. Mäkynen K, Jitsaardkul S, Tachasamran P, Sakai N, Puranachoti S, Nirojsinlapachai N et al (2013) Cultivar variations in antioxidant and antihyperlipidemic properties of pomelo pulp (*Citrus grandis* [L.] Osbeck) in Thailand. Food Chem 139(1):735–743
81. Xu G, Liu D, Chen J, Ye X, Ma Y, Shi J (2008) Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chem 106(2):545–551
82. Ong P, Acree T, Lavin E (1998) Characterization of volatiles in rambutan fruit (*Nephelium lappaceum* L.) J Agric Food Chem 46(2):611–615
83. Thitilertdecha N, Teerawutgulrag A, Kilburn J, Rakariyatham N (2010) Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities. Molecules 15(3):1453–1465
84. Manaharan T, Ming C, Palanisamy U (2013) *Syzygium aqueum* leaf extract and its bioactive compounds enhances pre-adipocyte differentiation and 2-NBDG uptake in 3T3-L1 cells. Food Chem 136(2):354–363
85. Nayak C, Rastogi N, Raghavarao K (2010) Bioactive constituents present in *Garcinia indica* Choisy and its potential food applications: a review. Int J Food Prop 13(3):441–453
86. Crespy V, Williamson G (2004) A review of the health effects of green tea catechins in in vivo animal models. J Nutr 134(12):3431S–3440S
87. Gardner E, Ruxton C, Leeds A (2007) Black tea-helpful or harmful? A review of the evidence. Eur J Clin Nutr 61(1):3–18
88. Xie W, Zhang X, Wang T, Hu J (2012) Botany, traditional uses, phytochemistry and pharmacology of *Apocynum venetum* L.(Luobuma): a review. J Ethnopharmacol 141(1):1–8
89. Heck C, De Mejia E (2007) Yerba mate tea (*Ilex paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. J Food Sci 72(9): R138–RR51
90. Bracesco N, Sanchez A, Contreras V, Menini T, Gugliucci A (2011) Recent advances on *Ilex paraguariensis* research: minireview. J Ethnopharmacol 136(3):378–384
91. Luo J-P, Deng Y-Y, Zha X-Q (2008) Mechanism of polysaccharides from *Dendrobium huoshanense*. On Streptozotocin-induced diabetic cataract. Pharm Biol 46(4):243–249
92. Chang C-C, Ku A, Tseng Y-Y, Yang W-B, Fang J-M, Wong C-H (2010) 6, 8-Di-C-glycosyl flavonoids from *Dendrobium huoshanense*. J Nat Prod 73(2):229–232
93. Liu C-L, Chen Y-S, Yang J-H, Chiang B-H (2007) Antioxidant activity of tartary (*Fagopyrum tataricum* (L.) Gaertn.) and common (*Fagopyrum esculentum* Moench) buckwheat sprouts. J Agric Food Chem 56(1):173–178
94. Kim S-J, Zaidul I, Suzuki T, Mukasa Y, Hashimoto N, Takigawa S et al (2008) Comparison of phenolic compositions between common and tartary buckwheat (*Fagopyrum*) sprouts. Food Chem 110(4):814–820
95. Jain S, Patil U (2010) Phytochemical and pharmacological profile of *Cassia tora* Linn – an overview. Indian J Nat Prod Resour 1:430–437
96. Akubugwo I, Obasi A, Ginika S (2007) Nutritional potential of the leaves and seeds of black nightshade-*Solanum nigrum* L. Var virginicum from Afikpo-Nigeria. Pak J Nutr 6(4): 323–326
97. Ikeda T, Tsumagari H, Nohara T (2000) Steroidal oligoglycosides from *Solanum nigrum*. Chem Pharm Bull 48(7):1062–1064
98. Zhu T, Kim S-H, Chen C-Y (2008) A medicinal mushroom: *Phellinus linteus*. Curr Med Chem 15(13):1330–1335

99. Jayaprakasha G, Jena BS, Negi PS, Sakariah K (2002) Evaluation of antioxidant activities and antimutagenicity of turmeric oil: a byproduct from curcumin production. Zeitschrift für Naturforschung C 57(9–10):828–835
100. Miquel J, Bernd A, Sempere J, Dıaz-Alperi J, Ramırez A (2002) The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. Arch Gerontol Geriatr 34(1):37–46
101. Kuroda M, Mimaki Y, Nishiyama T, Mae T, Kishida H, Tsukagawa M et al (2005) Hypoglycemic effects of turmeric (*Curcuma longa* L. rhizomes) on genetically diabetic KK-Ay mice. Biol Pharm Bull 28(5):937–939
102. Sridhar K, Bhat R (2007) Lotus – A potential nutraceutical source. J Agricult Technol 3(1): 143–155
103. Huang B, Ban X, He J, Tong J, Tian J, Wang Y (2010) Hepatoprotective and antioxidant activity of ethanolic extracts of edible lotus (*Nelumbo nucifera* Gaertn.) leaves. Food Chem 120(3):873–878
104. Mukherjee P, Mukherjee D, Maji A, Rai S, Heinrich M (2009) The sacred lotus (*Nelumbo nucifera*)–phytochemical and therapeutic profile. J Pharm Pharmacol 61(4):407–422
105. Rong X, Peng G, Suzuki T, Yang Q, Yamahara J, Li Y (2009) A 35-day gavage safety assessment of ginger in rats. Regul Toxicol Pharmacol 54(2):118–123
106. Ali B, Blunden G, Tanira M, Nemmar A (2008) Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food Chem Toxicol 46(2):409–420
107. Ani V, Varadaraj M, Naidu K (2006) Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (*Cuminum nigrum* L.) Eur Food Res Technol 224(1):109–115
108. Johri R (2011) *Cuminum cyminum* and *Carum carvi*: An update. Pharmacogn Rev 5(9):63–72
109. Gurib-Fakim A (2006) Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Asp Med 27(1):1–93
110. Jirovetz L, Buchbauer G, Stoilova I, Stoyanova A, Krastanov A, Schmidt E (2006) Chemical composition and antioxidant properties of clove leaf essential oil. J Agric Food Chem 54(17): 6303–6307

Nutraceutical Potential of Apiaceae

44

Milica G. Aćimović

Contents

Abstract

Apiaceae family is large, with over 3.000 species worldwide cultivated for many purposes. Some plants in this family such as carrots, parsley, parsnip and celery are common vegetable crops, while other members like anise, caraway, coriander, cumin, fennel, lovage, angelica and dill are famous for their medicinal and

M. G. Aćimović (✉)
Department of Alternative Crops and Organic Agriculture, Institute of Field and Vegetable Crops, Novi Sad, Serbia
e-mail: acimovicbabicmilica@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_17

aromatic properties. Usage of these plants is very popular in everyday diet because of their documented health benefits. Apiaceae are a very important source of phytochemicals – chemicals with biological activity. However, phytochemicals are non-nutritive plant chemicals, also called nutraceuticals. They are widely used for prevention, treatment or cure of conditions or diseases. Bioactive compounds with nutraceutical potential are polyphenolic compounds, polyacetylenes and terpenoids. The aim of this review is to represent selected plants of Apiaceae family currently used as nutraceuticals and describe their nutritional benefits.

Keywords
Vegetable · Spices · Biological activity · Food · Nutrition · Phenolics · Polyacetylenes · Terpenoids

Abbreviations

CAE	Caffeic acid equivalent
CE	Catechine equivalent
DW	Dry weight
FW	Fresh weight
GAE	Gallic acid equivalents
QE	Quercetin equivalent
TFC	Total flavanoids content
TPC	Total phenolic contents

1 Introduction

Apiaceae (Umbelliferae) family is large, with over 3.000 species worldwide cultivated for food, as vegetables, herbs, spices or for medicinal purposes. Some plants in this family such as carrots, parsley, parsnip and celery are common vegetable crops, while other members like anise, caraway, coriander, cumin, fennel, lovage, angelica and dill are famous for their medicinal and aromatic properties [1].

The plants from Apiaceae family are mainly temperate herbaceous annual (anise, caraway, coriander, cumin, dill, sweet fennel), biannual (carrot, parsley, parsnip, celery) or perennial (angelica, lovage, bitter fennel). Leaves are alternate on stem or arranged in leaf rosette (mainly in the first year of development in biannual and perennial members). In all the mentioned species, stem is erect and hollow, and in the upper part is branch. Each branch finishes with an inflorescence. The small and simple flowers are generally arranged into compound umbels. They have five petals that are usually white or yellow, with five stamens, and an ovary with two carpels. The fruit that develops from this ovary varies considerably between the spices. Generally the fruit are schizocarps, which contain two seeds.

All Apiaceae plants contain a well-developed secretory system in all plant parts, such as schizogeneus secretory cavities in the root, phloem in the stem and leaves and clearly-delimited tissue known as vittae in the fruit. These structures are important for depositing essential oils, which give the specific odor and flavor to

each plant. Due to their flavor, a large number of plants from this family are used as vegetables or spices [2].

Plants from the Apiaceae family are a very important source of nutraceuticals. Their usage is very popular in everyday diet because of their documented health benefits. The figure below shows members of Apiaceae family and their parts which are usually used. For example, caraway, cumin, aniseed are exclusively used as seed, while dill and coriander are used as seed and leaves, and from carrot seed and root. In case of fennel, apart from seed and leaves, succulent leaf stalks are also used. Celery is used in a similar way. From lovage, parsley and angelica, the root is also used together with above-ground parts (Fig. 1).

2 Bioactive Compounds of Apiaceae

Bioactive compounds can be divided into two groups: phytochemicals which are non-nutritive plant chemicals, also called nutraceuticals, and nutrients which include minerals, proteins, fibers, carbohydrates, fats, etc. However, nutraceuticals possess biological activity, while nutrients affect the growth, development and function of the human body.

Nutraceuticals can be designated as food with medical benefits; as indicated by its name which is derived from “nutrition” and “pharmaceutics”. They include polyphenolic compounds, polyacetylenes and terpenoids [3]. Nutraceuticals are widely used for prevention, treatment or cure of conditions or diseases [4, 5, 6]. The high antioxidant activity of nutraceuticals is the basis for many potential benefits, among which many for degenerative and chronic diseases [7].

2.1 Phenolic Compounds

Phenolic compounds, as one of major bioactive nutraceutical ingredients in plants, are responsible for the organoleptic characteristics of plant-derived foods and beverages, particularly color and taste properties and they also contribute to the nutritional qualities of fruits and vegetables [8]. The phenolic metabolites include: anthocyanins, anthochlors, benzofurans, chromones, coumarins, flavonoids, flavonones and flavonols, isoflavonoids, lignans, phenols and phenolic acids, phenolic ketones, phenylpropanoids, quinonoids, stilbenoids, tannins and xanthones [9]. The antioxidant property in many plants is related to the presence of phenolic compounds. Nutritionally, these compounds are responsible for increasing the shelf life of foods as well as slowing the lipid, protein and enzymatic oxidation, as well as for providing protection against development of cancers, cardiovascular and liver diseases, diabetes, osteoporosis and neurodegenerative diseases in humans [10, 11, 12, 13, 14]. However, the content of biologically active substances, among them polyphenols in plants, depends on various factors such as: area in which the plant is grown (agrochemical characteristic of soil), climatic conditions in the region during the growing season, cultivation technology but also the variety [15]. Apart from this,

Fig. 1 Plants from Apiaceae family with their parts which are usually used in nutrition

total phenolic contents depend also on postharvest processing (fresh, dry, freeze herb), solvents used for extraction (water, ethanol, acetone, etc.) and significantly varies due to plant material (seed, herb, root) etc. Review of total phenolic content according to literature is shown in Table 1.

Table 1 Phenolic compound from selected plants from Apiaceae family

Plant	Phenolic compounds
Anethum graveolens	TPC in dill seed methanolic extract is 773.14 mg GAE 100 g^{-1} dw, while TFC is 231.84 mg QE 100 g^{-1} dw [16]. On the other side, TPC in fresh dill herb acetone extract is 35.23 mg GAE g^{-1} dw, and content of TFC is 30.39 mg CAE g^{-1} dw [17]. However, TPC in dry dill herb varied from 55.46 to 71.29 mg GAE g^{-1} dw depend on solvents [18]. Further, fresh dill herb acetone extract contains 19.49 mg QE g^{-1} dw phenolic acids, and the dominant are chlorogenic and benzoic acids [17]
Angelica archangelica	TPC in angelica roots is 11.8–17.3 mg GAE g^{-1} extract, while content of coumarins is 0.91 mg 100 g^{-1} extract [19]. The dominant are coumarin derivatives among which isoimperatorin, oxypeucedanin, imperatorin, ostruthol, angelicin, bergapten, scopoletin, isopimpinellin, and xanthotoxin [20, 21]
Apium graveolens	TPC in celery seed methanol extract is 825.85 mg GAE 100 g^{-1} dw, and TFC is 177.57 mg CE 100 g^{-1} dw, while content of tanins is 243.36 mg CE 100 g^{-1} dw [22]. The dominant flavonoids are apigenin, luteolin, and kaempferol, while the dominant phenolic acids are caffeic, p-coumaric and ferulic acid [23]
Carum carvi	TPC in caraway seeds is 3.99 mg GAE g^{-1} dw with 42 phenolic compounds [24]. The dominant flavonoid constituent from caraway seed is quercetin 3-glucuronide, isoquercitrin, quercetin 3-O-caffeylglucoside and kaempferol 3-glucoside [25]. Phenolic acid content in caraway seed is about 65 μg g^{-1} dw, with dominant chlorogenic, p-coumaric and caffeic acid [24]
Coriandrum sativum	TPC in coriander leaves extracts is 1.12 mg GAE 100 mL^{-1} [26], and the main flavonoids are quercetin-3-*O*-rutinoside, kaempferol and acacetin, while identified phenolic acids are vanilic, ferulic and p-coumaric acid [27, 28]. Coriander seed contain TPC 0.72 g GAE 100 g^{-1} extract [29]. Rutin, quercetin, chlorogenic and caffeic acid were separated and identified flavonoids in the methanolic and ethanolic extracts of coriander seed [30]
Cuminum cyminum	Acetone extract of cumin seed contains TPC between 16.50 and 18.60 mg GAE g^{-1} dw, TFC between 4.99 and 5.91 mg CE g^{-1} dw and tannin 80.23–83.23 mg CE g^{-1} dw [31]. The main phenolic compounds from cumin are quercetin, p-coumaric, rosmarinic, vanillic and trans-2-dihydrocinnamic acids, as well as resorcinol [32]
Daucus carota	TPC in the carrot root varied considerably from 19.8 to 342.2 mg GAE 100 g^{-1} fw depend on root color [33], and from 81.25–113.69 mg GAE kg^{-1} fw depend on variety [15]. However, carrot contained high amounts of phenolic acids, flavonoids, and carotenoids. Content of ß-carotenes vary between 24.58 and 124.28 mg kg^{-1} fw [15] while total ascorbic acid ranged from 41.12 to 58.36 mg 100 g^{-1} fw, whereas 5-caffeolquinic acid ranged from 30.26 to 65.39 mg 100 g^{-1} fw [34]
Foeniculum vulgare	TPC in seed methanolic extract was 1017.29 mg GAE 100 g^{-1} dw, while TFC is 695.52 mg QE 100 g^{-1} dw [16]. Further, also TFC in seed methanolic extract is 9.325 mg QE g^{-1} dw, with dominant gallic acid (277.131 μg g^{-1} dw), caffeic acid (166.062 μg g^{-1} dw), ellagic acid (99.476 μg g^{-1} dw), quercetin (781.986 μg g^{-1} dw) and kaempferol (92.856 μg g^{-1} dw) [35]. From the other side, fennel herb contains two phenolic compounds, 3.4-dihydroxy-phenethylalchohol-6-*O*-caffeoyl-β-D-glucopyranoside and 3′0.8′-binaringenin [11]

(*continued*)

Table 1 (continued)

Plant	Phenolic compounds
Levisticum officinale	TPC in lovage leaves ranged between 359.75 and 1601.87 mg GAE 100 g^{-1} dw, while TFC varied between 551.01–3548.33 mg CE 100 g^{-1} dw, depending on postharvest treatment (fresh, frozen, dry). Phenolic compounds present in lovage leaves are: rutine, catechin, caffeic, chlorogenic, coumaric, sinapic, and ferulic acid [36]
Pastinaca sativa	The total phenolic acid in parsnip is 5.7 mg 100 g^{-1} fw, while the major soluble phenolic acid is chlorogenic acid [37]. Total content of coumarins ranged from 115.7 to 408.5 mg 100 g^{-1} dw. In vegetative plant parts the dominant are isopimpinellin and psoralen, while imperatorin was dominant in fruit [38]
Petroselinum crispum	TPC in parsley seed is 0.62 g GAE 100 g^{-1} extract, while in leaves it is 0.92 g GAE 100 g^{-1} extract [29]. However, another study shows that TPC in parsley leaves ranged from 15.20 to 54.20 mg CE g^{-1} extract, while TFC is between 4.50 and 42.1 mg QE g^{-1} extract [39]. Flavonoids isolated from aqueous extract of parsley leaves: apigenin, apigenin-7-O-glucoside or cosmosiin, apigenin-7-O-apiosyl-(1–2)-O-glucoside or apiin and the coumarin 2,3-dihydroxyfuranocoumarin or oxypeucedanin hydrate [40]
Pimpinella anisum	TPC in anise seed is 46.17 mg GAE 100 g^{-1} dw, while TFC is 17.43 mg CE 100 g^{-1} dw [41]. Another study shows that TPC is 42.09 mg g^{-1} extract, and identified mainly flavonoids (28.08 mg g^{-1} extract) and phenolic acids (14.01 mg g^{-1} extract). Morerover, apigenin and luteolin derivatives, as well as caffeoylquinic acid derivatives were determined [12]

2.2 Polyacetylenes

Polyacetylenes are a group of phytochemicals that have attracted significant interest in medicine and pharmaceutical industry in recent years due to their range of potential health-promoting bioactivities. Polyacetylenes isolated from Apiaceae plants include antifungal and antibacterial activity [42, 43], as well as anti-inflamatory [44] and anticancer [45, 46] properties. They also display antidiabetic [47] effects and have potential in the treatment of endotoxemia and inflammation accompanied by the overproduction of NO [48]. Apart from this, they possess neurotoxic, anti-platelet-aggregatory activity and are responsible for allergic skin reactions [49, 50, 51]. However, polyacetylenes have a major impact on the bitter taste in roots of parsnip, celeriac, parsley and carrot, as well as fennel bulbs [52, 53, 54, 55, 56, 57, 58, 59]. The review of polyacetilenes present in plants from Apiaceae family according to literature is shown in Table 2.

2.3 Terpenoids

Terpenoids, especially monoterpenes and sesquiterpenes are the main constituents in essential oils which, as volatile compounds, give fragrance to many aromatic plants [64]. Some terpenoids are very typical for plant species from the Apiaceae family as

Table 2 Polyacetylenes from selected plants from Apiaceae family

Plant	Polyacetylenes
Anethum graveolens	Dill roots contain polyacetylenes such as panaxynol ($C_{17}H_{24}O$) and falcarindiol ($C_{17}H_{24}O_2$) [60]
Angelica archangelica	Many species from genus Angelica contain polyacetylenes. For example, polyacetylene from *A. purpuraefolia* is (+)-9(Z), 17-octadecadiene-12,14-diyne-1,11,16-triol [61], while *A. furcijuga* contain (−)-falcarinol and falcarindiol [62]. *A. gigas* contains: octadeca-1, 9-dien-4, 6-diyn-3, 8, 18-triol (1), 18-acetoxy-octadeca-1, 9-dien-4, 6-diyn-3, 8-diol (2) and 3, 8, 18-triacetoxy-octadeca-1, 9-dien-4, 6-diyn (3) [48]
Apium graveolens	Celery root contains polyacetylenes: falcarindiol, falcarinol, panaxydiol and 8-*O*-methylfalcarindiol [44, 49]
Carum carvi	Aliphatic C17-polyacetylenes of the caraway are falcarinol, falcarindiol, falcarinolone and falcarindione [52]
Coriandrum sativum	In coriander, polyacetylenes are detected but not identified [52]
Cuminum cyminum	No data in the available literature
Daucus carota	The most abundant polyacetylenes in cultivated orange carrots is falcarindiol (16–84 mg kg^{-1} fw, followed by falcarinol (8–27 mg kg^{-1} fw) and falcarindiol-3-acetate (8–40 mg kg^{-1} fw) [59]
Foeniculum vulgare	The amount of polyacetylenes in fennel bulb ranged from 0.04 to 0.24 mg g^{-1} of freeze-dried plant material [49]. Polyphenoles detected in fennel bulb are falcarindiol, falcarindiol-3-acetate, and falcarinol [57]
Levisticum officinale	Polyacetylenes from lovage roots are: 3(*R*)-Falcarinol (3(*R*)-(−)-1,9-heptadecadien-4,6-diin-3-ol] (1) and 3(*R*)-8(*S*)-falcarindiol [3(*R*)-8(*S*)-(+)-1,9-heptadecadien-4,6-diin-3,8-diol] (2) [43]
Pastinaca sativa	Polyacetylene compounds from parsnip root are falcarinol and falcarindiol occurring in the highest concentrations (1600 and 5770 mg kg^{-1} freeze-dried material, respectively), followed by falcarinone and falcarinolone. Moreover, parsnip seeds contain polyacetylenic C18 ketoaldehyde [55]
Petroselinum crispum	Polyacetylene from parsley root are falcarindiol (up to 2320 mg kg^{-1} freeze-dried material), falcarinol, 8-O-methylfalcarindiol (350 mg kg^{-1} freeze-dried material) and panaxydiol (120 mg kg^{-1} freeze-dried material) [55]
Pimpinella anisum	Polyacetylenes have been reported to be present in some Pimpinella species, such as falcarinol in *P. pruatjan*, pentadeca-2,4,6,8-tetraene (1), 2,8-decadiene-4,6-diene-1-al (2), 2,8-tridecadiene-4,6-diene-10-ol (3) 2,8,10-tridecatriene-4,6-diene (4) in *P. major*, and 2-tridecaene-4,6-diene-8-ol-10-on in *P. saxifraga* [63]

they are the source of the familiar taste, for example carotol in carrot, *trans*-anethole in anise and fennel, carvone in caraway and dill [65, 66, 67]. Due to their aromatic qualities, these plants are used as supplements in everyday food in order to enhance the smell, taste and biological values. For this reason this group will be in the focus. Essential oils have been known to possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products [68, 69]. Anti-oxidative properties of essential oils are responsible for healing or improving degradation processes in many diseases such as cancer, rheumatoid arthritis,

Table 3 Terpenoids from selected plants from Apiaceae family

Plant	
Anethum graveolens	Dill seed essential oil contains carvone and limonene as the dominant compounds [73], while the main compounds in the herb essential oil are *α*-phellandrene, apiole, dill ether, limonene, geraniol and *p*-cymene [74]
Angelica archangelica	The main components of angelica roots essential oil are *α*-pinene, *δ*-3-carene, *β*-phellandrene and limonene, while in seed it is *β*-phellandrene, *α*-phellandrene, *α*-pinene, myrcene and *α*-copaene [75, 76]
Apium graveolens	Celery essential oil contains limonene and selinene. However, the important flavor constituents of the oil responsible for the typical aroma are phtalides (3-n-butyl-4-5-dihydrophthalide (sedanenolide), 3-n-butyl phthalide, sedanolide, and sedanonic anhydride) [77]
Carum carvi	Caraway seed essential oil is comprised from carvone and limonene, constituting more than 90% [66, 78, 79]
Coriandrum sativum	Coriander seed essential oil contains mainly linalool [80, 81, 82], while coriander herb oil has a significantly different composition with decanal, *trans*-2-decenal, 2-decen-1-ol, cyclodecane and *cis*-2-dodecenal as the main compounds [83]
Cuminum cyminum	The distinctive flavor and aroma are originate from essential oil, the dominant compounds of which are γ-terpinene-7-al, cumin-aldehyde, β-pinene and γ-terpinene [84, 85, 86, 87]
Daucus carota	Carrot root essential oil contains mainly geranyl, linalool, myristicine, pentacosane, spathulenol and *trans*-*γ*-bisabolene. The major compounds of aerial parts' essential oil is alismol, *trans*-*β*-caryophyllene, myrcene, *α*-humulene and *β*-ionone [88]. The major constituents of essential oil from carrot seeds are carotol, sabinene, *α*-pinene followed by aromadendrene, *β*-farnesene, sesquisabinene, *trans*-caryophyllene and myrcene [67]
Foeniculum vulgare	The dominant compound in fennel seed and herb essential oil is *trans*-anethole which gives them similar scent as to aniseed [89]. However, fennel has two varieties: sweet and bitter. Sweet fennel (var. *dulce*) apart of *trans*-anethole (more than 80%), contains estragole (less than 10%) and fenchone (less than 7.5%). Bitter fennel (var. *vulgare*) apart of *trans*-anethole (55–75%) contains fenchone (12–25%) [90]
Levisticum officinale	The lovage flavor, like the celery one, originates from essential oil where the dominant compound is *β*–phellandrene, while phthalides are present in small amounts and give the characteristic fragrance [91]
Pastinaca sativa	Root essential oil had the two major constituents, myristicine and terpinolene [92]. Aerial parts contain essential oil with dominant *cis*-*β*-ocimene, hexyl butanoate, *trans*-*β*-farnesene and lavandulyl acetate [93]
Petroselinum crispum	Major compounds in parsley essential oil are apiol, myristicin and *β*-phellandrene [94, 95]
Pimpinella anisum	Anise contains essential oil with the most abundant component, *trans*-anethole, above 96% which exhibits sweet and licorice taste with a herbal anise and fennel nuance [89, 96]

cirrhosis and arteriosclerosis as well as in degenerative processes associated with aging [70]. Essential oils also showed antimicrobial properties, which makes them efficient alternative antibiotics and antimycotic agents [71, 72]. Review of terpenoids present in Apiaceae according to literature is shown in Table 3.

3 Nutraceutical Potential of Apiaceae

The aim of this review is to represent members of Apiaceae family currently used as nutraceuticals and describe their nutritional benefits. Investigation of nutraceuticals from this family is very attractive because of their extensive application in everyday diet. Review of their chemical composition and biological activity highlighted their importance as food with health benefits.

3.1 *Anethum graveolens*

Dill seed and leaf are the parts which are mainly used. Dill seed is used in pickled cucumbers, bread, processed meats, sausages, cheese and condiments. Dill leaves are used in pickles, while fresh are used for garnish or to flavor salads, vegetable dishes, sea food, soups, yogurt and mayonnaise [2]. Dill has been used in traditional medicines worldwide since the ancient times. It is used to relieve colic pain in babies and flatulence in young children, as carminative (improves appetite), mild diuretic, galactogogue, stimulant and stomachic. It is also used for treatment of diarrhoea, astma, neuralgia, dysuria, dysmenorrhoea, gallbladder disease and insomnia [97, 98]. However, a great number of pharmacological studies show that dill possesses significant biological activity. Because of the high antioxidative potential [18, 99], dill can be used to improve biochemical processes in patients who suffer from diseases in relation to metabolic syndrome [100]. Studies show that dill can be used for managing diabetes and cardiovascular diseases because it possesses hypoglycemic properties [101, 102, 103]. It is documented that dill decreased total cholesterol without any side effects [104, 105, 106]. Apart from this, dill has hepatoprotective properties [107, 108, 109, 110, 111, 112]. Dill exhibited great anti-cancer activity on oral cavities and breast cancer cells lines [113]. Dill is a good antimicrobial agent [114, 115], which makes it a very significant plant in herbal medicine, especially as a base for the development of novel antimicrobial phytoremedies [116].

3.2 *Angelica archangelica*

Angelica root is used in herbal liqueurs and bitter spirits, in flavoring meat and canned vegetables, while the seed is used in alcoholic distillates. However, chopped angelica green parts (leaves and herb) can be added to fruit salads, fish dishes and cottage cheese, they are used for decorating cakes and pastry and to flavor jams and jellies, confectionaries and liqueurs [2]. The species is well known and has been cultivated since the ancient times for treating certain diseases, such as gastrointestinal problems, like a carminative or in flatulent colic, as well as diaphoretic and diuretic [117]. Applied externally, angelica is good as a counter-irritant, for treatment of rheumatic diseases [118]. New investigations of this plant show that it possesses good antioxidative [119, 120, 121] and antimicrobial activity [72, 75, 122, 123]. This indicates

that angelica can be used as a botanical preservative against molds, aflatoxin contamination and oxidative deterioration of walnut samples [124], as well as a control agent for plant pathogenic fungi in natural formulations [125]. Angelica root water extraction revealed a significant antioxidant role beside its chelation potency of lead ions, so it can be used as a natural chelator in case of lead poisoning [126]. Clinical investigations show that angelica expressed hepatoprotective activity [119, 127] as well as cytotoxicity in human pancreas cancer cells and mouse breast cancer cells [128]. Angelica also shows anxiolytic activity, so it can be used for nervous disorders and cerebral diseases, for example for epilepsy treatment [129, 130].

3.3 *Apium graveolens*

Celery has different forms and uses. Turnip-rooted celery, also called celeriac, is used mainly as grated raw salad, as well as cooked vegetable in stews and soups. Leaf celery, called smallage, is chopped and used for garnishing and flavoring, either fresh or dried. The succulent leafstalk, often with a part of leafblade, is used for the preparation of sauces, vegetable juices, stews, soups, salads, etc. Celery seed is used as condiment, in pickling vegetables, salad dressings, breads, biscuits, soups, spice mixed with salt, as bouquet garnish [2]. In traditional medicine, celery is used to treat many diseases. Traditionaly, celery is mainly used as a diuretic and as a treatment for arthritis and rheumatism. Celery also has sedating effect and has been used in herbal medicine to treat nervousness, hysteria and various other conditions [131, 132]. However, investigations show that celery possesses good antioxidant activity [133, 134, 135] as well as antimicrobial activity [136, 137]. Significant hepatoprotective activity [138, 139, 140, 141, 142], and anti-inflammatory effect of celery are reported [143, 144]. Hypoglycemic effect of celery is also reported, as well as that its potent role in ameliorating stressful complications accompanied by diabetes mellitus [145, 146]. Celery acts as an intestinal smooth muscle relaxant in the digestive tract [147]. Also, it has antihypertensive properties, and can be considered as an antihypertensive agent in chronic treatment of elevated blood pressure [148]. Celery showed a significant diuretic effect that accentuates the excretion of urinary calcium [149].

3.4 *Carum carvi*

Caraway seed is used in creams, cakes, baked goods, cheese, confections, fresh cabbage, meat dishes, rye bread, salads, while essential oil obtained from seed is used to flavor chewing gum, candy, soft drinks and alcoholic beverages [2, 150]. Caraway seed essential oil has been reported to have potential therapeutic effects, mainly due to its high antioxidant activity [151]. Considering the radical scavenging [152] and good antioxidant profile [153, 154], it has been recommended for its multifaceted pharmacological properties [155]. Caraway has been used to treat digestive disorders for a long time. It can be used successfully for establishing normal intestinal motility, for healing chronic constipation, gastric ulcer, dysbiosis,

dyspeption and heartburn [156, 157, 158, 159, 160, 161, 162]. The application of caraway fruits significantly inhibits the increase of total cholesterol and levels of triglycerides [163, 164, 165]. Apart from this it can be used in the treatment of hyperglycemia [166, 167]. Caraway fruit shows strong antibacterial and antifungal activities [168, 169, 170]. It also has anti-inflammatory properties [157] and anti-stress activity [171]. Caraway significantly increases urine output, and the total volume of urine excreted [172]. In addition, the aqueous extract of caraway fruits decreases the level of glucose in serum, urea, creatinine, total urinary protein and microalbuminuric levels. Caraway possesses strong anti-oxidant activity which provides renoprotection against diabetes and its complications [173]. It is established that the essential oil also protects the kidneys from damage which occur as a consequence of diabetic nephropathy [174].

3.5 *Coriandrum sativum*

Coriander leaf is used to make chutneys and sauces, green salsas, dips, snacks, soups, while the seed is used in couscous, stews and salads, as a condiment in pickle spices, seasonings, curry powders, sausages, cakes, pastries, biscuits and buns. Seed essential oil is used in beverages, baked goods, condiments, relishes and meat products [2]. However, leaf and seed have different aromas because of the different chemical composition, but both oils possess good antioxidative [175, 176], as well as antimicrobial activity [177, 178]. Studies indicated that coriander enabled development of a novel broad spectrum of antibacterial herbal formulations, and that it has potential for new natural antifungal formulations [179]. However, bioactive compounds present in coriander are used in traditional and modern medicine, as well as in everyday nutrition [69, 180, 181]. In folk medicine, coriander seed is used as an aromatic, carminative, stomachic, antispasmodic and against gastrointestinal complaints such as dyspepsia, flatulance and gastralgia [179]. It is often recomanded for insomnia and anxiety [182, 183, 184]. Its use is recommended for healing the urinary system, ie uretritis, cystitis and urinary tract infections [185]. It has also been used in heavy metal detoxification [186]. It is used as an analgetic and antirheumatic agent [187]. Coriander is effective against hyperlipidemia [188, 189, 190] and hyperglycemia [191, 192]. It also acts as a hepatoprotectant [193, 194, 195, 196, 197, 198] and anticancer agent [199, 200, 201]. Apart from this, coriander can also be used as an anthelmintic [202, 203].

3.6 *Cuminum cyminum*

Cumin seed is used as a flavoring component in beverages, confectioneries, baked goods, meat and meat products, condiments and relishes, gravies, snack foods, gelatins and puddings [2]. It is generally used as a food additive, popular spice, and flavoring agent in many cuisines [204]. Cumin seeds have also been widely used in traditional medicine for treatment of several health disorders and diseases, such as

toothaches, dyspepsia, diarrhea, epilepsy and jaundice. Hovewer, the literature presents ample evidence for the biological and biomedical activities of cumin, among which strong antioxidative [205, 206] and antimicrobial activity [207, 208]. Because of this, it is an important natural food preservative. Further, there is scientific proof that cumin shows antistress, antioxidant, and memory-enhancing activities, that its traditional use as a culinary spice in foods is beneficial in combating stress and related disorders [209]. Cumin possesses hypolipidemic [210, 211], as well as hypoglycaemic potential [212, 213]. Investigations show that cumin possesses hepatoprotective properties against drugs and chemically induced hepatotoxicities, by increasing the level of antioxidant enzymes in the liver [214, 215, 216]. The finding also suggests that it possesses anticancer activity against several carncer cell lines. These studies convey the use of cumin as a helper in the therapy or the control of colon, liver and prostrate cancer. Also, the use of cumin in diet may reduce the risk of cancer [217, 218]. Apart from this, phytochemical constituents from cumin seed show analgesic and anti-inflammatory activities [219].

3.7 *Daucus carota*

The carrot is mainly consumed as a root vegetable, primarily raw, in juices, salads, or for pickling, while it is used cooked in soups and stews, as well as for cakes. Carrot seed is used for essential oil distillation. Obtained oil contains carotol as the dominant compound, and is used as a flavoring agent in food products, mainly in beverages, baked goods, condiments, relishes and meat products [2]. Lately, a number of studies pointed out that the aerial parts are also a source of phytochemicals and could be economically important, for example antioxidants and for blood pressure lowering [220, 221, 222]. The ethnobotanical uses of this species also included applications in the treatment of cough, diarrhea, dysentery, cancer, malaria, tumors, as an antiseptic, abortifacient, aphrodisiac, carminative, stimulant, stomachic and tonic [223]. Studies show that root, as the part mainly used, act as an antioxidant [224, 225, 226], hepatoprotectant [227, 228, 229] and gastroprotectant [230, 231, 232, 233, 234]. Carrot is a good antiinflammatory [235, 236] and anticancer agent [46, 237, 238, 239, 240]. Also, it possesses hypoglycaemic and hypolipidemic properties [47, 241, 242, 243]. Carrot seeds appear to be a promising candidate for improving memory and it would be worthwhile to explore the potential of this plant in the management of Alzheimer patients [243]. Apart from this, carrot seed shows antinoceptive and antiinflamatory [244], as well as hypoglycaemic and hypolipidemic properties [245, 246, 247].

3.8 *Foeniculum vulgare*

Fennel seed is used in meat dishes, curries, spice blends, soups, vegetables, breads. Fresh and chopped leaves can be used as garnish for fish dishes, sauces, salads, stews and curries. Leaf stalk, also called pseudobulb, is used raw in salads, stuffing, soups, sauces; it can be baked or blanched. Seed and herb essential oil is used in beverages,

condiments, relishes, baked goods, frozen dairy, gelatines, puddings, meat products and candies [2]. Fennel has a wide range of bioactivity and has proved to be a good source for traditional medicine. Mainly, it is used as galactagogue and emmenagogue. Because of its diuretic activity, it is useful for kidney and bladder diseases. It is also relieves nausea and vomiting. It is useful for chronic fever and eliminates obstructions of internal organs especially those in liver, intestine and respiratory tract [248]. It provides a noteworthy basis in pharmaceutical biology for the development and formulation of new drugs and future clinical uses [249, 250]. However, investigations show that fennel has efficient antimicrobial activity against bacteria [35, 251, 252], fungi [253, 254, 255] and viruses [256]. Fennel also possesses good antioxidative potential [257, 258], because of hat it is used as food additive to provide protection against oxidative degradation of foods by free radicals, but also used to protect humans from oxidative stress damage [249, 259]. Fennel also possesses anti-inflammatory [260, 261], anticancer [262] and hepatoprotective activity [263, 264]. Further, hypolipidemic and hypoglycaemic potential are also proven [263, 265], as well as diuretic potential and beneficial effect on renal function [266, 267].

3.9 *Levisticum officinale*

The lovage root is used for producing soup seasonings, finished flavorings in liqueurs; the leaf is used for seasoning sauces, meat dishes, while the seed is used as spice, for flavoring cakes, soups, salads, for pickled vegetables (especially cabbage and cucumbers) [2]. Lovage has the strong, characteristic seasoning-like principle of the herb, aromatic odor and taste [268]. Lovage is one of the herbs that have been traditionally used for treatment of many diseases, as diaphoretic, expectorant, stomachic and stimulant [269]. Apart from this, lovage is used in treatment of kidney stones and urinary tract infections [270]. These uses are approved by pharmacological studies, i.e. lovage significantly decreased levels of urine cysteine, creatinine and volume [271]. Hovewer, clinical experience shows that lovage in combination with other plants can be used for treatment of urogenital and gestrointestinal diseases [272]. Lovage exhibited significant antimicrobial [43, 273, 274] and antioxidative activity [36, 275]. Investigations show that lovage is an inexpensive source of natural antibacterial substances for use in pathogenic systems to prevent the growth of bacteria and extend the shelf life of processed foods [276]. Apart from this, lovage possesses anticancer activity, i.e. inhibits human head and neck squamous carcinoma cells growth [277] as well as human liver cancer cell and breast cancer cell lines [278]. Lovage also shows neuroprotective activities, i.e. alcoholic extract has both repair and restoration effects on peripheral nerves [270], as well as antiinflamatory activity [278].

3.10 *Pastinaca sativa*

The parsnip is a root vegetable resembling white carrot. The root is used in soups, stews, cakes, pies and puddings, while leaves and young leafstalks can be used

cooked with other greens as a vegetable or added to soups. The seed is used as a condiment in making beer, wine or distilled spirits [91, 279]. Parsnip has a sweet, distinct, aromatic flavor, similar to nutmeg and cinnamon. This plant is used in traditional medicine worldwide, mainly as a carminative, spasmolytic and diuretic. Also, their usage in treatment of epilepsy is mentioned. Investigations show that parsnip shows anticonvulsant activity due to the presence of a furanocoumarin compound, xanthotoxin [280]. Apart from this, parsnip has anticancer activity, because it contains falcarinol which proved to be the most active compound with a pronounced toxicity against acute lymphoblastic leukemia cell line [49]. Also, parsnip possesses antimicrobial activity against the most common human gastrointestinal pathogenic microbial strains [93]. Parsnip shows significant antibacterial activities on phytopathogenic bacteria, so it might potentially be used as a biological pesticide [92]. The results showed that the addition of parsnip could effectively reduce lipid oxidation, maintain or improve sensory attributes and extend the shelf-life of beef burgers during refrigerated storage. Therefore, it is suggested that parsnip, as a natural herb, could be used to extend the shelf-life of meat products, providing the consumer with food containing natural additives, which might be seen as a healthier option than those of synthetic origin [281].

3.11 *Petroselinum crispum*

Parsley, like celery, has different forms and uses. Roothy form is mainly used as a vegetable, while the leafy form has two varieties, broad-leaved and curly-leaved. The parsley root is used as a vegetable to enhance flavor in soups, stews and condiments, while the leaf is used as a garnish (for salads, soups, boiled potatoes and egg dishes), blended in dips and cooked sauces. Seed is usually used for essential oil extraction. Obtained oil is used to flavor meat sauces, pickles, spice blends, baked goods, oils and fats, processed vegetables, soups, gelatines and puddings [2]. Parsley can be used fresh and dried. Parsley has a pungent, warm, spicy, herbaceous-scent. However, smell and taste in dried spices are different from the fresh ones due to changes in volatile profile during the drying process [282]. Parsley is mainly used in food industry as a vegetable and spice, while in etnopharmacology application as an appetizer, carminative and diuretic. However, investigations show that parsley shows potential to prevent oxidative stress-related diseases and can be developed into functional food or alternative natural antioxidants [283, 284]. Parsley showed a hepatoprotective effect against acute liver injury induced by chemical agents [285, 286, 287, 288], as well as injuries due to the complication of diabetes [289] or chronic changes induced in non-alcoholic fatty liver disease [290]. Parsley is a good diuretic and has antihypertensive effect [291, 292]. Apart from this, parsley shows antiurolithic effects against calcium oxalate stones [293].

3.12 *Pimpinella anisum*

Anise is used in beverages, baked goods, condiments, relishes, oils and fats, frozen dairy, gravies, meat products and soft candy. Seed essential oil is used in chewing gums, gelatines, puddings, soft and hard candies [2, 150]. Anise, like fennel, has an aromatic and sweet taste. It is used in folk medicine in many countries for treatment of digestive, respiratory and neurological diseases, as well as natural estrogen [294, 295]. New investigations show that anise is rich in phytochemical contents, which possess high antioxidant [296, 297] and antimicrobial activities [71, 299]. A new interesting approach to develop plants as natural source and preservative for the food industry is considered. Treatment with anise is effective in reducing the level of some of biochemical parameters and ameliorate behavior of intoxicated by lead [298]. Apart from this, anise possesses strong anticancer activity on human prostate cancer cell line [300], as well as on gastric cancer cell line [301]. Thus, anise could be one of the foods that attribute to cancer prevention and treatment. It could also be a natural source of novel anticancer compounds with anti proliferative and/or apoptotic properties. Anise also possesses hypoglycaemic and hypolipidemic properties [302, 303]. Investigation shows hepatoprotective activity, too [304, 305]. High range of active potentials leads to various applications of anise, such as health supplement and pharmaceutical benefits [41]. This is conditioned by significant content of synergistic action of the bioactive compounds present in the seed [12, 306].

4 Conclusion

Phytochemical screening of spices and vegetables which are usually used in diet, shows that they possess bioactive constituents of pharmaceutical importance. Therapeutic activity is especially important in prevention and treatment of modern diseases which are directly related to oxidative stress, such as aging, cancer etc. Nutritionally induced acute and chronic diseases such as diabetes, hyperlipidemia, liver diseases and others, can be prevented or relieved by using plants. Thereby, promoting optimal health, longevity and quality of life can all be achieved by plants, i.e. nutraceuticals present in them.

It is known that many plants have been used as food additives and in folk medicine for treating numerous diseases worldwide since the ancient times. However, only recently has there been a huge number of pharmacological and clinical studies about the positive effects that plants have on the human health. These studies have awakened people's interest in the plant's usage. Plants from Apiaceae family are used in almost all national cuisines, both as a vegetable (carrot, celery, parsley, parsnip, fennel) or as spice or condiment (dill, cumin, anise, coriander, lovage, angelica). Due to the complex chemical composition, they show significant therapeutic activity, while their aromatic properties enable their wide application in

everyday nutrition. All economic studies imply that nutraceuticals will play an important role in the future development of food with therapeutic properties.

References

1. Tunçtürk M, Özgökçe F (2015) Chemical composition of some Apiaceae plants commonly used in herby cheese in eastern Anatolia. Turk J Agric For 39:55–62
2. Aćimović M, Milić N (2017) Perspectives of the Apiaceae hepatoprotective effects – a review. Nat Prod Commun 12:309–317
3. Huang WY, Cai YZ, Zhang Y (2010) Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutr Cancer 62:1–20
4. Siró I, Kápolna E, Kápolna B, Lugasi A (2008) Functional food. Product development, marketing and consumer acceptance – a review. Appetite 51:456–467
5. Jain N, Ramawat K (2013) Nutraceuticals and antioxidants in prevention of diseases. In: Ramawat KG, Mérillon JM (eds) Natural products, Phytochemistry, botany and metabolism of alkaloids, Phenolics and terpenes. Springer, Berlin/Heidelberg
6. Wang J, Guleria S, Koffas M, Yan Y (2016) Microbial production of value-added nutraceuticals. Curr Opin Biotechnol 37:97–104
7. Cornelli U (2009) Antioxidant use in nutraceuticals. Clin Dermatol 27:175–194
8. Tapas AR, Sakarkar DM, Kakde RB (2008) Flavonoids as nutraceuticals: a review. Trop J Pharm Res 7:1089–1099
9. Dillard C, German B (2000) Review phytochemicals: nutraceuticals and human health. J Sci Food Agric 80:1744–1756
10. Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Med Cell Longev 2:270–278
11. Ghanem M, Radwan H, Mahdy ES, Elkholy Y, Hassanein H, Shahat A (2012) Phenolic compounds from *Foeniculum vulgare* (Subsp. *piperitum*) (Apiaceae) herb and evaluation of hepatoprotective antioxidant activity. Pharm Res 4:104–108
12. Martins N, Barros L, Santos-Buelgac C, Ferreira I (2016) Antioxidant potential of two Apiaceae plant extracts: a comparative study focused on the phenolic composition. Ind Crop Prod 79:188–194
13. Ereifej KI, Feng H, Rababah TM, Tashtoush SH, Al-U'datt MH, Gammoh S, Al-Rabadi GJ (2016) Effect of extractant and temperature on phenolic compounds and antioxidant activity of selected spices. Food Nutr Sci 7:362–370
14. Saleem F, Sarkar D, Ankolekar C, Shetty K (2017) Phenolic bioactives and associated antioxidant and anti-hyperglycemic functions of select species of Apiaceae Family targeting for type 2 diabetes relevant nutraceuticals. Ind Crop Prod. https://doi.org/10.1016/j.indcrop.2017.06.023
15. Bystrická J, Kavalcová P, Musilová J, Vollmannová A, Tóth T, Lenková M (2015) Carrot (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) as source of antioxidants. Acta Agri Slovenica 105:303–311
16. Nagy M, Tofană M, Socaci SA, Pop AV, Bors MD, Farcas A, Moldovan O (2014) Total phenolic, flavonoids and antioxidant capacity of some medicinal and aromatic plants. Bull UASVM Food Sci Technol 71:209–210
17. Świeca M, Gawlik-Dziki U (2008) Influence of thermal processing on phenolics compounds level and antiradical activity of dill (*Anethum graveolens* L.) Herba Polonica 54:59–69
18. Isbilir SS, Sagiroglu A (2011) Antioxidant potential of different dill (*Anethum graveolens* L.) leaf extracts. Int J Food Prop 14:894–902
19. Molnar M, Jerković I, Suknović D, Bilić-Rajs B, Aladić K, Šubarić D, Jokić S (2017) Screening of six medicinal plant extracts obtained by two conventional methods and supercritical CO2 extraction targeted on coumarin content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity and total phenols content. Molecules 22:348. https://doi.org/10.3390/molecules22030348

20. Harmala P, Vuorela H (1990) Optimization of the high-performance liquid chromatography of coumarins in *Angelica archangelica* with reference to molecular structure. J Chromatogr 507:367–380
21. Kumar D, Bhat ZA, Kumar V, Shah MY (2013) Coumarins from *Angelica archangelica* Linn. And their effects on anxiety-like behavior. Prog Neuro-Psychopharmacol Biol Psychiatry 40:180–186
22. Dellal A, Benali FT, Hamel A, Dif MM, Bouazza S, Douaoui A, Rahmani H (2016) Optimization of the extraction conditions of phenolic compounds from (*Apium graveolens*) seeds by response surface methodology. Adv Environ Biol 10:155–163
23. Yao Y, Sang W, Zhou M, Ren G (2010) Phenolic composition and antioxidant activities of 11 celery cultivars. J Food Sci 75:C9–13
24. Vallverdú-Queralt A, Regueiro J, Alvarenga JFR, Martinez-Huelamo M, Leal LN, Lamuela-Raventos RM (2015) Characterization of the phenolic and antioxidant profiles of selected culinary herbs and spices: caraway, turmeric, dill, marjoram and nutmeg. Food Sci Technol Campinas 35:189–195
25. Kunzemann J, Herrmann K (1977) Isolation and identification of flavon(ol)-O-glycosides in caraway (*Carum carvi* L.), fennel (*Foeniculum vulgare* mill.), anise (*Pimpinella anisum* L.), and coriander (*Coriandrum sativum* L.), and of flavon-C-glycosides in anise. I. Phenolics of spices. Z Lebensm Unters Forsch 164:194–200
26. Al-Juhaimi F, Ghafoor K (2011) Total phenols and antioxidant activities of leaf and stem extracts from coriander, mint and parsley grown in Saudi Arabia. Pak J Bot 43:2235–2237
27. Nambiar VS, Daniel M, Guin P (2010) Characterization of polyphenols from coriander leaves (*Coriandrum sativum*), red amaranthus (*A. paniculatus*) and green amaranthus (*A. frumentaceus*) using paper chromatography: and their health implications. J Herb Med Toxicol 4:173–177
28. Barros L, Dueñas M, Dias MI, Sousa MJ, Santos-Buelga C, ICFR F (2012) Phenolic profiles of *in vivo* and *in vitro* grown *Coriandrum sativum* L. Food Chem 132:841–848
29. Farah H, Elbadrawy E, Al-Atoom AA (2015) Evaluation of antioxidant and antimicrobial activities of ethanolic extracts of parsley (*Petroselinum crispum*) and coriander (*Coriandrum sativum*) plants grown in Saudi Arabia. Int J Adv Res 3:1244–1255
30. Rajeshwari U, Andallu B (2011) Isolation and simultaneous detection of flavonoids in the methanolic and ethanolic extracts of *Coriandrum sativum* L. seeds by RP-HPLC. Pak J Food Sci 21:13–21
31. Bettaieb-Rebey I, Bourgou S, Debez IBS, Jabri-Karoui I, Sellami IH, Msaada K, Limam F, Marzouk B (2012) Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Food Bioprocess Technol 5:2827–2836
32. Bettaieb I, Bourgou S, Wannes WA, Hamrouni I, Limam F, Marzouk B (2010) Essential oils, phenolics, and antioxidant activities of different parts of cumin (*Cuminum cyminum* L.) J Agric Food Chem 58:10410–10418
33. Leja M, Kamińska I, Kramer M, Maksylewicz-Kaul A, Kammerer D, Carle R, Baranski R (2013) The content of phenolic compounds and radical scavenging activity varies with carrot origin and root color. Plant Foods Hum Nutr 68:163–170
34. Faisal NA, Chatha SAS, Hussain AI, Ikram M, Bukhari SA (2016) Liaison of phenolic acids and biological activity of escalating cultivars of *Daucus carota*. Int J Food Prop. https://doi.org/10.1080/10942912.2016.1252390
35. Dua A, Garg G, Mahajan R (2013) Polyphenols, flavonoids and antimicrobial properties of methanolic extract of fennel (*Foeniculum vulgare* miller). Eur J Exp Biol 3:203–208
36. Tomsone L, Kruma Z, Talou T, Zhao TM (2015) Natural antioxidants of horseradish and lovage extracted by accelerated solvent extraction. J Hyg Eng Desig 10:16–24
37. Kaushik P, Andújar I, Vilanova S, Plazas M, Gramazio P, Herraiz FJ, Brar NS, Prohens J (2015) Breeding vegetables with increased content in bioactive phenolic acids. Molecules 20:18464–18481
38. Ekiert H, Gomółka E (2000) Furanocoumarins in Pastinaca Sativa L. in vitro culture. Pharmazie 55:618–620

39. Trifunschi S, Ardelean D (2012) Quantification of phenolics and flavonoids from *Petroselinum crispum* extracts. J Med Ar 15:83–86
40. Chaves D, Frattani F, Assafim M, de Almeida AP, Zingali R, Costa S (2011) Phenolic chemical composition of *Petroselinum crispum* extract and its effect on haemostasis. Nat Prod Comm 6:961–964
41. Christova-Bagdassarian VL, Bagdassarian KS, Atanassova MS (2013) Phenolic compounds and antioxidant capacity in Bulgarian plans (dry seeds). Int J Adv Res 1:186–197
42. Garrod B, Lewis BG, Coxon DT (1978) Cis-heptadeca-1,9-diene-4,6-diyne-3,8-diol, an antifungal polyacetylene from carrot root tissue. Physiol Plant Pathol 13:241–246
43. Schinkovitz A, Stavri M, Gibbons S, Bucar F (2008) Antimycobacterial polyacetylenes from *Levisticum officinale*. Phytother Res 22:681–684
44. Metzger B, Waksmonski J, Thompson A, Barnes D (2013) Supercritical fluid extraction (SFE) of anti-inflammatory polyacetylenes from celeriac (*Apium graveolens* L.) FASEB J 27:1079.34
45. Dembitsky V, Levitsky D (2006) Acetylenic terrestrial anticancer agents. Nat Prod Commun 1:405–429
46. Zaini RG, Brandt K, Clench MR, Le Maitre CL (2012) Effects of bioactive compounds from carrots (*Daucus carota* L.), polyacetylenes, beta-carotene and lutein on human lymphoid leukaemia cells. Anti Cancer Agents Med Chem 12:640–652
47. El-Houri RB, Kotowska D, Christensen KB, Bhattacharya S, Oksbjerg N, Wolber G, Kristiansen K, Christensen LP (2015) Polyacetylenes from carrots (*Daucus carota*) improve glucose uptake in vitro in adipocytes and myotubes. Food Funct 6:2135–2144
48. Choi YE, Ahn H, Ryu JH (2000) Polyacetylenes from *Angelica gigas* and their inhibitory activity on nitric oxide synthesis in activated macrophages. Biol Pharm Bull 23:884–886
49. Zidorn C, Jöhrer K, Ganzera M, Schubert B, Sigmund EM, Mader J, Greil R, Ellmerer EP, Stuppner H (2005) Polyacetylenes from the Apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. J Agric Food Chem 53:2518–2523
50. Christensen LP (2011) Aliphatic C(17)-polyacetylenes of the falcarinol type as potential health promoting compounds in food plants of the Apiaceae Family. Recent Pat Food Nutr Agric 3:64–77
51. Chen Y, Peng S, Luo Q, Zhang J, Guo Q, Zhang Y, Chai X (2015) Chemical and pharmacological progress on polyacetylenes isolated from the family Apiaceae. Chem Biodivers 12:474–502
52. Christensen LP, Brandt K (2006) Bioactive polyacetylenes in food plants of the Apiaceae Family: occurrence, bioactivity and analysis. J Pharm Biomed Anal 41:683–693
53. Kreutzmann S, Christensen L, Edelenbos M (2008) Investigation of bitterness in carrots (*Daucus carota* L.) based on quantitative chemical and sensory analyses. LWT 41:193–205
54. Schulz-Witte J, Nothnagel T, Schulz H (2010) Comparison of different clean-up methods for simultaneous HPLC determination of carotenoids and polyacetylenes in carrot root. J Appl Bot Food Qual 83:123–127
55. Roman M, Baranski R, Baranska M (2011) Nondestructive Raman analysis of polyacetylenes in Apiaceae vegetables. J Agric Food Chem 59:7647–7653
56. Kramer M, Bufler G, Nothnagel T, Carle R, Kammerer DR (2012) Effects of cultivation conditions and cold storage on the polyacetylene contents of carrot (*Daucus carota* L.) and parsnip (*Pastinaca sativa* L.) J Hortic Sci Biotechnol 87:101–106
57. Rawson A, Hossain M, Patras A, Tuohy M, Brunton N (2013) Effect of boiling and roasting on the polyacetylene and polyphenol content of fennel (*Foeniculum vulgare*) bulb. Food Res Int 50:513–518
58. Petrache P, Rodino S, Butu M, Pribac G, Pentea M, Butnariu M (2014) Polyacetylene and carotenes from *Petroselinum sativum* root. Dig J Nanomater Biostruct 9:1523–1527
59. Dawid C, Dunemann F, Schwab W, Nothnagel T, Hofmann T (2015) Bioactive C17-polyacetylenes in carrots (*Daucus carota* L.): current knowledge and future perspectives. J Agric Food Chem 63:9211–9222

60. Nakano Y, Matsunaga H, Saita T, Mori M, Katano M, Okabe H (1998) Antiproliferative constituents in Umbelliferae plants II. Screening for polyacetylenes in some Umbelliferae plants, and isolation of panaxynol and falcarindiol from the root of *Heracleum moellendorffii*. Biol Pharm Bull 21:257–261
61. Min BS (2006) Coumarins and a polyacetylene from the roots of *Angelica purpuraefolia*. Nat Prod Sci 12:129–133
62. Matsuda H, Kageura T, Ninomiya K, Toguchida I, Nishida N, Yoshikawa M (1998) Hepatoprotective and nitric oxide production inhibitory activities of coumarin and polyacetylene constituents from the roots of *Angelica furcijuga*. Bioorg Med Chem Lett 8:2191–2196
63. Nurcahyanti A, Nasser I, Sporer F, Graf J, Bermawie N, Reichling J, Wink M (2016) Chemical composition of the essential oil from aerial parts of Javanian *Pimpinella pruatjan* Molk. And its molecular phylogeny. Diversity 8:15. https://doi.org/10.3390/d8030015
64. El Sohaimy SA (2012) Functional foods and nutraceuticals-modern approach to food science. World Appl Sci J 20:691–708
65. Aćimović M, Korać J, Jaćimović G, Oljača S, Đukanović L, Vuga-Janjatov V (2014a) Influence of ecological conditions on seeds traits and essential oil contents in anise (*Pimpinella anisum* L.) Not Bot Horti Agrobot Cluj Napoca 42:232–238
66. Aćimović M, Oljača S, Tešević V, Todosijević M, Đisalov J (2014b) Evaluation of caraway essential oil from different production areas of Serbia. Hort Sci (Prague) 41:122–130
67. Aćimović M, Stanković J, Cvetković M, Ignjatov M, Lj N (2016b) Chemical characterization of essential oil from seeds of wild and cultivated carrots from Serbia. Bot Serb 40:55–60
68. Tongnuanchan P, Benjakul S (2014) Essential oils: extraction, bioactivities, and their uses for food preservation. J Food Sci 79:1231–1249
69. Aćimović M, Lj K, Popović S, Dojčinović N (2015a) Apiaceae seeds as functional food. J Agric Sci (Belgrade) 60:237–246
70. Saleh MA, Clark S, Woodard B, Deolu-Sobogun SA (2010) Antioxidant and free radical scavenging activities of essential oils. Ethn Dis 20:78–82
71. Kosalec I, Pepeljnjak S, Kuštrak D (2005) Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., Apiaceae). Acta Pharma 55:377–385
72. Fraternale D, Flamini G, Ricci D (2014) Essential oil composition and antimicrobial activity of *Angelica archangelica* L. (Apiaceae) roots. J Med Food 17:1043–1047
73. Bailer J, Aichinger T, Hackl G, de Hueber K, Dachler M (2001) Essential oil content and composition in commercially available dill cultivars in comparison to caraway. Ind Crop Prod 14:229–239
74. Rana VS, Blazquez AM (2014) Chemical composition of the essential oil of *Anethum graveolens* aerial parts. J Essent Oil Bear Pl 17:1219–1223
75. Aćimović M, Pavlović S, Varga A, Filipović V, Cvetković M, Stanković J, Čabarkapa I (2017a) Chemical composition and antibacterial activity of *Angelica archangelica* root essential oil. Nat Prod Commun 12:205–206
76. Aćimović M, Cvetković M, Stanković J, Filipović V, Nikolić L, Dojčinović N (2017b) Analysis of volatile compounds from Angelica seeds obtained by headspace method. AJMAP 3:10–17
77. Sowbhagya HB (2014) Chemistry, technology, and nutraceutical functions of celery (*Apium graveolens* L.): an overview. Crit Rev Food Sci Nutr 54:389–398
78. Meshkatalsadat MH, Salahvarzi S, Aminiradpoor R, Abdollahi A (2012) Identification of essential oil constituents of caraway (*Carum carvi*) using ultrasonic assist with headspace solid phase microextraction (UA-HS-SPME). Dig J Nanomater Biostruct 7:637–640
79. Seidler-Łożykowska K, Kędzia B, Karpińska E, Bocianowski J (2013) Microbiological activity of caraway (*Carum carvi* L.) essential oil obtained from different origin. Acta Sci Agron 35:495–500

80. Aćimović M, Oljača S, Jaćimović G, Dražić S, Tasić S (2011) Benefits of environmental conditions for growing coriander in Banat region, Serbia. Nat Prod Commun 6:1465–1468
81. Aćimović M, Stanković J, Cvetković M (2016) Effect of weather conditions, location and fertilization on coriander fruit essential oil quality. J Essent Oil Bear Plant 19:1208–1215
82. Aćimović M, Grahovac M, Stanković J, Cvetković M, Maširević S (2016) Essential oil composition of different coriander (*Coriandrum sativum* L.) accessions and their influence on mycelial growth of *Colletotrichum* ssp. Acta Scientiarum Polonorum Hortorum Cultus 15:35–44
83. Mandal S, Mandal M (2015) Coriander (*Coriandrum sativum* L.) essential oil: chemistry and biological activity. Asian Pac J Trop Biomed 5:421–428
84. Aliniana S, Razmjooa J, Zeinali H (2016) Flavonoids, anthocyanins, phenolics and essential oil produced in cumin (*Cuminum cyminum* L.) accessions under different irrigation regimes. Ind Crop Prod 81:49–55
85. Ma M, Mu T, Sun H, Zhang M, Chen J, Yan Z (2015) Optimization of extraction efficiency by shear emulsifying assisted enzymatic hydrolysis and functional properties of dietary fiber from deoiled cumin (*Cuminum cyminum* L.) Food Chem 179:270–277
86. Topal U, Sasaki M, Goto M, Otles S (2008) Chemical compositions and antioxidant properties of essential oils from nine species of Turkish plants obtained by supercritical carbon dioxide extraction and steam distillation. Int J Food Sci Nutr 59:619–634
87. Wanner J, Bail S, Jirovetz L, Buchbauer G, Schmidt E, Gochev V, Girova T, Atanasova T, Stoyanova A (2010) Chemical composition and antimicrobial activity of cumin oil (*Cuminum cyminum*, Apiaceae). Nat Prod Commun 5:1355–1358
88. Zatla AT, Dib MEA, Djabou N, Tabti B, Meliani N, Costa J, Muselli A (2017) Chemical variability of essential oil of *Daucus carota* subsp. *sativus* from Algeria. J Herbs Spices Med Plant 23:216–230
89. Aćimović M, Tešević V, Todosijević M, Đisalov J, Oljača S (2015c) Compositional characteristics of the essential oil of *Pimpinella anisum* and *Foeniculum vulgare* grown in Serbia. Bot Serb 39:9–14
90. Aćimović M, Lj K, Stanković J, Cvetković M, Filipović V (2015) Essential oil composition from sweet and bitter fennel fruits from Serbia. Med Raw Mat 35:121–129
91. Aćimović M, Cvetković M, Stanković J, Malenčić Đ, Kostadinović L (2015b) Compound analysis of essential oils from lovage and celery fruits obtained by headspace extraction. Ann Agron (Novi Sad) 39:44–51
92. Nikolić M, Markovic T, Ćirić A, Glamočlija J, Marković D, Soković M (2015) Susceptibility of oral *Candida* spp. reference strains and clinical isolates to selected essential oils of Apiaceae species. Med Raw Mat 35:151–162
93. Matejić J, Džamić A, Mihajilov-Krstev T, Ranđelović V, Krivošej Z, Marin P (2014) Antimicrobial potential of essential oil from *Pastinaca sativa* L. Biologica Nyssana 5:31–35
94. Linde GA, Gazim ZC, Cardoso BK, Jorge LF, Tešević V, Glamoćlija J, Soković M, Colauto NB (2016) Antifungal and antibacterial activities of *Petroselinum crispum* essential oil. Genet Mol Res 15(3). https://doi.org/10.4238/gmr.15038538
95. Borges IB, Cardoso BK, Silva ES, de Oliveira JS, da Silva RF, de Rezende CM, Gonçalves JE, Junior RP, de Souza SGH, Gazim ZC (2016) Evaluation of performance and chemical composition of Petroselinum Crispum essential oil under different conditions of water deficit. Afr J Agric Res 11:480–486
96. Gende LB, Maggi MD, Fritz R, Eguaras MJ, Bailac PN, Ponzi MI (2009) Antimicrobial activity of *Pimpinella anisum* and *Foeniculum vulgare* essential oils against *Paenibacillus larvae*. J Essent Oil Res 21:91–93
97. Jana S, Shekhawat GS (2010) *Anethum graveolens*: an Indian traditional medicinal herb and spice. Pharmacogn Rev 4:179–184
98. Aćimović M, Milić N (2015) Dill in traditional medicine and modern phytotherapy. Med Raw Mat 35:23–35

99. El Mansouri L, Bousta D, Balouiri M, Ouedrhiri W, Elyoubi-El HA (2015) Antioxidant activity of aqueous seed extract of *Anethum graveolens* L. Int J Pharm Sci Res 7:1219–1223
100. Mansouri M, Nayebi N, Keshtkar A, Hasani-Ranjbar S, Taheri E, Larijani B (2012) The effect of 12 weeks *Anethum graveolens* (dill) on metabolic markers in patients with metabolic syndrome; a randomized double blind controlled trial. Daru 20:47. https://doi.org/10.1186/2008-2231-20-47
101. Mishra N (2013) Haematological and hypoglycemic potential *Anethum graveolens* seeds extract in normal and diabetic Swiss albino mice. Vet World 6:502–507
102. Goodarzi MT, Khodadadi I, Tavilani H, Oshaghi EA (2016) The role of *Anethum graveolens* L. (dill) in the management of diabetes. J Trop Med 2016:1098916. https://doi.org/10.1155/2016/1098916
103. Mobasseri M, Payahoo L, Ostadrahimi A, Bishak YK, Jafarabadi MA, Mahluji S (2014) *Anethum graveolens* supplementation improves insulin sensitivity and lipid abnormality in type 2 diabetic patients. Pharm sci 20:40–45
104. Yazdanparast R, Bahramikia S (2008) Evaluation of the effect of *Anethum graveolens* L. crude extracts on serum lipids and lipoproteins profiles in hypercholesterolaemic rats. Daru 16:88–94
105. Bano F, Ikram H, Akhtar N (2013) Aqueous extract of *Anethum graveolens* L. seeds decrease LDL-C:HDL-C ratio in over weight rats. Pak J Biochem Mol Biol 46:26–29
106. Mirhosseini M, Baradaran A, Rafieian-Kopaei M (2014) *Anethum graveolens* and hyperlipidemia: a randomized clinical trial. J Res Med Sci 19:758–761
107. Yazdanparast R, Alavi M (2001) Antihyperlipidaemic and antihypercholesterolaemic effects of *Anethum graveolens* leaves after removal of furocoumarins. Cytobios 105:185–191
108. Tamilarasi R, Sivanesan D, Kanimozhi P (2012) Hepatoprotective and antioxidant efficacy of *Anethum graveolens* Linn in carbon tetrachloride induced hepatotoxicity in albino rats. J Chem Pharm Res 4:1885–1888
109. Thuppia A, Jitvaropas R, Saenthaweesuk S, Somparn N, Kaulpiboon J (2011) Hepatoprotective effect of the ethanolic extract of *Anethum graveolens* L. on paracetamol-induced hepatic damage in rats. Planta Med 77:PF18
110. Ali WSH (2013) Hypolipidemic and antioxidant activities of *Anethum graveolens* against acetaminophen induced liver damage in rats. WJMS 8:387–392
111. Rabeh NM, Aboraya AO (2014) Hepatoprotective effect of dill (*Anethum graveolens* L.) and fennel (*Foeniculum vulgare*) oil on hepatotoxic rats. PJN 13:303–309
112. Oshaghi EA, Khodadadi I, Tavilani H, Goodarzi MT (2016) Effect of dill tablet (*Anethum graveolens* L) on antioxidant status and biochemical factors on carbon tetrachloride-induced liver damage on rat. Int J App Basic Med Res 6:111–114
113. Peerakam N, Wattanathorn J, Punjaisee S, Buamongkol S, Sirisa P, Chansakaow S (2014) Chemical profiling of essential oil composition and biological evaluation of *Anethum graveolens* L. (seed) grown in Thailand. J Nat Sci Res 4:34–41
114. Kaur GJ, Arora DS (2009) Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. BMC Complement Altern Med 9:30. https://doi.org/10.1186/1472-6882-9-30
115. Chen Y, Zeng H, Tian J, Ban X, Ma B, Wang Y (2013) Antifungal mechanism of essential oil from *Anethum graveolens* seeds against *Candida albicans*. J Med Microbiol 62:1175–1183
116. Dahiya P, Purkayastha S (2012) Phytochemical analysis and antibacterial efficacy of dill seed oil against multi-drug resistant clinical isolates. Asian J Pharm Clin Res 5:62–64
117. Sava-Sand C, Antofie MM (2016) New improvements in plant quality of *Angelica archangelica* l. as a crop species of food and pharmaceutical interest. Sci Paper Series Manag Eco Eng Agri Rural Develop 16:477–480
118. Bhat ZA, Kumar D, Shah MY (2011) *Angelica archangelica* Linn. Is an angel on earth for the treatment of diseases. Int J Nutri Pharmacol Neurol Diseases 1:36–50

119. Elgohary AA, Shafaa MW, Raafat BM, Rizk RA, Metwally FG, Saleh AM (2009) Prophylactic effect of *Angelica archangelica* against acute lead toxicity in albino rabbits. Romanian. J Biophys 19:259–275
120. Wojcikowski K, Stevenson L, Leach D, Wohlmuth H, Gobe G (2007) Antioxidant capacity of 55 medicinal herbs traditionally used to treat the urinary system: a comparison using a sequential three-solvent extraction process. J Alternat Complement Med 13:103–109
121. Sezer-Senol F, Skalicka-Woźniak K, Khan MTH, Orhan IE, Głowniak K (2011) An *in vitro* and *in silico* approach to cholinesterase inhibitory and antioxidant effects of the methanol extract, furanocoumarin fraction, and major coumarins of *Angelica officinalis* L. fruits. Phytochem Lett 4:462–467
122. Nemeth S, Paşca B, Teodorescu A, Coita I, Teaha D (2015) Coumarins isolated from the dry roots of *Angelica archangelica* L. and their antibacterial activity. Analele Universităţii din Oradea, Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimntară B 14:355–362
123. Rather RA, Rehman SU, Naseer S, Lone SH, Bhat KA, Chouhan A (2013) Flash chromatography guided fractionation and antibacterial activity studies of *Angelica archangelica* root extracts. IOSR-JAC 4:34–38
124. Prakash B, Singh P, Goni R, Raina AK, Dubey NK (2015) Efficacy of *Angelica archangelica* essential oil, phenyl ethyl alcohol and α- terpineol against isolated molds from walnut and their antiaflatoxigenic and antioxidant activity. J Food Sci Technol 52:2220–2228
125. Fraternale D, Flamini G, Ricci D (2016) Essential oil composition of *Angelica archangelica* L. (Apiaceae) roots and its antifungal activity against plant pathogenic fungi. Plant Biosyst 150:558–563
126. Raafat BM, Zahrany SM, Al-Zahrani AS, Tawifiek E, Al-Omery AM (2012) Angelica Archangelica roots water extraction as a natural antioxidant tolerating ROS production in lead poisoning. RJPBCS 3:795–806
127. Yeh ML, Liu CF, Huang CL, Huang TC (2003) Hepatoprotective effect of *Angelica archangelica* in chronically ethanol-treated mice. Pharmacology 68:70–73
128. Sigurdsson S, Ogmundsdottir HM, Gudbjarnason S (2005) The cytotoxic effect of two chemotypes of essential oils from the fruits of *Angelica archangelica* L. Anticancer Res 25:1877–1880
129. Kumar D, Ali Bhat Z (2012) Anti-anxiety activity of methanolic extracts of different parts of *Angelica archangelica* Linn. J Tradit Complement Med 2:235–241
130. Pathak S, Wanjari MM, Jain SK, Tripathi M (2010) Evaluation of antiseizure activity of essential oil from roots of *Angelica archangelica* Linn. In mice. Indian J Pharm Sci 72:371–375
131. Asif HM, Akram M, Usmanghani K, Akhtar N, Shah PA, Uzair M, Ramzan M, Ali Shah SM, Rehman R (2011) Monograph of *Apium graveolens* Linn. JMPR 5:1494–1496
132. Fazal SS, Singla RK (2012) Review on the pharmacognostical and pharmacological characterization of Apium Graveolens Linn. IGJPS 2:36–42
133. Kooti W, Daraei N (2017) A review of the antioxidant activity of celery (*Apium graveolens* L.). J Evid Based Complementary Altern Med. https://doi.org/10.1177/2156587217717415
134. Sameh B, Ibtissem B, Mahmoud A, Boukef K, Boughattas NA (2011) Antioxidant activity of *Apium graveolens* extracts. JBAPN 1:340–343
135. Kooti W, Ali-Akbari S, Asadi-Samani M, Ghadery H, Ashtary-Larky D (2014) A review on medicinal plant of *Apium graveolens*. Adv Herb Med 1:48–59
136. Shanmugapriya R, Ushadevi T (2014) In vitro antibacterial and antioxidant activities of *Apium graveolens* L. seed extracts. Int J Drug Dev Res 6:165–170
137. Uddin Z, Shad AA, Bakht J, Ullah I, Jan S (2015) In vitro antimicrobial, antioxidant activity and phytochemical screening of Apium Graveolens. Pak J Pharm Sci 28:1699–1704
138. Singh A, Handa SS (1995) Hepatoprotective activity of *Apium graveolens* and *Hygrophila auriculata* against paracetamol and thioacetamide intoxication in rats. J Ethnopharmacol 49:119–126

139. Sultana S, Ahmed S, Jahangir T, Sharma S (2005) Inhibitory effect of celery seeds extract on chemically induced hepatocarcinogenesis: modulation of cell proliferation, metabolism and altered hepatic foci development. Cancer Lett 221:11–20
140. Kolarovic J, Popovic M, Mikov M, Mitic R, Gvozdenovic L (2009) Protective effects of celery juice in treatments with doxorubicin. Molecules 14:1627–1638
141. Belal NM (2011) Hepatoprotective effect of feeding celery leaves mixed with chicory leaves and barley grains to hypercholesterolemic rats. Asian J Clin Nutri 3:14–24
142. Osman N (2013) The role of antioxidant properties of celery against lead acetate induced hepatotoxicity and oxidative stress in irradiated rats. Arab J Nucl Sci Appl 46:339–346
143. Ramezani M, Nasri S, Yassa N (2009) Antinociceptive and anti-inflammatory effects of isolated fractions from *Apium graveolens* seeds in mice. Pharm Biol 49:740–743
144. Choosri N, Tanasawet S, Chonpathompikunlert P, Sukketsiri W (2017) *Apium graveolens* extract attenuates adjuvant induced arthritis by reducing oxidative stress. J Food Biochem 41 (1):12276. https://doi.org/10.1111/jfbc.12276
145. Mansi K, Abushoffa AM, Disi A, Aburjai T (2009) Hypolipidemic effects of seed extract of celery (*Apium graveolens*) in rats. Phcog Mag 5:301–305
146. Al-Saaidi JAA, Alrodhan MNA, Ismael AK (2012) Antioxidant activity of n-butanol extract of celery (Apium Graveolens) seed in streptozotocin-induced diabetic male rats. Res Pharmaceut Biotechnol 4:24–29
147. Branković S, Gočmanac-Ignjatović M, Kostić M, Veljković M, Miladinović B, Milutinović M, Radenković M (2015) Spasmolytic activity of the aqueous and ethanol celery leaves (*Apium graveolens* l.) extracts on the contraction of isolated rat ileum. Acta Medica Medianae 54:11–16
148. Moghadam MH, Imenshahidi M, Mohajeri SA (2013) Antihypertensive effect of celery seed on rat blood pressure in chronic administration. J Med Food 16:558–563
149. Al Jawad FH, Al Razzuqi RAM, Al Jeboori AA (2011) Apium Graveolens accentuates urinary ca+2 excretions in experimental model of nephrocalcinosis. Int J Green Pharm 5:100–102
150. Aćimović M, Dolijanović Ž, Oljača S, Kovačević D, Oljača M (2015) Effect of organic and mineral fertilizers on essential oil content in caraway, anise and coriander fruits. Acta Scientiarum Polonorum Hortorum Cultus 14(1):95–103
151. Najda A, Dyduch J, Brzozowski N (2008) Flavonoid content and antioxidant activity of caraway roots (*Carum carvi* L.) Veget Crops Res Bull 68:127–133
152. Foti MC, Ingold KU (2003) Mechanism of inhibition of lipid peroxidation by γ-terpinene, an unusual and potentially useful hydrocarbon antioxidant. J Agric Food Chem 51: 2758–2765
153. Damašius J, Škėmaitė M, Kirkilaitė G, Vinauskienė R, Venskutonis PR (2007) Antioxidant and antimicrobial properties of caraway (*Carum carvi* L.) and cumin (*Cuminum cyminum* L.) extracts. Vet Med Zoot 40:9–13
154. Samojlik I, Lakić N, Mimica-Dukić N, Đaković-Švajcer K, Božin B (2010) Antioxidant nad hepatoprotective potential of essential oils of coriander (*Coriandrum sativum* L.) and caraway (*Carum carvi* L.) (Apiaceae). J Agric Food Chem 58:8848–8853
155. Johri KR (2011) *Cuminum cyminum* and *Carum carvi*: an update. Pharmacogn Rev 5:63–72
156. Hawerelak JA, Cattley T, Myers SP (2009) Essential oils in the treatment of intestinal dysbiosis: a preliminary *in vitro* study. Altern Med Rev 14:380–384
157. Keshavarz A, Minaiyan M, Ghannadi A, Mahzouni P (2013) Effects of *Carum carvi* L. (caraway) extract and essential oil on TNBS-induced colitis in rats. Research in pharmaceutical. Science 8:1–8
158. Khayyal MT, Seif-El-Nasr M, El-Ghazaly MA, Okpanyi SN, Kelber O, Weiser D (2006) Mechanisms involved in the gastro-protective effect of STW5 (Iberogast®) and its components against ulcers and rebound acidity. Phytomedicine 13:56–66
159. Al-Essa MK, Shafagoj YA, Mohammed FI, Afifi FU (2010) Relaxant effect of ethanol extr act of *Carum carvi* on dispersed intestinal smooth muscle cells of the guinea pig. Pharm Biol 48:76–80

160. Sadeghian S, Neyestani T, Shirazi MH, Ranjabarian P (2005) Bacteriostatic effect of dill, fennel, caraway and cinnamon extracts against *Helicobacter pylori*. J Nutr Environ Med 15:47–55
161. Villarini M, Fatigoni C, Cerbone B, Dominici L, Moretti M, Pagiotti R (2011) In vitro testing of a laxative herbal food supplement for genotoxic and antigentoxic properties. J Med Plants Res 5:2533–2539
162. Yosefi SS, Sadeghpour O, Sohrabvand F, Atarod Z, Askarfarashah M, Ateni TR, Yekta NH (2014) Effectiveness of *Carum carvi* on early return of bowel motility after caesarean section. Eur. J Exp Biol 4:258–262
163. Haidari F, Sayed-Sadjadi N, Taha-Jalali M, Mohammed-Shahi M (2011) The effect of oral administration of *Carum carvi* on weight, serum glucose, and lipid profile in streptozotocin-induced diabetic rats. Saudi Med J 32:695–700
164. Lemhadri A, Hajji L, Michel JB, Eddouks M (2006) Cholesterol and triglycerides lowering activities of caraway fruits in normal and streptozotocin diabetic rats. J Ethnopharmacol 106:321–326
165. Saghir MR, Sadiq S, Nayak S, Tahir MU (2012) Hypolipidemic effect of aqueous extract of *Carum carvi* (black zeera) seeds in diet induced hyperlipidemic rats. Pak J Pharm Sci 25:333–337
166. Eidi A, Eidi M, Rohani HA, Basati F (2010) Hypoglycemic effect of ethanolic extract of *Carum carvi* L. seeds in normal and streptozotocin-induced diabetic rats. J Med Plants 9:106–113
167. Moubarz G, Taha MM, Mahdy-Abdallah H (2014) Antioxidant effect of *Carum carvi* on the immune status of streptozotocin-induced diabetic rats infested with *Staphylococcus aureus*. World Appl Sci J 30:63–69
168. Iacobellis NS, Cantore PL, Capasso F, Senatore F (2005) Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J Agric Food Chem 53:57–61
169. Simic A, Rančić A, Sokolović MD, Ristić M, Grujić-Jovanović S, Vukojevic J, Marin PD (2008) Essential oil composition of *Cymbopogon winterianus* and *Carum carvi* and their antimicrobial activities. Pharm Biol 46:437–441
170. Škrinjar M, Mandić A, Mišan A, Sakač M, Lj Š, Zec M (2009) Effect of mint (*Mentha piprita* L.) and caraway (*Carum carvi* L.) on growth of some toxigenic *Aspergillus* species and aflatoxin B1 production. J Nat Sci Matica Srpska Novi Sad 116:131–139
171. Koppula S, Kopalli SR, Sreemantula S (2009) Adaptogenic and nootropic activities of aqueous extracts of *Carum carvi* Linn (caraway) fruit: an experimental study in Wistar rats. Aus J Med Herbal 21:72–78
172. Lahlou S, Tahraoui A, Israili Z, Lyoussi B (2007) Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. J Ethnopharmacol 110:458–463
173. Sadiq S, Nagi AH, Shahzad M, Zia A (2010) The reno-protective effect of aqueous extract of *Carum carvi* (black zeera) seeds in streptozotocin induced diabetic nephropathy in rodents. Saudi J Kidney Dis Transpl 21:1058–1065
174. El-Soud NH, El-Lithy NA, El-Saeed G, Wahby MS, Khalil MY, Morsy F, Shaffie N (2014) Renoprotective effects of caraway (*Carum carvi* L.) essential oil in streptozotocin induced diabetic rats. J Appl Pharmaceut Sci 4:27–33
175. Marangoni C, de Moura NF (2011) Antioxidant activity of essential oil from *Coriandrum sativum* L. in Italian salami. Ciênc Tecnol Aliment 31:124–128
176. Darughe F, Barzegar M, Sahari MA (2012) Antioxidant and antifungal activity of coriander (*Coriandrum sativum* L.) essential oil in cake. Int Food Res J 19:1253–1260
177. Matasyoh JC, Maiyo ZC, Ngure RM, Chepkorir R (2009) Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. Food Chem 113: 526–529
178. Silva F, Ferreira S, Queiroz JA, Domingues FC (2011) Coriander (*Coriandrum sativum* L.) essential oil: its antibacterial activity and mode of action evaluated by flow cytometry. J Med Microbiol 60:1479–1486

179. Aćimović M, Oljača S, Dražić S (2012) Uses of coriander (*Coriandrum sativum* L.) Med Raw Mat 31:67–82
180. Aćimović M, Kostadinović L, Puvača N, Popović S, Urošević M (2016a) Phytochemical constituents of selected plants from Apiaceae family and their biological effects in poultry. Food Feed Res 43:35–41
181. Momin AH, Acharya SS, Gajjar AV (2012) *Coriandrum sativum* – review of advances in phytopharmacology. IJPSR 3:1233–1239
182. Emamghoreishi M, Heidari-Hamedani GH (2008) Effect of extract and essential oil of Coriandrum sativum seed against pentylenetetrazole-induced seizure. Pharm Sci 7:1–10
183. Mahendra P, Bisht S (2011) Anti-anxiety activity of Coriandrum Sativum assessed using different experimental anxiety models. Indian J Pharmacol 43:574–577
184. Pathan AR, Kothawade KA, Logade MN (2011) Anxiolytic and analgetic effect of seeds of Coriandrum Sativum Linn. International journal of research in pharmacy and. Chemistry 1:1087–1099
185. Aissaoui A, El-Hilaly J, Israili ZH, Lyoussi B (2008) Acute diuretic effect of continuous intravenous infusion of an aqueous extract of *Coriandrum sativum* L. in anesthetized rats. J Ethnopharmacol 115:89–95
186. Millet J (2005) Cilantro, chlorella, and heavy metals. Med Herbal 14:17–20
187. Rajeshwari U, Andallu B (2011) Medicinal benefits of coriander (*Coriandrum sativum* L.) Spatula DD 1:51–58
188. Lal AA, Kumar T, Murthy PB, Pillai KS (2004) Hypolipidemic effect of *Coriandrum sativum* L. in triton-induced hyperlipidemic rats. Indian J Exp Biol 42:909–912
189. Dhanapakiam P, Mini Joseph J, Ramaswamy VK, Moorthi M, Senthil Kumar A (2008) The cholesterol lowering property of coriander seeds (*Coriandrum sativum*): mechanism of action. J Environ Biol 29:53–56
190. Joshi SC, Sharma N, Sharma P (2012) Antioxidant and lipid lowering effects of *Coriandrum sativum* in cholesterol fed rabbits. Int J Pharm Pharm Sci 4:231–234
191. Yibru E, Menon MKC, Belayneh Y, Seyifu D (2015) The effect of *Coriandrum sativum* seed extract on hyperglycemia, lipid profile and renal function in streptozotocin induced type- 2 diabetic Swiss albino mice. IJHSR 5:166–177
192. Aissaoui A, Zizi S, Israili ZH, Lyoussi B (2011) Hypoglycemic and hypolipidemic effects of *Coriandrum sativum* L. in Meriones Shawi rats. J Ethnopharmacol 137:652–661
193. Kansal L, Sharma V, Sharma A, Lodi S, Sharma SH (2011) Protective role of *Coriandrum sativum* (coriander) extracts against lead nitrate induced oxidative stress and tissue damage in the liver and kidney in male mice. Int J Appl Biol Pharm 2:65–83
194. John NAA, Shobana G, Keerthana K (2014) Protective effect of *Coriander sativum* L. on cadmium induced toxicity in albino rats. World J Pharm Pharm Sci 3:525–534
195. Moustafa AH, Ali EMM, Moselhey SS, Tousson E, El-Said KS (2014) Effect of coriander on thioacetamide-induced hepatotoxicity in rats. Toxicol Ind Health 30:621–629
196. Sreelatha S, Padma PR, Umadevi M (2009) Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced hepatotoxicity in rats. Food Chem Toxicol 47:702–708
197. Ramadan MM, Algader NNEA, El-Kamali HH, Ghanem KZ, Farrag ARH (2013) Chemopreventive effect of *Coriandrum sativum* fruits on hepatic toxicity in male rats. WJMS 8:322–333
198. Pandey A, Bigoniya P, Raj V, Patel KK (2011) Pharmacological screening of *Coriandrum sativum* Linn. For hepatoprotective activity. J Pharm Bioallied Sci 3:435–441
199. Nithya TG, Sumalatha D (2014) Evaluation of invitro anti-oxidant and anticancer activity of *Coriandrum sativum* against human colon cancer HT-29 cell lines. Int J Pharm Pharm Sci 6:421–424
200. Tang EL, Rajarajeswaran J, Fung SY, Kanthimathi MS (2013) Antioxidant activity of *Coriandrum sativum* and protection against DNA damage and cancer cell migration. BMC Complement Altern Med 13:347. https://doi.org/10.1186/1472-6882-13-347
201. Gomez-Flores R, Hernández-Martínez H, Tamez-Guerra P, Tamez-Guerra R, Quintanilla-Licea R, Monreal-Cuevas E, Rodríguez-Padilla C (2010) Antitumor and immunomodulating

potential of *Coriandrum sativum*, *Piper nigrum* and *Cinnamomum zeylanicum*. J Nat Prod 3:54–63
202. Chandan HS, Tapas AR, Sakarkar DM (2011) Anthelmintic activity of extracts of Coriandrum Sativum Linn. In Indian earthworm. Int J Phytomed 3:36–40
203. Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E (2007) In vitro and in vivo anthelmintic activity of crude extracts of Coriandrum Sativum against Haemonchus contortus. J Ethnopharmacol 110:428–343
204. Mnif S, Aifa S (2015) Cumin (*Cuminum cyminum* L.) from traditional uses to potential biomedical applications. Chem Biodivers 12:733–742
205. Moghadam ARL (2016) Chemical composition and antioxidant activity *Cuminum cyminum* L. essential oils. Int J Food Prop 19:438–442
206. Rebey IB, Zakhama N, Karoui IJ, Marzouk B (2012) Polyphenol composition and antioxidant activity of cumin (*Cuminum cyminum* L.) seed extract under drought. J Food Sci 77: C734–C739
207. Abbaszadegan A, Gholami A, Ghahramani Y, Ghareghan R, Ghareghan M, Kazemi A, Iraji A, Ghasemi Y (2016) Antimicrobial and cytotoxic activity of *Cuminum cyminum* as an intracanal medicament compared to chlorhexidine gel. Iran Endod J 11:44–50
208. Saee Y, Dadashi M, Eslami G, Goudarzi H, Taheri S, Fallah F (2016) Evaluation of antimicrobial activity of *Cuminum cyminum* essential oil and extract against bacterial strains isolated from patients with symptomatic urinary tract infection. NBM 4:147–152
209. Koppula S, Choi DK (2011) Cuminum Cyminum extract attenuates scopolamine-induced memory loss and stress-induced urinary biochemical changes in rats: a noninvasive biochemical approach. Pharm Biol 49:702–708
210. Dhandapani S, Subramanian VR, Rajagopal S, Namasivayam N (2002) Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. Pharmacol Res 46:251–255
211. Srivastava V, Dubey S, Sharma SB, Chaddha V (2013) Studies on hypolipidemic activity of seeds of *Cuminum cyminum* Linn. Indo am. J Pharm Res 3:8260–8265
212. Willatgamuwa SA, Platel K, Saraswathi G, Srinivasan K (1998) Antidiabetic influence of dietary cumin seeds (*Cuminum cyminum*) in streptozotocin induced diabetic rats. Nutr Res 18:131–142
213. Keihan GS, Gharib MH, Momeni A, Hemati Z, Sedighin R (2016) A comparison between the effect of *Cuminum cyminum* and vitamin E on the level of leptin, paraoxonase 1, HbA1c and oxidized LDL in diabetic patients. Int J Mol Cell Med 5:229–235
214. Mushtaq A, Ahmad M, Jabeen Q, Saqib A, Wajid M, Akram MA (2014) Hepatoprotective investigations of *Cuminum cyminum* dried seeds in nimesulide intoxicated albino rats by phytochemical and biochemical methods. Int J Pharm Pharm Sci 6:506–510
215. Abbas N, Naz M, Alyousef L, Ahmed ES, Begum A (2017) Comparative study of hepatoprotective effect produced by *Cuminum cyminum*, fruits of *Phyllanthus emblicus* and silymarin against cisplatin-induced hepatotoxicity. Int J Pharm Sci Res 8:2026–2032
216. Kumar A, Kumar R, Kumar N, Nath A, Singh JK, Ali M (2011) Protective effect of *Cuminum cyminum* and *Coriandrum sativum* on profenofos induced liver toxicity. Int J Pharm Biol Arch 2:1405–1409
217. Mekawey AAI, Mokhtar MM, Farrag RM (2009) Antitumor and antibacterial activities of [1-(2-ethyl, 6-Heptyl) phenol] from *Cuminum cyminum* seeds. J Appl Scis Res 5:1881–1888
218. Prakash E, Gupta DK (2014) Cytotoxic activity of ethanolic extract of *Cuminum cyminum* Linn against seven human cancer cell line. Univers J Agric Res 2:27–30
219. Bhat SP, Rizvi W, Kumar A (2014) Effect of *Cuminum cyminum* L. seed extracts on pain and inflammation. J Nat Remedies 14:186–192
220. Gilani AH, Shaheen E, Saeed SA, Bibi S, Irfanullah SM, Faizi S (2000) Hypotensive action of coumarin glycosides from *Daucus carota*. Phytomedicine 7:423–426
221. Sudewi S, Wahyuono S, Astuti P (2014) Isolation and identification of free radicals scavenger from *Daucus carota* L leaves. Trad Med J 19:142–148

222. Mohammedi H, Mecherara-Idjeri S, Foudil-Cherif Y, Hassani A (2015) Chemical composition and antioxidant activity of essential oils from Algerian *Daucus carota* L. subsp. carota aerial parts. J Essent Oil Bear Pl 18:873–883
223. Al-Snafi AE (2017) Nutritional and therapeutic importance of Daucus Carota- a review. IOSR J Pharm 7:72–88
224. Zhang D, Hamauzu Y (2004) Phenolic compounds and their antioxidant properties in different tissues of carrots (*Daucus carota* L.) J Food Agric Environ 2:95–100
225. Sun T, Simon PW, Tanumihardjo SA (2009) Antioxidant phytochemicals and antioxidant capacity of biofortified carrots (*Daucus carota* L.) of various colors. J Agric Food Chem 57:4142–4147
226. Mueller L, Boehm V (2011) Antioxidant activity of β-carotene compounds in different *in vitro* assays. Molecules 16:1055–1069
227. Shoba S, Patil PA, Vivek V (2008) Hepatoprotective activity of *Daucus carota* L. aqueous extract against paracetamol, isoniazid and alcohol induced hepatotoxicity in male Wistar rats. Pharmacologyonline 3:776–787
228. Jain PK, Khurana N, Pounikar Y, Patil S, Gajbhiye A (2012) Hepatoprotective effect of carrot (*Daucus carota* L.) on paracetamol intoxicated rats. IJPPT 1:115–120
229. Singh K, Singh N, Chandy A, Manigauha A (2012) *In vivo* antioxidant and hepatoprotective activity of methanolic extracts of *Daucus carota* seeds in experimental animals. Asian Pac J Trop Biomed 2:385–388
230. Khatib N, Angel G, Nayna H, Kumar JR (2010) Gastroprotective activity of the aqueous extract from the roots of *Daucus carota* L in rats. IJRAP 1:112–119
231. Jiin WH, Hidayat EM, Lukman K (2014) Gastroprotective effect of carrot (*Daucus carota* L.) juice in rat models. Althea Medical Journal 1:35–39
232. Patil MV, Kandhare A, Bhise S (2012) Anti-inflammatory effect of *Daucus carota* root on experimental colitis in rats. Int J Pharm Pharm Sci 4:337–343
233. Chandra P, Kishore K, Ghosh AK (2015) Assessment of antisecretory, gastroprotective, and *in-vitro* antacid potential of *Daucus carota* in experimental rats. Osong Public Health Res Perspect 6:329–335
234. Wehbe K, Mroueh M, Daher CF (2009) The potential role of *Daucus carota* aqueous and methanolic extracts on inflammation and gastric ulcers in rats. J Complement Integr Med 6(1):7. https://doi.org/10.2202/1553-3840.1159
235. Valente J, Resende R, Zuzarte M, Goncalves MJ, Lopes MC, Cavaleiro C, Pereira C, Saiguerio L, Cruz MT (2015) Bioactivity and safety profile of *Daucus carota* subsp. maximus essential oil. Ind Crop Prod 77:218–224
236. Kamiloglu S, Grootaert C, Capanoglu E, Ozkan C, Smagghe G, Raes K, Van Camp J (2016) Anti-inflammatory potential of black carrot (*Daucus carota* L.) polyphenols in a co-culture model of intestinal Caco-2 and endothelial EA.hy926 cells. Mol Nutr Food Res 00:1–11. https://doi.org/10.1002/mnfr.201600455
237. Diab-Assaf M, El-Sharif S, Mroueh M (2007) Evaluation of anti-cancer effect of *Daucus carota* on the human promyelocytic leukemia HL-60 cells. Clin Cancer Res 13:56
238. Najm PI (2014) The anti-cancer activity of 2 himachalene-6-ol extracted from *Daucus carota* ssp. *carota*. Dissertation, Lebanese American University
239. Shebaby WN, El-Sibai M, Smith KB, Karam MC, Mroueh M, Daher CF (2013) The antioxidant and anticancer effects of wild carrot oil extract. Phytother Res 27:737–744
240. Shebaby WN, Mroueh M, Bodman-Smith K, Mansour A, Taleb RI, Daher CF, El-Sibai M (2014) *Daucus carota* pentane-based fractions arrest the cell cycle and increase apoptosis in MDA-MB-231 breast cancer cells. BMC Complement Altern Med 14:387. https://doi.org/10.1186/1472-6882-14-387.
241. Ranjbar B, Pouraboli I, Mehrabani M, Dabiri S, Javadi A (2010) Effect of the methanolic extract of *Daucus carota* seeds on the carbohydrate metabolism and morphology of pancreas in type I diabetic male rats. Physiol Pharmacol 14:85–93

242. Vasudevan M, Parle M (2006) Pharmacological evidence for the potential of *Daucus carota* in the management of cognitive dysfunctions. Biol Pharm Bull 29:1154–1161
243. Jaffat HS, Semysim EA (2016) Hypo-lipidemic effects of aqueous extract of *Daucus carota* seeds (*Daucus carota* L.) induced atherogenic diet in wister male rats. Res J Pharm Biol Chem Sci 7:2714–2720
244. Mani V, Parle M, Ramasamy K, Majeed ABA (2010) Anti-dementia potential of *Daucus carota* seed extract in rats. Pharmacologyonline 1:552–565
245. Vasudevan M, Gunnam KK, Parle M (2006) Antinociceptive and anti-imflammatory properties of *Daucus carota* seeds extract. J Health Sci 52:598–606
246. Pouraboli I, Ranjbar B (2015) The effect of *Daucus carota* seeds extract on lipid profile, LFT and kidney function indicators in streptozocin-induced diabetic rats. Int J Plant Sci Ecol 1:84–87
247. Singh K, Dhongade H, Singh N, Kashyap P (2010) Hypolipidemic activity of ethanolic extract of *Daucus carota* seeds in normal rats. IJBAR 1:73–80
248. Rahimi R, Ardekani MR (2013) Medicinal properties of Foeniculum Vulgare mill. In traditional Iranian medicine and modern phytotherapy. Chin J Integr Med 19:73–79
249. Badgujar SB, Patel VV, Bandivdekar AH (2014) *Foeniculum vulgare* mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. Biomed Res Int 2014:842674. https://doi.org/10.1155/2014/842674
250. Rather MA, Dar BA, Sofi SN, Bhat BA, Qurishi MA (2016) *Foeniculum vulgare*: a comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arab J Chem 9:S1574–S1583
251. Diao WR, QP H, Zhang H, JG X (2014) Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (*Foeniculum vulgare* mill.) Food Control 35:109–116
252. Mota AS, Martins MR, Arantes S, Lopes VR, Bettencourt E, Pombal S, Gomes AC, Silva LA (2015) Antimicrobial activity and chemical composition of the essential oils of Portuguese *Foeniculum vulgare* fruits. Nat Prod Commun 10:673–676
253. Mimica-Dukić N, Kujundžić S, Soković M, Couladis M (2003) Essential oil composition and antifungal activity of *Foeniculum vulgare* mill. Obtained by different distillation conditions. Phytother Res 17:368–371
254. Skrobonja J, Delić D, Karaman M, Matavulj M, Bogavac M (2013) Antifungal properties of *Foeniculum vulgare*, *Carum carvi* and *Eucalyptus* sp. essential oils against *Candida albicans* strains. J Nat Sci Matica Srpska Novi Sad 124:195–202
255. Thakur N, Sareen N, Shama B, Jagota K (2013) Studies on in vitro antifungal activity of *Foeniculum vulgare* Mill. against spoilage fungi. GJBB 2:427–430
256. Shukla HS, Dubey P, Chaturvedi RV (1989) Antiviral properties of essential oils of *Foeniculum vulgare* and *Pimpinella anisum* L. Agron EDP Sci 9:277–279
257. Shahat AA, Ibrahim AY, Hendawy SF, Omer EA, Hammouda FM, Abdel-Rahman FH, Saleh MA (2011) Chemical composition, antimicrobial and antioxidant activities of essential oils from organically cultivated fennel cultivars. Molecules 16:1366–1377
258. Chang S, Bassiri A, Jalali H (2013) Evaluation of antioxidant activity of fennel (*Foeniculum vulgare*) seed extract on oxidative stability of olive oil. JCHR 3:53–61
259. El Ouariachi E, Lahhit N, Bouyanzer A, Hammouti B, Paolini J, Majidi L, Desjobert JM, Costa J (2014) Chemical composition and antioxidant activity of essential oils and solvent extracts of Foeniculum Vulgare mill. From Morocco. J Chem Pharm Res 6:743–748
260. Choi EM, Hwang JK (2004) Antiinflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. Fitoterapia 75:557–565
261. Yang IJ, Lee DU, Shin HM (2015) Anti-inflammatory and antioxidant effects of coumarins isolated from *Foeniculum vulgare* in lipopolysaccharide-stimulated macrophages and 12-O-tetradecanoylphorbol-13-acetate-stimulated mice. Immunopharmacol Immunotoxicol 37:308–317

262. Mohamad RH, El-Bastawesy AM, Abdel-Monem MG, Noor AM, Al-Mehdar HA, Sharawy SM, El-Merzabani MM (2011) Antioxidant and anticarcinogenic effects of methanolic extract and volatile oil of fennel seeds (*Foeniculum vulgare*). J Med Food 14:986–1001
263. Özbek H, Ugras S, Dulger H, Bayram I, Tuncer I, Ozturk G, Ozturk A (2003) Hepatoprotective effect of *Foeniculum vulgare* essential oil. Fitoterapia 74:317–319
264. Özbek H, Ugras S, Bayram I, Uygan I, Erdogan E, Öztürk A, Huyut Z (2004) Hepatoprotective effect of *Foeniculum vulgare* essential oil: a carbon-tetrachloride induced liver fibrosis model in rats. Scand J Lab Anim Sci 1:9–17
265. Parsaeyan N (2016) The effect of *Foeniculum vulgare* (fennel) extract on lipid profile, lipid peroxidation and liver enzymes of diabetic rat. IJDO 8:24–29
266. Beaux D, Fleurentin J, Mortier F (1997) Diuretic action of hydroalcohol extracts of *Foeniculum vulgare* var *dulce* (D.C.) roots in rats. Phytother Res 11:320–322
267. Sadrefozalayi S, Farokhi F (2014) Effect of the aqueous extract of *Foeniculum vulgare* (fennel) on the kidney in experimental PCOS female rats. Avicenna J Phytomed 4: 110–117
268. Blank I, Schieberle P (1993) Analysis of the seasoning-like flavour substances of a commercial lovage extract (*Levisticum officinale* Koch.) Flav Frag J 8:191–195
269. Reza VRM, Abbas H (2007) The essential oil composition of Levisticum Officinale from Iran. Asian J Biochem 2:161–163
270. Mahmoudzehi S, Dorrazehi GM, Jamalzehi S, Khabbaz AHH, Ghorbani F, Hooti A, Dadkani AG, Souran MM (2017) The neuroprotective effects of alcoholic extract of *Levisticum officinale* on alpha motoneurons' degeneration after sciatic nerve compression in male rats. Biomed Pharmacol J 10:633–640
271. Mohammadi M, Parvaneh E, Ghamari ZT (2016) Clinical investigation of *Levisticum officinale* (lovage) effectiveness' in patients with cystinuria. J Urol Res 3:1071
272. Naber KG (2013) Efficacy and safety of the phytotherapeutic drug Canephron® N in prevention and treatment of urogenital and gestational disease: review of clinical experience in Eastern Europe and Central Asia. Res Rep Urol 5:39–46
273. Mirjalili MH, Salehi P, Sonboli A, Hadian J, Ebrahimi SN, Yousefzadi M (2010) The composition and antibacterial activity of the essential oil of *Levisticum officinale* Koch flowers and fruits at different developmental stages. J Serb Chem Soc 75:1661–1669
274. Ebrahimi A, Eshraghi A, Mahzoonieh MR, Lotfalian S (2016) Antibacterial and antibiotic-potentiation activities of *Levisticum officinale* L. extracts on pathogenic bacteria. Int J Inf Secur 4:e38768. https://doi.org/10.17795/iji-38768
275. Mohamadi N, Rajaei P, Moradalizadeh M, Amiri MS (2017) Essential oil composition and antioxidant activity of *Levisticum officinale* Koch. At various phenological stages. J Med Plants 16:45–55
276. Mirjalili MH, Salehi P, Sonboli A, Hadian J, Ebrahimi SN, Yousefzadi M (2010) The composition and antibacterial activity of the essential oil of Levisticum Officinale Koch flowers and fruits at different developmental stages. J Serb Chem Soc 75:1661–1669
277. Sertel S, Eichhorn T, Plinkert P, Efferth T (2011) Chemical composition and antiproliferative activity of essential oil from the l of a medicinal herb, *Levisticum officinale*, against UMSCC1 head and neck squamous carcinoma cells. Anticancer Res 31:185–192
278. El-Hamid SRA, Abeer YI, Hendawy SF (2009) Anti-inflammatory, antioxidant, anti-tumor and physiological studies on *Levisticum officinale* Koch plant. Planta Med 75:PE62. https://doi.org/10.1055/s-0029-1234623
279. Cain N, Darbyshire SJ, Francis A, Nurse RE, Simard MJ (2010) The biology of Canadian weeds. 144. *Pastinaca sativa* L. Can J Plant Sci 90:217–240
280. Skalicka-Woźniak K, Zagaja M, Głowniak K, Łuszczki J (2014) Purification and anticonvulsant activity of xanthotoxin (8-methoxypsoralen). Cent Eur J Biol 9:431–436
281. Akbarmivehie M, Baghaei H (2016) The effect of addition parsnip herb and its extract on momtaze hamburger shelf life. Eur Online J Nat Soc Sci 5:132–146

282. Mangkoltriluk W, Srzednicki G, Craske J (2005) Preservation of flavour components in parsley (*Petroselinum crispum*) by heat pump and cabinet drying. Pol J Food Nutr Sci 14:63–66
283. Zhang H, Chen F, Wang X, Yao HY (2006) Evaluation of antioxidant activity of parsley (*Petroselinum crispum*) essential oil and identification of its antioxidant constituents. Food Res Int 39:833–839
284. Tang EL, Rajarajeswaran J, Fung S, Kanthimathi MS (2015) *Petroselinum crispum* has antioxidant properties, protects against DNA damage and inhibits proliferation and migration of cancer cells. J Sci Food Agric 95:2763–2771
285. Al-Howiriny TA, Al-Sohaibani MO, El-Tahir KH, Rafatullah S (2003) Preliminary evaluation of the anti-inflammatory and anti-hepatotoxic activities of 'parsley' *Petroselinum crispum* in rats. J Nat Remedies 3:54–62
286. Kamal T, Abd-Elhady E, Sadek K, Shukry M (2014) Effect of parsley (*Petroselium crispum*) on carbon tetrachloride-induced acute hepatotoxicity in rats. Res J Pharm Biol Chem Sci 5:1524–1534
287. Troncoso L, Guija E (2007) *Petroselinum sativum* (perejil) antioxidant and hepatoprotective effects in rats with paracetamol-induced hepatic intoxication. Anales de la Facultad de Medicina Universidad Nacional Mayor de San Marcos 68:333–343
288. Jassim AM (2013) Protective effect of *Petroselinum crispum* (parsley) extract on histopathological changes in liver, kidney and pancreas induced by sodium valproate in male rats. Kufa J Veteri Med Sci 4:20–27
289. Bolkent S, Yanardag R, Ozsoy-Sacan O, Karabulut-Bulan O (2004) Effects of parsley (*Petroselinum crispum*) on the liver of diabetic rats: a morphological and biochemical study. Phytother Res 18:996–999
290. Nair VY, Balakrishanan N, Antony Santiago JV (2015) *Petroselinum crispum* extract attenuates hepatic steatosis in rats fed with fructose enriched diet. Bratislava Med J 116:547–553
291. Campos KE, Balbi APC, Alves MJQF (2009) Diuretic and hipotensive activity of aqueous extract of parsley seeds (*Petroselinum sativum* Hoffm.) in rats. Rev Bras Farmacogn 19:41–45
292. Vargas JLZ, Lujan EGT, Pachas LCC, Lujan EPT, Lujan MT, Lujan PE (2016) Determination of diuretic activity of *Petroselinum sativum* (parsley). J Hypertens 34:431. https://doi.org/10.1097/01.hjh.0000501115.12030.e5
293. Moram GSE (2016) Evaluation of anti-urolithiatic effect of aqueous extract of parsley (*Petroselinum sativum*) using ethylene glycol-induced renal calculi. WJPR 5:1721–1735
294. Shojaii A, Fard MA (2012) Review of pharmacological properties and chemical constituents of *Pimpinella anisum*. ISRN Pharmaceutics. https://doi.org/10.5402/2012/510795
295. Aćimović M, Dojčinović N (2014) A review of pharmacological properties of anise (*Pimpinella anisum* L.) Med Raw Mat 34:3–17
296. Gülçın I, Oktay M, Kıreçcı E, Küfrevıoğlu I (2003) Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. Food Chem 83:371–382
297. Tavallali V, Rahmati S, Bahmanzadegan A (2017) Antioxidant activity, polyphenolic contents and essential oil composition of Pimpinella Anisum L. as affected by zinc fertilizer. J Sci Food Agric. https://doi.org/10.1002/jsfa.8360
298. Bekara A, Aithamadouhe N, Kahloula K, Sadi N, Aoues AK (2016) Effect of *Pimpinella anisum* L aqueous extract against oxidative stress induced by lead exposure in young rats brain. J Appl Environ Biol Sci 6:85–93
299. Akhtar A, Deshmukh AA, Bhonsle AV, Kshirsagar PM, Kolekar MA (2008) In vitro antibacterial activity of *Pimpinella anisum* fruit extracts against some pathogenic bacteria. Vet World 1:272–274
300. Kadan S, Rayan M, Rayan A (2013) Anticancer activity of anise (*Pimpinella anisum* L.) seed extract. Open Nutraceuticals J 6:1–5
301. Rahamooz-Haghighi S, Asadi MH (2016) Anti-proliferative effect of the extracts and essential oil of Pimpinella Anisum on gastric cancer cells. J Herb Med Pharmacol 5:157–161

302. Shobha RI, Rajeshwari CU, Andallu B (2013) Anti-peroxidative and anti diabetic activities of aniseeds (*Pimpinella anisum* l) and identification of bioactive compounds. AJPCT 1:516–527
303. Rajeshwari U, Shobha I, Andallu B (2011) Comparison of aniseeds and coriander seeds for antidiabetic, hypolipidemic and antioxidant activities. Spatula DD 1:9–16
304. El-Sayed MGA, Elkomy A, Sahar S, El-Banna AH (2015) Hepatoprotective effect of *Pimpinella anisum* and *Foeniculum vulgare* against carbon tetrachloride induced fibrosis in rats. World. J Pharm Sci 4:78–88
305. Jamshidzadeh A, Heidari R, Razmjou M, Karimi F, Moein MR, Farshad O, Akbarizadeh AR, Shayesteh MRH (2015) An *in vivo* and *in vitro* investigation on hepatoprotective effects of *Pimpinella anisum* seed essential oil and extracts against carbon tetrachloride-induced toxicity. Iran J Basic Med Sci 18:205–211
306. Aćimović M, Tešević V, Mara D, Stanković J, Cvetković M, Djuragić O (2016) Influence of fertilization on total polyphenole content in aniseed postdistillation waste material. AJMAP 3:57–67

Functional Components and Medicinal Properties of Food

45

Christian Izuchukwu Abuajah

Contents

Abstract

There is growing evidence that functional components (bioactives and phytochemicals) of food play an integral role in the link between food and the prevention of diseases. Although some functional components were labelled anti-nutrients, their role as potential healthy biochemical components of diets for the prevention of degenerative pathologies have been scientifically elucidated.

C. I. Abuajah (✉)
Department of Food Science and Technology, University of Uyo, Uyo, Nigeria
e-mail: izychuks@yahoo.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_39

Some properties which link functional components to potential health-modulating roles and functions can be classified into anti-oxidation, anti-cancer, anti-diabetic, anti-inflammatory, cardiovascular, anti-microbial, immunomodulatory and anti-hypertensive. However, the mechanisms through which they impact on human health are not completely clear. Food processing techniques exercise effects on functional components of food. While some processing techniques increase their concentration in food, others decrease them. Therefore, in this era when the role of a healthy diet in preventing degenerative, non-communicable and chronic diseases is well accepted, the borderline between food and medicine is becoming very thin. Thus, the concept of food has obviously gone beyond basic nutrition only. While products intended to cure diseases are classified as medicine, a healthy diet consisting of foods with functional components can help optimize health and promote well-being as well as reduce or prevent the risk of developing certain diseases.

Keywords
Bioactives · Diet · Food · Functional components · Health · Medicine · Nutrition · Phytochemicals · Well-being

Abbreviations

AK1	Adenylate kinase1 encoding gene
CAT	Catalase
CLA	Conjugated linoleic acid
CR3	Complementary receptor3
DF	Dietary fiber
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DP	Degree of polymerization
EGC3G	Epigallocatechin-3-gallate
EPA	Eicosapentaenoic acid
FOS	Fructo-oligosaccharide
GP_X	Glutathione peroxidase
GR	Glutathione reductase
HD	High density lipoprotein
HG-2	Human hepatoma cells G2
HPP	High pressure processing
HTLV-1	Human T-celllymphotroic virus type1
HWE	Hot water extract
IL-1	Interleukin-1
LAB	Lactic acid bacteria
LacCer	Lactocylceramide (a bioactive lipid)
LDL	Low density lipoprotein
MAPK	Mitogen activated protein kinases
MP2	Microphage inflammatory protein2
*m*RNA	Messenger ribonucleic acid

MyD88	Myeloid differentiation primary receptor gene 88
NFAT	Nuclear factor of activated T cells
NF-kB	Nuclear factor kappa light-chain-enhancer of activated β cells.
NK	Natural killer
Nrf2	Nuclear factor2 pathway
NTP	Non-thermal processing
PEF	Pulsed electric field
Pi3k	Phosphatidyl inositol-4,5-biphosphate-3-kinase
PKC	Protein kinase C
PUFA	Poly-unsaturated fatty acid
RNS	Reactive Nitrogen species
ROS	Reactive oxygen species
SIGNR3	Specific ICAM (intracellular adhesion molecule)-3 grabbing non-intergrin-related antigen
SOD	Superoxide dismutase
syk	Spleen tyrosine kinase encoding enzyme gene
TLR	Toll-like receptors
TNF-α	Tumor necrosis factor alpha
TRAF6	TNF receptor associated factor6
WBC	Water binding capacity

1 Introduction

Food is any substance consumed to nourish and refresh the body [1]. It is the most imaginable sets of complicated biochemical substances ingested into the body to provide nutritional support and refreshment necessary for the health, growth, and normal functions of every living organism. Essentially, food is a mixture of chemicals or nutrients which can be separated into different components with one or more different functions in the body including energy generation; growth and repair of worn out cells and tissues; regulation of processes and protection against diseases and invasion by pathogens. The two major components of food are nutritive or primary metabolites (consisting of the macronutrients that are present in relatively large amounts, such as proteins, carbohydrates, lipids and micronutrients present in small amounts, such as vitamins, minerals, and water which are essential for the sustenance of normal body functions, e.g., energy generation, growth, repair, regulation, etc.) and non-nutritive or secondary metabolites (these are bioactives or phytochemicals which possess properties with potentially positive effect on health beyond basic nutrition. These non-nutritve substances are known as functional components. A combination of the right types of food optimizes health and impacts positively on wellness. Dietary guidelines around the world recommend increased consumption of fruits and vegetables, as good sources of beneficial plant chemicals and essential nutrients to improve health and reduce the risk of chronic diseases [2]. Accordingly, daily intake of at least 400 g of vegetables and fruits have been recommended by experts [3].

The non-nutritive components of food are variously referred to in different contexts as functional or bioactive components (biomolecules present in food that exhibit the capacity to modulate one or more metabolic processes, which results to health benefits and promotion of well-being) or phytochemicals (plant-derived, biologically active chemicals that function in the body to prevent certain disease processes) [4, 5]. There are over a 1000 phytochemicals found in foods, and one serving of about 120 g of a fruit or vegetable may have as many as 100 different phytochemicals [6]. Research has established a relationship between functional components in food, health and well-being [7]. Consequently, functional components have health-promoting roles at various stages of disease control that are associated with multiple progressive steps, from initiation to development. Thus, they can be effectively applied in the treatment and prevention of diseases [8]. These days, as a result of the acceptance of the role of a healthy nutrition based on the right choice of diet in preventing diseases, the difference between food and medicine is no longer obvious [9].

Previously, it was thought that functional components occur only in plant foods including whole grains, nuts, seeds, spices, fruits, and vegetables. However, probiotics, polyunsaturated fatty acids (PUFA) such as conjugated linolenic acid (CLA), long-chain omega-3, -6 and -9 fatty acids including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and bioactive peptides are equally found in animal products such as milk, fermented milk products and cold-water fish. They also have multiple metabolic activities allowing for beneficial effects on several diseases and target tissues in the body [5]. Table 1 presents some functional components of food, their common sources, and potential benefits.

This chapter therefore presents functional components of food in the light of their medicinal properties, impact and mode of action in optimizing health and well-being. In addition, the different types, nature, functions, source, and the effect of processing techniques on functional components of food are discussed.

2 An Overview of Functional Components of Food

Functional components are mainly the non-nutritive secondary metabolites in food. Although some functional components are labelled as anti-nutrients, their role as potential healthy biochemical constituents of diets for the prevention of degenerative pathologies have been scientifically elucidated [10]. Functional components were mostly ignored until recently when their potential metabolic effects were first detected. For example, flavones were found to protect against heart diseases and soy-based estrogens against cancer [10]. The bioactivity and chemistry of other non-nutritive compounds in foods such as red wine, coffee, nuts, seeds, whole grains, spices, herbs, fruits and vegetables have been investigated to determine their biochemical effects and potential health benefits [11]. They are essential for normal growth, development and defense of plants [12] and seem to replicate the same functions in humans. To date, different types of secondary metabolites have been identified in plants [13]. Chemically, these compounds are either nitrogen-containing alkaloids or nitrogen-deficient terpenoids and phenolics [14].

Table 1 Some functional ingredients of food, their sources, and potential benefits

Bioactive components	Source	Potential benefits
Carotenoids		
Alpha-carotene/beta-carotene	Carrots, fruits, vegetables	Neutralize free radicals which may cause damage to cells
Lutein	Green vegetables	Reduce the risk of muscular degeneration
Lycopene	Tomato products (ketchup, sauces)	Reduce the risk of prostate cancer
Non-starchy polysaccharides		
Fucoidan	Mushrooms (maitake and reshi), brown seaweeds	Immune modulation; apoptosis of cancer cells; stimulates brain development; anticlotting effect; lower blood cholesterol levels; decrease high blood pressure, stabilize blood sugar
Insoluble dietary fiber	Wheat bran	Reduces risk of breast or colon cancer
Soluble dietary fiber (β-glucans)	Oats, barley, psyllium	Reduces risk of cardiovascular disease; protects against heart disease and some cancers; lower LDL and total cholesterol
Fatty acids		
Long chain omega-3 fatty acids (DHA/EPA),	Salmon and other fish oils	Reduce risk of cardiovascular disease; improve mental and visual functions.
Conjugated linoleic acid (CLA)	Cheese, meat products	Improve body composition; decrease risk of certain cancers
Phenolics		
Anthocyanidins	Fruits	Neutralize free radicals; reduce risk of cancer
Catechins	Tea	Neutralize free radicals; reduce risk of cancer
Flavonones	Citrus	Neutralize free radicals; reduce risk of cancer
Flavones	Fruits/vegetables	Neutralize free radicals; reduce risk of cancer
Lignans	Flax, rye, vegetables	Prevention of cancer; renal failure.
Tannins (proanthocyanidins)	Cranberries, cranberry products, cocoa, chocolate	Improve urinary tract health; reduce risk of cardiovascular disease
Phytosterols		
Stanol ester	Corn, soy, wheat, wood oils	Lower blood cholesterol levels by inhibiting cholesterol absorption
Prebiotics and probiotics		
Fructo-oligosaccharides (FOS);	Jerusalem artichokes, shallots, onion powder,	Improve quality of intestinal microflora; gastrointestinal health
Lactobacillus; Biofidobacterium	Yogurt, other dairy products	Improve quality of intestinal microflora; gastrointestinal health
Soy phytoestrogens		
Isoflavones: Daidzein genistein	Soybeans and soy-based foods	Menopause symptoms such as hot flashes; protection against heart disease and some cancers; lowering of LDL and total cholesterol

Groups of plant secondary metabolites are diverse and usually occur in multiple and complex forms such as methylated, ethylated, glycosylated, esterified, thiolated, or hydroxylated [15]. These include about 8000 varieties of phenolics including flavonoids and non-flavonoids; about 25,000 terpenoids, carotenoids, xanthophylls, and iridoids; about 12,000 alkaloids; and several sulfate-containing compounds such as isothiocyanates [11]. To date, numerous studies of the capacities of functional components as phytochemicals including glucosinolates, phytosterols, tocopherols, tocotrienols, allyl sulfides, and non-starch carbohydrates including soluble and insoluble dietary fiber and fucoidan, as antioxidation, anticancerous, anti-inflammatory, antiviral, immune modulating, and antihypertensive agents are widely reported in literature [16–18]. The list is never exhaustive as new compounds beneficial to human health are constantly being discovered [19].

Functional components exhibit the capacity to modulate one or more metabolic processes, which results to optimal health benefits and promotion of wellness in humans. In addition, they have been shown to exert multiple bioactive functions including scavenging of reactive oxygen species (ROS), dynamic regulation of metabolic functions of proteins, enzymes, transporters, receptors, and signal transduction processes related to various lifestyle-related diseases. Thus, allowing for beneficial effects on several degenerative and non-communicable and chronic diseases as well as target tissues [11, 15]. Therefore, functional components have potential positive impact on health beyond basic nutrition. An overview of the types, functions, medicinal properties and sources of some food functional components as reported by Abuajah et al. [20] is as follows:

2.1 Non-starch Carbohydrates (Examples Dietary Fibers, Fucoidans)

Basically, these are structural and storage carbohydrate polymers of simple sugars including glucose, galactose, fructose, xylose, arabinose, etc., but are not starchy in nature (i.e. their sugar units are not linked by either α-(1 → 4) or α-(1 → 6) glycosidic bonds. Thus, they are not hydrolysable by the human digestive enzymes but undergo fermentative modification by the probiotic microbes in the colon. There are several kinds of non-starch carbohydrates including dietary fibers and fucoidans.

(a) **Dietary fibers**: Dietary fibers (DF), which could be either soluble or insoluble, are non-starchy polysaccharides and structural materials of the cell walls of cereals and microorganisms. They are the indigestible part of plant foods composed of long linear and branched chains of glucose molecules held together by bonds that cannot be hydrolyzed by human digestive enzymes. Chemically, DFs are glucose polymers in hetero-structural configuration of β-(1 → 3:1 → 4) or β-(1 → 3:1 → 6) bonds depending on their sources (Fig. 1). Cereal and bacterial DFs are primarily linear with large regions of β-(1 → 4) linkages

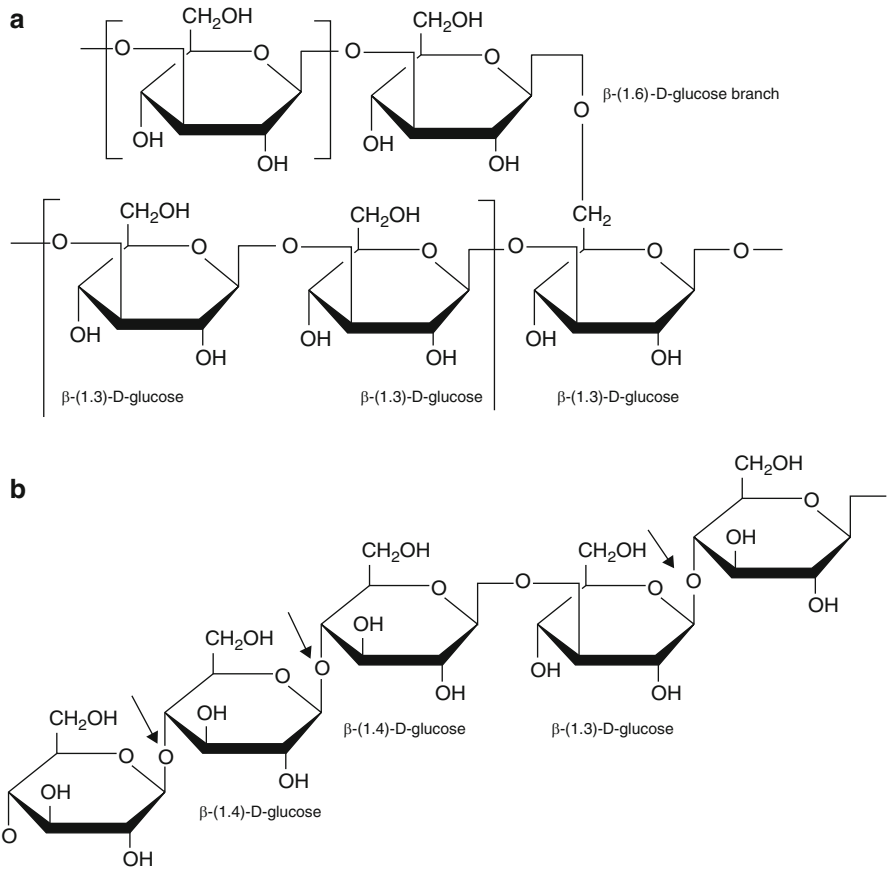


Fig. 1 Structures of β-glucans. (**a**) Fungi β-glucan (**b**) Cereal β-glucan

separating shorter stretches of β-(1 → 3) structures. Fungi β-glucans (e.g., mushroom) have short β-(1 → 6)-linked branches coming off the β-(1 → 3) backbone whereas those of yeast have β-(1 → 6) branches that are further elaborated with additional β-(1 → 3) regions. These structural differences do have large implications and impact on their properties, e.g., water solubility.

Also, differences in the length of the polysaccharide chain, extent of branching, and the length of those branches result in differences in hot water extracts (HWE), level of viscosity and differences in molecular weights [9, 21–23]. The water-soluble fibers are mainly β-glucans, gums, pectin, mucilage and arabinoxylans while the water-insoluble fibers are composed of lignin, cellulose and hemicellulose [24–26]. Specifically, the mixed-linkage structure of β-glucans and length of the polysaccharide chain or degree of polymerization (DP) are responsible for their physical properties and characteristics including viscosity, solubility, molecular weight,

water binding capacity (WBC), and foamability (foam capacity and stability). These linkages prevent compact folding of β-glucan chains, making them soluble in water [27, 28]. To maintain their functional attributes, it is important that the processing of β-glucans does not destroy their structure because these large macromolecules are mechanically sensitive and can be broken at high shear rates [28].

(b) **Fucoidans**: Fucoidans are non-starchy but sulfated polysaccharides which occur in various species of brown algae and brown seaweed such as mozuku, kombu and bladderwrack. Different forms of fucoidan have also been discovered in animal species, including sea cucumber [29]. Fucoidans have a complex structure which varies according to its source. It is primarily a polymer of α-(1 → 3) linked fucose pyranose sugar units with sulfate groups substituted at C-2 and C-4 positions on some fucose residues. In addition, fucoidan backbone could be of α-(1 → 3)-linked α-*l*-fucopyranose sugar or of alternating (1 → 3)- and (1 → 4)-linked α-*l*-fucopyranose sugar residues, which may also include sulfated galactofucans with backbones built of β-(1 → 6)-*d*-galacto- and/or β-(1 → 2)-*d*-mannopyranose sugar units with fucosepyranose or fuco-oligosaccharide units forming branched points (one for every 2–3 fucosepyranose residues within the chain) with glucuronic acid, xylose or glucose substitutions [29, 30]. Two structural features which distinguish fucosepyranose from other six-carbon pyranose sugars present in mammals are the lack of a hydroxyl group on the carbon at the 6-position (C-6) and its l-configuration [31]. Basically, fucosepyranose is equivalent to 6-deoxy-*l*-galactose. A simple structure of fucoidan polymer and a non-substituted fucosepyranose sugar unit are shown in Fig. 2.

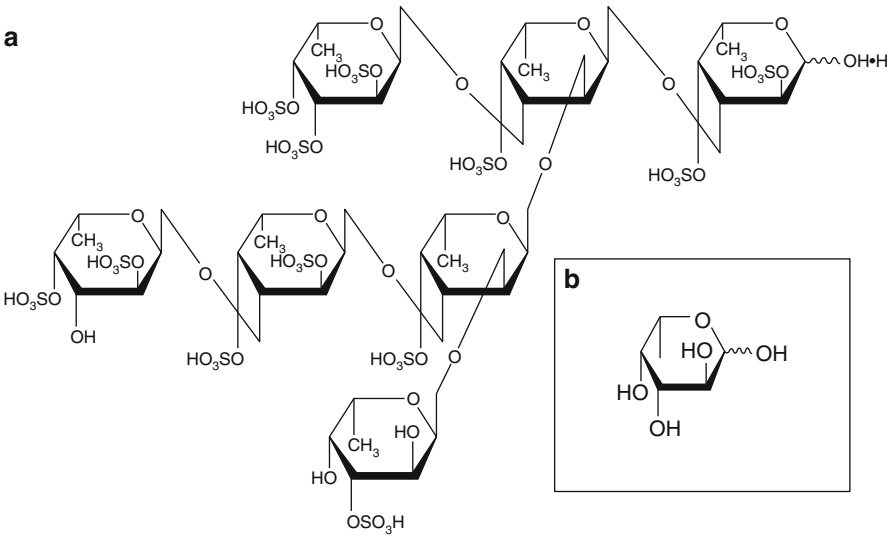


Fig. 2 Structures of (**a**) Fucoidan (**b**) Non-substituted fucose pyranose unit

2.1.1 Functions

The long fibrous structures of dietary fiber allow them to entrap harmful toxins and carcinogens in the digestive tract. Cereal β-glucan (a soluble dietary fiber) has gained special attention for their many health benefits including its viscous nature that slows down the passage of food through the gut thereby reducing the rate of glucose absorption in the intestine, thus lowering the glycemic index (blood glucose level) and serum cholesterol. In addition it possesses the characteristics of good water retention capacity, gelling ability and hydro-colloidality and these properties have influenced their use as substitutes for fat [32]. β-Glucans act as anticytotic, antimutagenic, antitumoregenic and immunomodulatory agents in the human body. Fungi β-glucans, a family of diversified structures found in the cell wall of yeast and molds, enhance leucocytes activity that is responsible for enhancing body defense mechanism. They have been reported to act as immune system activators and cell response modifiers. Innulin has successfully replaced fat in dairy products [6, 22, 23, 28, 33]. Soluble dietary fiber can dissolve in or absorb water and is effective in binding toxins and cholesterol in the intestinal tract. Based on data from in vitro and in vivo animal studies, β-glucans enter the proximal small intestine rapidly and are captured by the macrophages after oral administration. The β-glucans are then internalized and fragmented into smaller sizes and are carried to the marrow and endothelial reticular system. The small β-glucans fragments are then released by the macrophages and taken up by the circulating granulocytes, monocytes and dendritic cells (Fig. 3). This turns on the immune response system [22, 34].

β-Glucans are captured by macrophages via the Dectin-1 receptor with or without toll-like receptors 2 and 6 (TLR-2 and 6). The large β-glucan molecules are then internalized and fragmented into smaller sizes within the macrophages. They are carried to the marrow and reticulo-endothelial system and subsequently released. These small β-glucan fragments are eventually taken up by the circulating

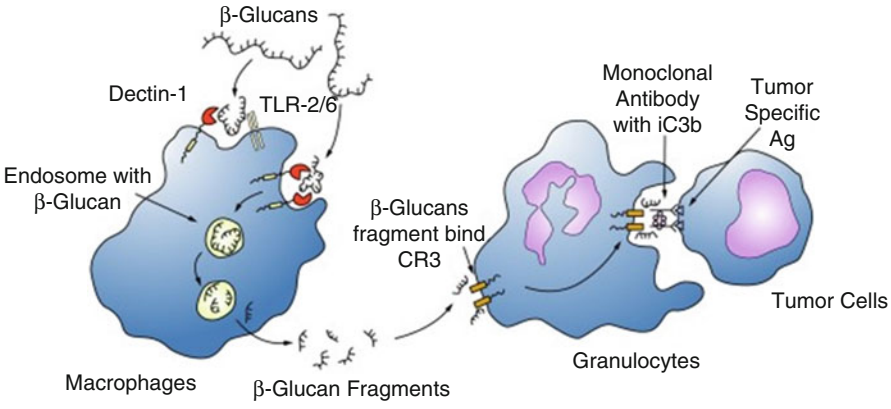


Fig. 3 β-Glucans and resistance to infections

granulocytes, monocytes or other macrophages via the complementary receptor 3 (CR-3). This turns on the immune response; one of its actions being the phagocytosis of the monoclonal antibody tagged tumor cells.

Similarly, β-glucans can bind and act on a diversity of immune specific and related receptors including Dectin-1, complement receptor cells (CR3), cytokines, chemokines, transcriptional factors and growth factors to trigger a wide spectrum of immune responses. The targeted immune cells of β-glucans include macrophages, neutrophils, monocytes, natural killer (NK) cells and dendritic cells. Thus, a cascade of information transduction system is initiated that stimulates the entire immune system against unwanted cell growth. The immunomodulatory functions induced by β-glucans involve both innate and adaptive immune responses [21, 22, 35, 36]. Simplified illustrations of these mechanisms are shown in Fig. 4. Insoluble dietary fiber, on the other hand, cannot dissolve in water and is effective in adding to fecal bulk and increasing the rate of passage of food through the intestinal tract. Insoluble dietary fiber also dilutes out potential carcinogens and decreases contact of toxins and carcinogens with the intestinal tract and speeds up their passage out of the body [23, 27, 28].

β-Glucans act on a variety of membrane receptors found on immune cells. By their actions various signaling pathways are activated and their respective simplified downstream signaling molecules are shown. The reactor cells include monocytes, macrophages, dendritic cells, natural killer cells and neutrophils. Their corresponding surface receptors are listed. β-Glucans also trigger a cascade of cytokines release, such as tumor necrosis factor alpha (TNF)-α and various types of interleukins (ILs). The immunomodulatory functions induced by β-glucans involve both innate and adaptive immune responses.

Fucoidans offer various potential bioactive and functional benefits. It is used as an ingredient in some dietary supplement products. The bioactive properties may vary depending on the source of seaweed, the compositional and structural traits, the content (charge density), distribution, and bonding of the sulfate substitutions, and the purity of the fucoidan product. Fucoidan inhibits the spread of cancerous cells by preventing the adhesion of tumor cells to the extracellular matrix as well as induce apoptosis, or programmed self-destruction, in human T-cell leukemia virus type I (HTLV-1) which is responsible for adult T-cell leukemia. The polysaccharide paves way for apoptosis by inactivating NF-kB, a naturally occurring substance that regulates anti-apoptotic proteins [31].

Fucoidan have also been shown to stimulate the phagocytic action of macrophages and synthesis of several immune cell types, which increase protection against infection [21, 22, 37]. The nutritional makeup of fucoidan could be likened to that of breast milk which is the most perfect immune-supporting food known. The polysaccharide gives the immune system a big boost by enhancing phagocytosis, the process through which white blood cells attack and destroy pathogens. Fucoidan also increases the number of mature white blood cells that are circulating in the body, activity against hepatopathy, uropathy and renalpathy, protective effects on gastric organ and gastric mucosa as well as therapeutic potential in surgery [29], bolstering the first line of defense against infections and diseases

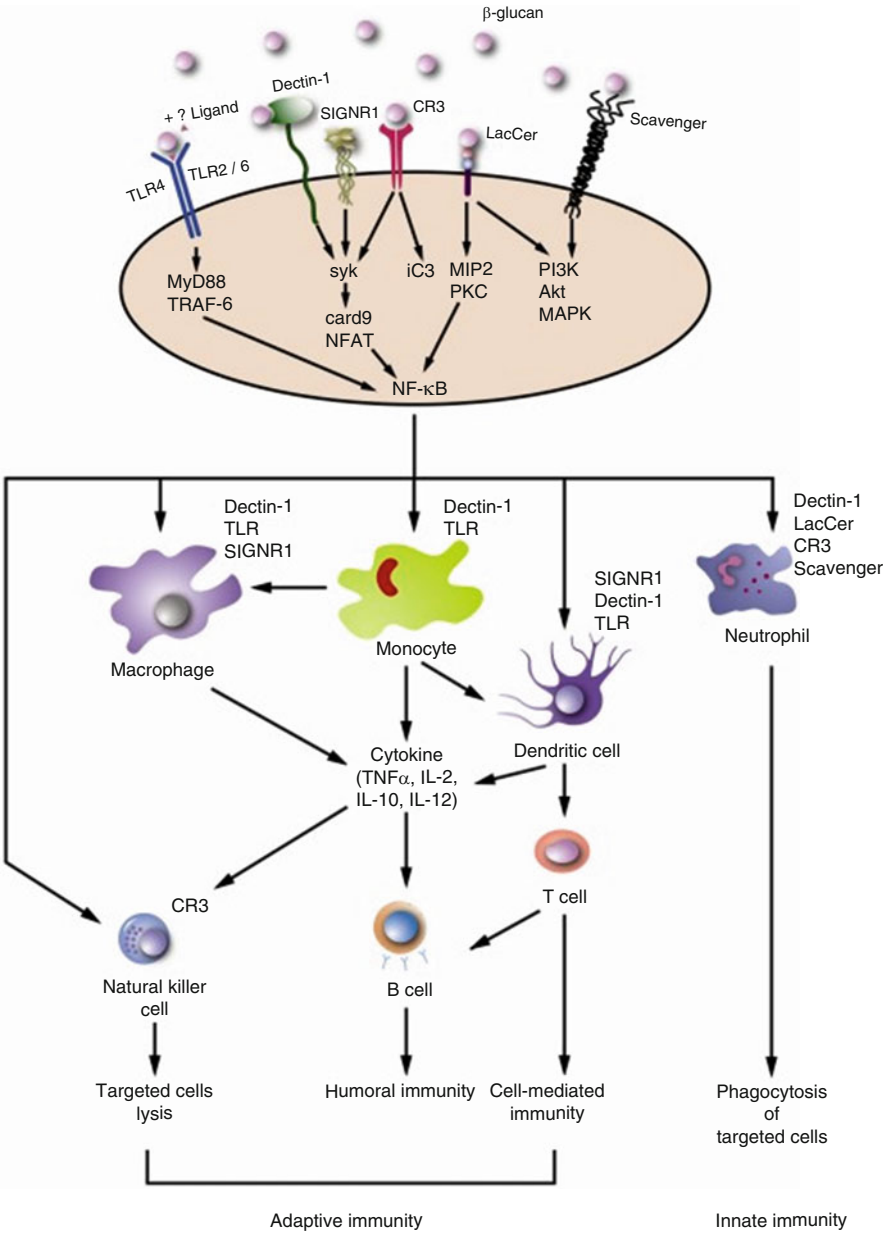


Fig. 4 Immune activation induced by β-glucans

[37]. In addition, several other bioactivities associated with fucoidan have been reported in literature including anti-coagulant and anti-thrombotic activity of blood plasma, antivirus, anti-inflammatory, reduction of blood lipids, anti-oxidation, and anti-complementary properties.

2.1.2 Sources

Foods rich in soluble dietary fiber (DF) include apples, cranberries, mango, oranges, asparagus, broccoli, carrots, peanuts, walnuts, most legumes, oats, and psyllium while those rich in insoluble dietary fiber are apples, bananas, berries, broccoli, green peppers, spinach, almonds, sesame seeds, most legumes, brown rice, whole-wheat breads and cereals. In addition, cereals (e.g., oats and barley) and bacteria are rich sources of β-(1 → 3:1 → 4)-glucan (a DF with strong colloidal properties which is considered as good functional ingredient in foods for its cholesterol-lowering and low-glycemic index functions). β-(1 → 3:1 → 4)-Glucan is present in cereal bran (e.g., 2.2–7.8% in oat and 2.5–11.3% in barley). Brown seaweeds and some medicinal mushrooms are high in fucoidan [38]. Polysaccharides of mushrooms and yeasts such as β-(1 → 3:1 → 6)-glucan have been in focus for their anti-tumor activity and the chemical diversity of these glycans ranges from homopolymers to highly complex heteropolymers. Varieties of sugars such as glucose, galactose, mannose, xylose, arabinose, sucrose ribose, glucouronic acid etc. are involved in the formation of such polysaccharides. Some of the glycans which form conjugates with proteins and peptides show higher potent anti-tumor activity [21, 22, 39].

2.2 Carotenoids, Phenolics, Phytosterols, Tocopherols/Trienols and Organo-Sulfur Compounds

Carotenoids, phenolics, phytosterols, tocopherols/trienols and organo-sulfur compounds are classes of phytochemicals commonly referred to as antioxidants. Antioxidants are bioactive compounds which neutralize free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) in the cell. A free radical is an atom that has an unpaired electron and is highly charged and unstable and causes damage to the cell. Free radicals can form in lipids, proteins, and carbohydrates. Examples of antioxidants are as follows:

(a) **Carotenoids (examples Lycopene, Lutein)**: The carotenoids are lipid-soluble plant pigments that are either oxygenated or non-oxygenated hydrocarbons containing at least forty carbons and an extensive conjugated double bond system. Alpha-carotene, beta-carotene, and lycopene are the predominant non-polar functional carotenoids and lutein is the primary polar functional carotenoid. Carotenoids can be found esterified to fatty acids or un-esterified in plant tissues. Lycopene are the most active oxygen neutralizer with potential chemopreventive activities (Fig. 5). The total carotenoid content of fruits and vegetables varies with age and storage [40].

(b) **Phenolics (examples Apigenin, Rutin)**: Phenolic compounds, commonly known as polyphenols, are the most numerous and widely distributed group of functional molecules. They are diverse groups of plant substances that contain one or more (benzene) rings and varying number of hydroxyl (OH), carbonyl (CO) and carboxylic acid (COOH) groups. They commonly exist in conjugated forms with one or more attached sugar residues. They are classified as flavonoids which comprise

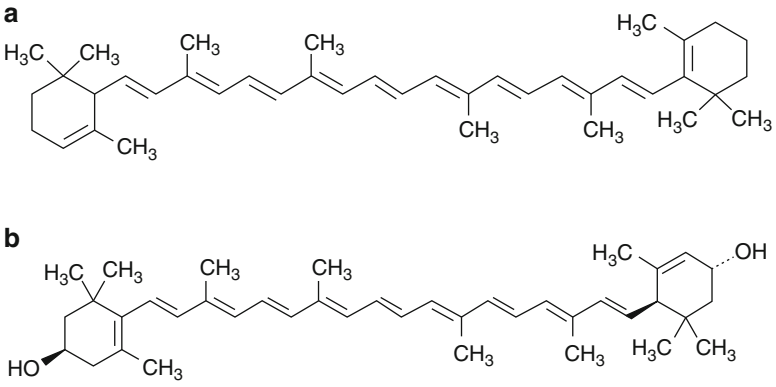


Fig. 5 Structures of carotenoids (**a**) Lycopene (**b**) Lutein

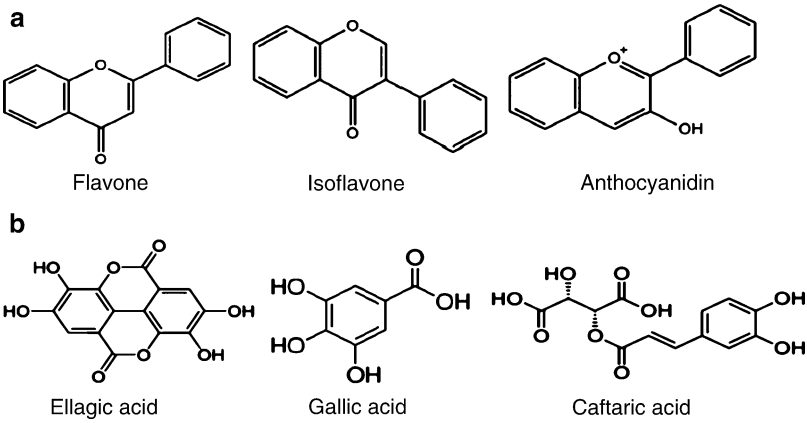


Fig. 6 Structures of polyphenols (**a**) Flavonoids (**b**) Nonflavonoids

of flavonols (kaempferol, quercetin), flavones (apigenin, luteolin), isoflavones (daiazein, genistein), flavanones (naringenin, hespertin), anthocyanidins (malvidin, cyaniding) and flavan-3-ols (catechin, epicatechin), and non-flavonoids including chlorogenic acids such as quinic acid and tartaric acid [40, 41] (Fig. 6). Phenolics content can vary tremendously between food sources and within foods of the same type. The following ranges were reported for total polyphenol content in some food materials and fruits: barley and millet (590–1500 mg 100 g^{-1} dry matter); oats and corns (8.7–30.9 mg 100 g^{-1} dry matter); fresh onions and leeks (20–20.25 mg 100 g^{-1} dry matter), fresh brussel sprouts (6–15 mg 100 g^{-1} dry matter), blueberries, strawberries, cranberries, and raspberries the total polyphenol content is about 37–429 mg 100 g^{-1} dry matter [42].

(c) **Phytosterols (example Stigmasterol)**: Phytosterols are the plant equivalent of cholesterol in animals. Their structures are similar. However, the side-chain in plant sterols contains additional double bonds and methyl and/or ethyl groups.

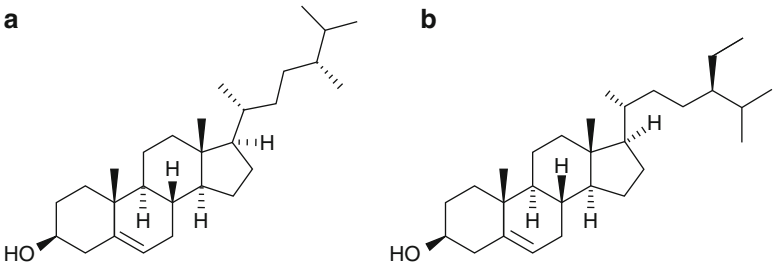


Fig. 7 Structures of phytosterols (**a**) campesterol (**b**) Beta-sitosterol

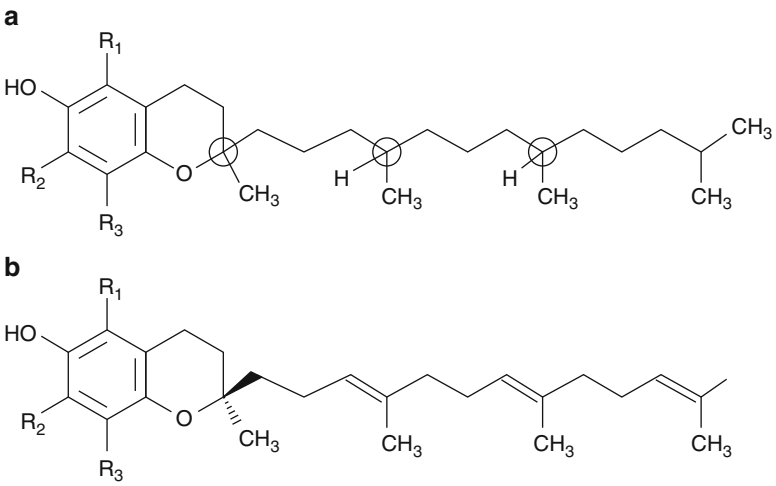


Fig. 8 Structures of (**a**) Tocopherol (**b**) Tocotrienol

The most common bioactive phytosterols are beta-sitosterol, kampesterol and stigmasterol (Fig. 7). A daily non-vegetarian diet contains approximately 250 mg of unsaturated phytosterols while a vegetarian diet contains over 500 mg. The saturated derivatives of plant sterols are plant stanols such as sitostanol [5].

(d) **Tocopherols/Tocotrienols (example Vitamin E)**: The tocopherols and tocotrienols are lipid-soluble functional components which contain a phenolic-chromanol ring linked to an isoprenoid side chain that is either saturated (tocopherols) or unsaturated (tocotrienols) (Fig. 8). There are also four primary forms of tocopherols and tocotrienols: alpha, beta, gamma, and delta that differ in the number and position of methyl groups on the phenolic-chromanol ring. In addition, tocopherols have three asymmetrical carbons at positions 2, 4, and 8 of the isoprenoid side chain. Consequently, there are eight isomeric forms of tocopherols, of which RRR-a-tocopherol has the greatest bioactivity and is also the most abundant in human blood and tissues [6, 40].

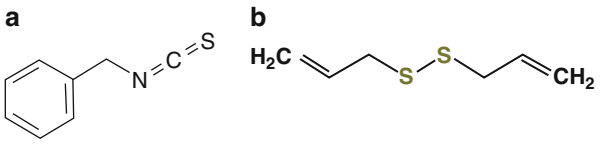


Fig. 9 Structures of organo-sulfur compounds (**a**) Benzyl isothiocyanate (**b**) Diallyl disulfide

(e) **Organo-sulfur compounds (example Diallyl sulphide)**: The organo-sulfur compounds are commonly found in cruciferous vegetables such as broccoli, cauliflower, and brussel sprouts or allium vegetables (vegetables in the same family or class with cabbage, onions and garlic) such as leeks. Organo-sulfur compounds contain sulfur atoms that are bound to a cyanate group or a carbon atom in a cyclic or non-cyclic configuration. The functional ingredients of foods containing organo-sulfur compounds are obtained only after cutting, chewing or crushing has disrupted the cells to expose them. In cruciferous vegetables, various isothiocyanates such as sulforaphane, phenyl isothiocyanate and benzyl isothiocyanate (Fig. 9a) are formed from glucosinolyates by the action of myrosinase. In alliums, allicin is formed from allin and then rapidly converted to diallyl sulfide, diallyl disulfide (Fig. 9b) or diallyl trisulfide by the action of allinase. In both cruciferous and allium vegetables, these hydrolytic breakdown products are the health-promoting functional components [5].

2.2.1 Functions

The primary functions of antioxidants include the regulation of the redox potential within a cell and the reduction of potential initiators of cell death and carcinogenesis. Hence antioxidants are anti-carcinogenic agents. The redox potential refers to the balance of the reducing and oxidizing reactions that occur within the cell. Redox changes within a cell are able to trigger various molecular responses such as induction of cell death and activation of signal transduction (the transfer of messages between cells and within a cell). Therefore, redox regulation of physiological and pathological processes is important in optimizing health and disease prevention [40, 43]. Other functional antioxidant compounds are able to bind to toxins or carcinogens in the intestinal tract, such as the binding of *N*-nitroso compounds by polyphenols in tea, thereby preventing their transformation or even absorption [40].

The lipid-lowering mechanism of phytosterol and stanols occurs by sequestering cholesterol in the intestinal tract and reducing its absorption. Epidemiological and experimental studies suggest that dietary phytosterols may offer protection from most of the common cancers in western societies such as colon, breast and prostate cancers [44]. The possible mechanism by which phytosterols offer this protection include its effects on membrane structure, tumor and host cell tissues, signal transduction pathways that regulate tumor growth and apoptosis, immune functions of the host and cholesterol metabolism by the host [21, 22]. Phytosterols are precursors for lactogogues. Lactogogues are generally prescribed to lactating mothers to augment milk production and also acts as a precursor for hormones required for

reproductive growth. Other phytosterols including stigmasterol, sitosterol and kampesterol are also precursors for hormones. These compounds increase the estrogen production, which in turn stimulates the proliferation of the mammary gland ducts to produce milk [45].

The structural similarity between several isoflavone metabolites and those of estrogens and estradiols suggests the possibility of estrogen-like biological activities in isoflavones. Isoflavones or phytoestrogens, however, exhibit antagonist estrogen activity resulting in lower overall exposure to estrogen in premenopausal women and reducing breast cancer risk [46–48]. In postmenopausal women, phytoestrogen-rich diets reduce hormone-sensitive plasma cholesterol levels and bone loss [49, 50].

Similarly, the induction of enzyme systems that detoxify toxic chemicals including the phase I (example, cytochrome P450 group of oxidases) and phase II (e.g., *N*-acetyl transferase, glutathione *S*-transferase, *UDP*-glucoronyl transferase, etc.) detoxifying enzymes is thought to reduce one's susceptibility to mutagenic effects. Functional food components with antioxidant functions are able to activate phase II detoxifying enzymes via the antioxidant responsive element pathway such as the nuclear factor [erythroid-derived 2]-like 2 also known as Nfe2l2 or Nrf2 signaling pathway [40, 42, 51]. Organo-sulfur compounds such as isothiocyanates, in particular sulforaphane, are potent mono-inducers of phase II detoxifying enzymes [52] while diallyl sulfides from garlic preparations are inducers of both phases I and II detoxifying enzymes [53].

A primary mechanism for immune-modulation is the multiple antioxidant capability of polyphenols, tocopherols, carotenoids, isothiocyanates, and allyl sulfides, lycopene being the most active oxygen neutralizer with potential chemo-preventive activities. Together, these compounds are able to reduce the deleterious effects of reactive oxygen species (ROS) and free radicals, which cause premature death of immune cells [54]. Garlic is found to be a superior phytochemical in the reduction of total cholesterol levels [55] and is also reported to control arterial stiffness by increasing the good cholesterol: high density lipoprotein (HDL) and decreasing the bad cholesterol: low density lipoprotein (LDL). It also inhibits inducible nitric oxide synthase by reducing the protein and *m*RNA and thus promotes vasodilatation of blood vessels. Garlic has strong immunopotential capacity and enhances the natural killer (NK) activity and proliferation of T-lymphocytes by delaying the hypersensitivity reaction [40].

Aged garlic extract is found to be a promising immune modifier with internal body regulatory functions, particularly in the control of sarcoma-180 and lung carcinoma as well as inhibition of platelet aggregation. Both the oil- and water-soluble components of garlic extract have shown health benefits. Specifically, its oil extract reduces serious mental disorder and prevents blood coagulation even in diabetics while its water extract is effective in cell cycle and viability of human hepatoma cells (HG2) [6]. Since garlic extract reverses oxidant responses it seems likely that it protects tissues from oxidative damages [56].

2.2.2 Sources

Carrots, squash, sweet potato, and spinach contain abundant beta-and alpha-carotene and the dark green leafy vegetables such as kale, spinach, mustard greens, and green

beans are good sources of lutein. Lycopene is found predominately in tomatoes. Other bioactive components in tomato are kaempferol or chlorogenic acid, which have anti-mutagenic activities. This suggests that tomato suspension have a protective effect on colon cancer which is mediated by the modulation of different biological pathways during carcinogenesis [57]. Typical dietary sources which are rich in tocopherol and tocotrienols include vegetable oils, nuts and the germ portion of grains [58].

Among the foods that have been shown to have beneficial immunomodulatory effects are broccoli, garlic, onions, vegetable oils, almonds, and walnuts [5, 6]. Similarly, garlic, soy bean, cabbage, ginger, licorice root extract (extract of the root of *Glycytthiza glabra*) and umbelliferous vegetables (vegetables that grow or produce its plant beneath the ground, e.g., carrots) have been identified as foods and herbs with the highest anticancer activity. Citrus in addition to providing an ample supply of vitamin C, folic acid, potassium and soluble fiber contains a host of active phytochemicals [58].

Green tea enhances humoral and cell-mediated immunity while decreasing the risk of certain cancers and cardio-vascular disease. On the other hand, ginseng enhances the production of macrophages and T-cells, natural killer cells and colony forming activity of bone marrow [59]. Soy bean, garlic, ginger and green tea which have been suggested, in epidemiological studies, to reduce the incidence of cancer may do so by inducing programmed apoptosis (cell death). Soybean extract has been shown to prevent the development of polycystic kidneys [60]. Turmeric is most potent against skin tumors [61].

Moringa oleifera is a rich source of a variety of functional components (phytochemicals) in its leaves, pods and seeds. Moringa is reported to contain 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach [62]. In addition, it contains other phytochemicals such as tannins, phenolics such as flavonoids, sterols, terpenoids, saponins, anthraquinones, alkaloids and reducing sugar as well as other anti-cancerous agents like glucosinolates, isothiocyanates, glycoside compounds, glycerol-1-9-octadecanoate and about 76% poly-unsaturated fatty acids such as linoleic acid, linolenic acid and oleic acid [45].

2.3 Probiotics and Prebiotics

(a) **Probiotics** are the beneficial live microorganisms, which, when administered in adequate amounts, confer health benefits on the host [63], examples Lactic acid bacteria (LAB), Biofidobacterium.

(b) **Prebiotics** are the non-digestible food ingredients that stimulate the growth and activity of probiotics in the digestive system in ways claimed to be beneficial to health. Thus, prebiotics are healthy non-digestible food ingredients that make their way through our digestive system and help the beneficial or good bacteria grow and flourish [63, 64].

2.3.1 Functions

Probiotics are believed to protect humans in two major ways. The first is the role that they play in human digestive tract. Human digestive tracts need a healthy balance between the good and bad microorganisms. But our lifestyles such as poor food choices, emotional stress, and lack of sleep, antibiotic over-use, other drugs, and environmental influences can shift the balance in favor of the bad microorganisms [65]. When the digestive tract is healthy, it filters out and eliminates things that can damage it, such as harmful microbes, toxins, chemicals, and other waste products. On the flip side, it takes in the things that the human body needs (nutrients from food and water) and helps deliver them to the cells where they are needed. The idea is not to kill off all of the microbes. Human bodies do have a need for the bad ones and the good ones. The problem is when the balance is shifted to have more bad than good. An imbalance has been associated with diarrhea, urinary tract infections, muscle pain, and fatigue [65].

The second major benefit of probiotics is the impact they have on the immune system. The immune system protects against pathogens. When it doesn't function properly, one can suffer from allergic reactions, autoimmune disorders (for example, ulcerative colitis, Crohn's disease, and rheumatoid arthritis), and infections (for example, infectious diarrhea, *Helicobacter pylori*, skin infections, and vaginal infections). By maintaining the correct balance from birth, these ailments are prevented [63, 66]. During delivery through the birth canal, a newborn picks up the beneficial bacteria from his/her mother. These good bacteria are not transmitted when a Caesarean section is performed and have been shown to be the reason why some infants born by Caesarean section have allergies, sub-optimal immune systems, and lower levels of gut micro-flora [65].

Some of the specific mechanisms by which probiotics exclude undesirable microorganisms include the production of inhibitory substances, blocking of adhesion sites, competition for nutrients, degradation of toxin receptors, and stimulation of immunity [66].

Prebiotics stimulate the growth of beneficial and healthy microorganisms (probiotics) in the gut with a resultant increased resistance to invading pathogens. This positive impact of prebiotics, in an unaltered form, in the human intestine is known as the *prebiotic effect*. However, such prebiotic effect is manifested when there is increase in the number and activity of probiotics. This effect is induced by consuming functional foods that contain prebiotics. The prebiotic definition does not emphasize a specific microbial group.

2.3.2 Sources

Dietary supplements and fermented food products have been advertised as containing beneficial cultures. These cultures are what would now be considered probiotics [67]. Other foods currently claimed to provide probiotics are cereal juice, frozen yogurt, granola, candy bars, and cookies. While they may contain probiotics, there is no guarantee that they have them in optimal levels. Only the manufacturer of the product can confirm if there are any studies to support his specific claims [65].

The most common types of prebiotics are non-starchy carbohydrates such as soluble dietary fiber (e.g., β-glucans, inulin, etc.) and other oligosaccharides such as fructo-oligosaccharide (fructans), galacto-oligosaccharide, etc. Many of the plants frequently eaten as vegetables – asparagus, garlic, leek, onion, artichoke – are excellent sources of inulin. β-Glucans and inulin are common in many plants containing dietary fiber and fructans. Traditional dietary sources of prebiotics also include soybeans, raw oats, unrefined wheat and unrefined barley. Some of the oligosaccharides that naturally occur in breast milk are believed to play an important role in the development of a healthy immune system in infants through the prebiotic-probiotic relationship.

3 Food as Medicine

Since ancient times, plants have always been the common sources of food and medicines, either in the form of traditional herbal preparations or as pure active principles [68]. Many herbal extracts have been tested in numerous systems to assess their chemopreventive and chemotherapeutic efficacy [69]. Similarly, there is growing evidence that functional components (phytochemicals) play an integral role in the link between food and health thereby providing disease-fighting foods for the prevention of chronic degenerative pathologies or in support of traditional remedies [18]. Most of the observed therapeutic effects of plants have been linked to their potent phytochemical content especially anti-oxidants. Healing of diseases or maintenance of a healthy lifestyle based on antioxidant activity could be the scientific basis of traditional herbal medicines [70].

In this regard, the eastern civilizations of Asia and the oriental cultures including the Chinese, Japanese, Indians and Egyptians were the few civilizations that explored these healing powers of food phytochemicals for thousands of years and provided evidences which supported claims that functional phytochemicals in foods can be effectively used to prevent the risk of developing diseases and promote well-being [71, 72]. However, a diet can only be adjudged healthy if its combination of individual food types is appropriate and their nutritive and non-nutritive metabolites are biologically available for cell absorption and utilization. Table 2 gives the effect of some physiological factors on the bioavailability of functional components. Limiting certain food types or components such as sugars, salts and saturated or trans-fatty acids alone or simply delivering an intake of pure nutrients alone may not be regarded entirely as a healthy diet. Therefore, while it is easy to recommend a healthy diet, it is much harder to define it for a particular need [9].

Hence, in this era when the role of a healthy diet in preventing non-communicable diseases are well accepted, the borderline between food and medicine is becoming very thin [9]. Thus, the concept of food has obviously gone beyond basic nutrition only. While products intended to cure diseases are classified as medicine, a healthy diet consisting of foods with functional components can help promote well-being and even reduce the risk of developing certain diseases [70].

Table 2 Effect of some physiological factors on the bioavailability of functional components[a]

S/N	Factors	Effect
1.	Food matrix	Functional components are compartmentalized, bound in cell walls and are unavailable
2.	Food preparation	Cutting, mashing, grinding, peeling, trimming activate enzymes, e.g., polyphenol oxidase, allinase. Improves availability of antioxidants
3.	Food processing: Thermal/non-thermal processing, (NTP example high pressure processing (HPP), pulsed electric field (PEF))	Mild heat treatment (steaming, blanching) over short period improves antioxidant content but this decreases with severe treatment (boiling, cooking). NTP has positive effect on polyphenols and negative effect on carotenoids
4.	Food mixtures	Enhances availability of polyphenols with sugars, ascorbic acid, fat and stabilizing effects of polyphenols on other phytochemicals
5.	Gastric digestion	De-polymerization of large polyphenols, hydrogen bonding of polyphenols to proteins
6.	Small intestine	Micelle formation, increased role of uptake and efflux transporters (cell to gut lumen to basolateral to cell), transition from matrix to oil phase
7.	Colon	Phase I/II interactions, influence of micro-flora on phytochemicals, colonic absorption, increased formation of metabolites and phase I/II products
8.	Tissues	Biotransformation, phase I and II metabolism, interaction with transporters in certain tissues (blood-brain barriers, placenta, testis) or excretory organs (liver, kidney)

[a]Bohn et al. [15]

4 Healing Powers of Functional Components

Some properties which link functional components to potential health-modulating roles and functions can be classified into antioxidation, anti-cancerous, anti-diabetic, anti-inflammatory, prevention of cardio-vascular diseases, anti-microbial, immune-modulatory and anti-hypertensive [20, 73, 74].

4.1 Anti-oxidation Properties

Free radicals in the form of reactive oxygen species (ROS) or reactive nitrogen species (RNS) are the usual by-products of body metabolic activities which involve

oxidation and reduction reactions in cells. It is expected that at low concentrations, these reactions have beneficial effects on cellular responses and immune functions [75]. However, at high concentrations they cause the condition known as oxidative stress which is harmful to cell structures. In simple terms, oxidative stress is defined as the condition whereby the balance between formation and removal of free radicals is shifted towards formation of more free radicals rather than their removal [76]. It is generally believed that oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, diabetes and cardio-vascular diseases [75, 77]. The human body has its own endogenous anti-oxidant system designed to combat oxidative stress which is supported and strengthened by exogenously supplied anti-oxidants in diets [75]. In this regard, phytochemicals such as phenolic compounds are believed to play key role in protecting the body against oxidative stress and its effects due to their well-known anti-oxidant properties. The antioxidant capacity or activity of phytochemicals, in vitro, is well reported in literature and serves as an indicator of the potential ability of dietary antioxidants to combat oxidative stress [78, 79]. This has been determined mostly through free-radical scavenging ability although other assays have been used such as inhibition of lipid peroxidation and metal ion chelating capacity. Similarly, the potential of anti-oxidants to combat oxidative stress have been demonstrated in vivo, mostly in rats. This involved the monitoring of activities of anti-oxidant enzymes or anti-oxidant molecules within the experimental animal on feeding it with the phytochemical of choice [79–81]. Overall, anti-oxidant supplementation reduced lipid peroxidation and enhanced anti-oxidant capacity of blood (plasma), heart, kidney, testes, lung and pancreas. It is therefore acceptable that oxidative stress is a precursor for the development of various chronic and degenerative diseases. It has been suggested that free radicals are involved in the pathology of more than 50 human diseases, including aging [82]. However, it is also important to note that free radicals are not harmful at all times, rather, their toxicity depends on several factors including the type of ROS/RNS, their concentration and localization, and the kinetics of their production and elimination [83].

4.2 Anti-cancer Properties

Various reports provide some evidence of potential anticancer properties of functional components of food. These properties have been demonstrated in vitro through assays including their effects on oxidative stress (free-radical scavenging activity), inhibition of DNA damage, anti-proliferative effects on cancer cell lines and phase II enzyme induction [84]. Methoxylated 3-deoxyanthocyanins was found to be the most potent inducer of quinine oxidoreductase, a phase II detoxifying enzyme [85]. The effects of functional components on oxidative DNA damage have been reported [86]. Higher total phenolic contents including flavonoids and non-flavonoids such as rutin and quercetin increased the inhibition of oxidative DNA damage. Similarly, the inhibition of hydroxyl radical-induced DNA damage by an anthocyanin has also been reported [87].

4.3 Anti-diabetic Properties

Diabetes is associated with various conditions including oxidative stress, impaired insulin secretion and insulin resistance due to malfunctioning of β-cells of the pancreatic islets of langerhan, leading to defective utilization of carbohydrates as energy source [88]. Due to their anti-oxidant properties, phenolic compounds may reduce oxidative stress conditions and also protect pancreatic β-cells. Another widely used way of demonstrating anti-diabetic effects of functional components is by determining their inhibitory effects against starch-degrading enzymes such as α-amylase and α-glucosidase. Phytochemicals have been shown to possess inhibitory effects against α-amylase and α-glucosidase [89, 90]. Some in vivo studies on the anti-diabetic effects of phytochemicals have also been reported. Diets high in phytochemicals could protect against hyperglycemia and alloxan-induced oxidative stress through the restoration of levels of endogenous enzymatic superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X), and glutathione reductase (GR) and non-enzymatic (vitamins E and C) anti-oxidants in diabetic rats [91].

4.4 Anti-inflammatory Properties

Inflammation may be described as an immune response to cellular or tissue injury or infection by pathogens [92]. The condition of inflammation itself is not considered a disease. However, if left unchecked to become chronic, there can be exacerbated tissue damage and modulation of various cell-signaling pathways [93]. Chronic inflammation causes life style diseases, such as obesity, metabolic syndrome, arterial sclerosis, and cancer [94–98]. The process of inflammation consists of a wide and complex range of cellular and molecular pathways and reactions involving a host of enzymes [92]. These enzymes include cyclooxygenase, lipoxygenase, phospholipase A2 and nitric oxide synthase. Cytokines such as IL-1, IL-2 and TNF-α and nitric oxide are important pro-inflammatory products of cellular reactions [16, 73]. Phytochemicals can modulate inflammatory processes by inhibiting pro-inflammatory enzymes [92] which influence the production of the cytokines and nitric oxide. The anti-inflammatory properties of dietary phytochemicals may be determined by their inhibitory activities against the pro-inflammatory enzymes and by monitoring the production of pro-inflammatory cytokines. Simple nitric oxide radical scavenging capacity could also be used as an indicator of anti-inflammatory properties. In vitro and in vivo anti-inflammatory activities have been widely reported [80, 81, 99].

4.5 Cardio-vascular Properties

The oxidation of LDL seem to trigger-off the onset of coronary heart disease [100]. The oxidized LDL is taken up by macrophages (foam cells) and smooth muscle cells leading to the formation of fatty streaks and eventual development of arteriosclerosis [101]. The prevention of the oxidation of LDL may be a potential prevention of

cardiovascular disease. The ability of functional components including phenolics to prevent LDL oxidation by exerting anti-oxidant effects such as cholesterol lowering effects through the suppression of LDL-cholesterol and the elevation of HDL cholesterol that is important for the prevention of cardiovascular disease has been reported in literature [102]. The effect of supplementation of phytochemicals from a pseudocereal (quinoa) in a fructose containing diet on some cardiovascular disease parameters in male Wistar rats was studied by Pasko et al. [103]. Rats fed with quinoa had reduced serum total cholesterol, LDL, triacylglycerol, glucose and plasma total protein. Those fed fructose had decreased HDL but this decrease was reversed on feeding with quinoa. The reduction in LDL and prevention in decrease in HDL on feeding with quinoa suggests potential ability of the phytochemicals in quinoa to prevent cardio-vascular disease [73]. The observed hypo-cholesterolemic effects may be due to synergistic effects of high crude fiber and high anti-oxidant concentrations such as quercetin. Thiol-containing compounds and phenolics were shown to inhibit rat microsomal lipid peroxidation which may be significant for the prevention of LDL oxidation and hence prevention of arteriosclerosis and cardiovascular disease [104]. Oxidative stress-mediated conditions such as cardiovascular disease are also accompanied by loss of endogenous antioxidant compounds such as glutathione-glutathione disulfide ratio [77].

4.6 Anti-hypertensive Properties

Functional components possess therapeutic capacity to decrease blood pressure [105]. These non-nutritive food factors such as flavonoid polyphenols (flavonols example quercetin; flavones example lutelin; and isoflavones example coumesterol) and carotenoids are scavengers of free radicals like superoxide anions, lipid proxy radicals, and lipid oxidation of LDL cholesterol, hence are anti-atherogenic (decreasing the formation of atherosclerotic plaques and arterial stiffness) thereby making arteries more responsive to vasodilation thereby lowering blood pressure [106]. Low risk of stroke is associated with high intake of plant food materials including fruits and vegetables and thus prevents hypertension [105]. In addition, several plant sources in the forms of herbs and spices including *Allium sativum* (Garlic), *Camellia sinensis* (Tea), *Hibiscus sabdariffa* (Roselle), *Nigella sativum* (Black cumin), *Panax* (Ginseng), *Cymbopogon citratus* (Lemon grass), *Zingiber officinale* (Ginger) contain non-nutritive secondary metabolites which have been demonstrated to possess vaso-relaxant, anti-oxidant, anti-proliferative, anti-inflammatory, and diuretic functions which play anti-hypertensive roles [107].

5 Optimal Health and Well-Being

Health and well-being or wellness are terms which are often used interchangeably, but their meanings are no doubt different. According to the World Health Organization, health is a state of "complete" physical, mental and social well-being and not

merely the absence of disease or infirmity. Health is therefore a dynamic condition resulting from a body's constant adjustment and adaptation in response to stress and changes in the environment for the maintenance of a homeostatic inner equilibrium [108]. Accordingly, the primary determinants of health include social, economic, and physical environments, as well as the individual's personal characteristics and behaviors. This definition has attracted serious criticisms for being overtly inclusive and practically unattainable, especially as it relates to the word "complete." However, "complete" medically expands the definition of health beyond the mere absence of diseases [109]. The maintenance and improvement of health, accordingly, depends not only on external or environmental factors (including the health care systems) but also on the efforts and lifestyle choices of the person involved [110]. On the other hand, optimal health is a level of health where the body's natural defense mechanisms are activated to function maximally. Optimal health turns off all chronic infections and oxidative stress. It prepares the body to respond quickly and effectively to any invasion by pathogens, toxins and other infections thereby preventing the onset of degenerative diseases including cancer, hypertension, stroke, diabetes, etc.

Although variously defined, depending on the context of usage, well-being generally depicts a healthy balance of the mind, body and spirit (state of comfortability or happiness) which results in an overall feeling of wholesomeness. However, according to the National Wellness Institute, wellness is considered as an active process through which people become aware of, and make choices towards, a more successful existence based on a conscious, positive and affirming, self-directed and evolving process of achieving full potential; a multidimensional and holistic lifestyle, encompassing mental and spiritual well-being, and the environment. Therefore, in understanding the difference between these two terms, health could be regarded as a state of being – both physical, mental, and social – whereas wellness aims to enhance this state of being by living a healthy lifestyle [110].

6 Mode of Action of Functional Components and How they Impact on Optimal Health and Well-Being

The mechanisms through which functional components impact on human health are not completely clear and seem to be multiple [18, 111]. Some identified multiple molecular targets of functional components include pro- and anti-apoptotic proteins, cell cycle proteins, cell adhesion molecules, protein kinases, transcription factors, metastasis and cell growth pathways [112–114]. Phytochemicals such as epigallocatechin-3-gallate (EGC-3-G) from green tea, curcumin from turmeric, and resveratrol from red wine tend to aim at a multitude of molecular targets. These multiple characteristics may have contributed to the unavailability of definitive mechanisms of action despite decades of research [115]. Specifically, the main mechanisms suggested for phenolics include an improvement in the lipid profile of plasma, an increase in its antioxidant activity as well as an enhancement of the endothelial function by exerting anti-inflammatory effects, reducing LDL oxidation, inhibiting

endothelial NADPH oxidase, modulating nitric oxide synthase activity/expression and increasing nitric oxide status [116, 117].

Data from in vitro studies suggest a protective interaction of flavonoids with lipid bilayers against oxidative damage, as a result of their localization in lipoprotein domains and cell membranes thus explaining the possible in vivo role phenolics in protecting LDL from oxidation. Moreover, it has been hypothesized that bioactive compounds, once absorbed and metabolized, may accumulate inside the cell membrane modifying the membrane composition, fluidity and functionality [83, 118]. In vivo, plant antioxidants are generally assessed for their effects on the activity of endogenous antioxidant enzymes or oxidative damage biomarkers before and after induction of oxidative stress in experimental animals. Some of these commonly used methods directly evaluate the enzymatic activity of endogenous anti-oxidants such as SOD, CAT, GPx and GR, while other methods involve quantification of oxidative damage biomarkers. The formation of specific end products resulting from interaction of ROS with biologically important macromolecules such as DNA, protein and lipids is measured by quantifying oxidative damage biomarker methods. DNA damage is determined by measuring the 8-hydroxydeoxyguanosine content. Carbonyl and aldehyde such as malondialdehyde contents are measured as biomarkers of protein and lipid oxidation, respectively [119]. Potential health benefits of functional components of food are summarized in Fig. 10.

Several claims have been made, evaluated and approved by various regulatory agencies in different parts of the world in respect of the health benefits and positive effects of functional components of food such as soluble DF including β-glucans on health and well-being [120]. Such approvals include those by the US Food and Drug Administration (FDA) in 1997 [121]; the Panel on Diabetic Products and Nutrition and Allergies of the European Commission in 2009 and 2011, respectively [122, 123]; the Joint Health Claims Initiative (JHCI) expert committee and council of the UK [124] and in Sweden [28]. These approvals were based on recommended dose of 3.0 g day^{-1} of β-glucans to attain health benefits including maintenance of gut health and normal blood cholesterol level, reduction of energy and post-prandial glycemic responses. Processed β-glucans are effective food supplement which has no side effects irrespective of level of consumption and therefore generally recognized as safe (GRAS) [125].

7 Effect of Processing on Functional Components of Food

Processing exercises some effects which could be positive or negative on phytochemicals and other functional components of food. While some food processing techniques increase the concentration of these functional components in food, others decrease them (Table 2). It has been shown that the length of post-harvest storage, steam blanching, and thermal processing all influence the retention of functional compounds in allium vegetables [126]. However, losses of about 30-80% of bioactive isothiocyanates through heat processing have been reported

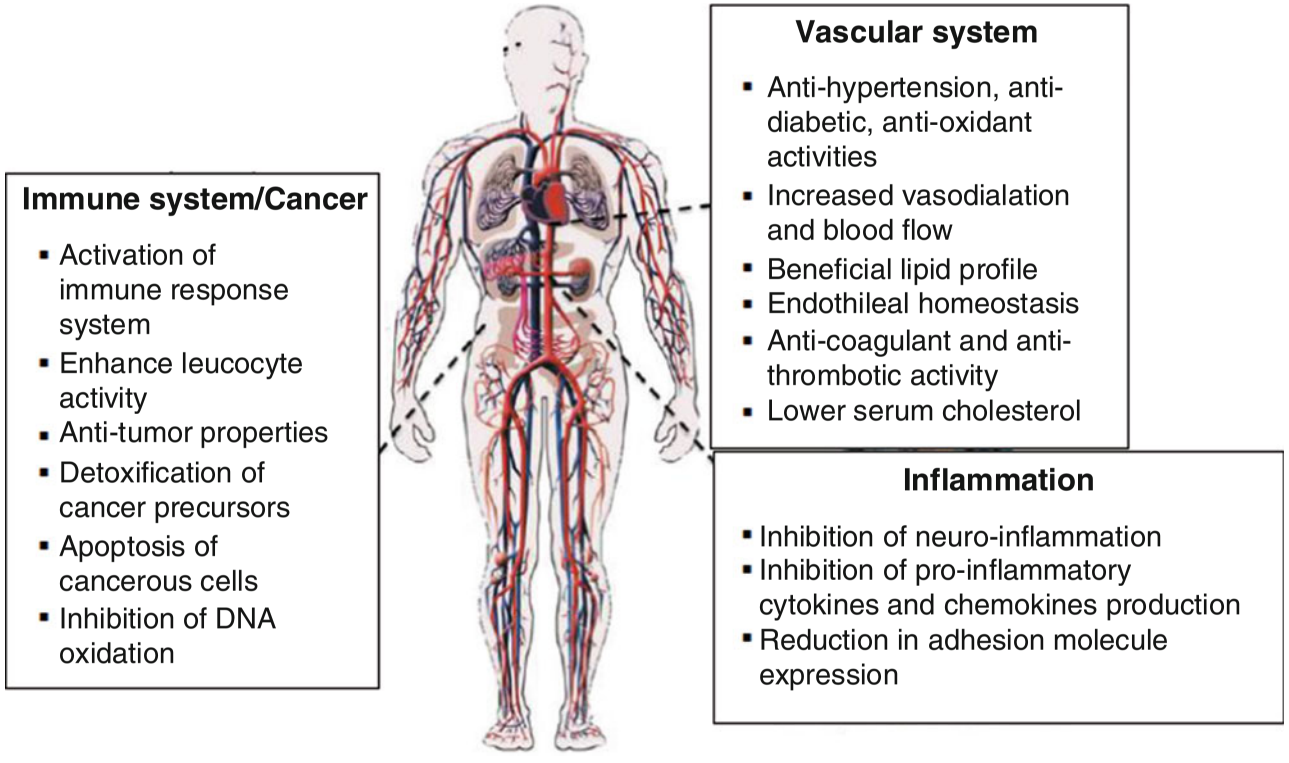


Fig. 10 Potential health benefits of functional components

[120]. In addition, high temperatures (100 °C and above) inactivates key enzymes such as myrosinase in cruciferous vegetables and allinase in allium vegetables, thereby reducing the amount of functional components in these food materials. However, temperatures associated with normal cooking have shown little evidence of substantial loss of isothiocyanates. Leaching of glucosinolates and their hydrolysis products also results in a reduction in total phytochemical content following cooking. Research has shown that heating garlic in a microwave oven for 30-60 s or heating it to a temperature of 60-100 °C results in significant losses of its anti-inflammatory, anti-cancerous, anti-microbial and anti-oxidant activities [127].

The bioavailability of carotenoids and other lipid-soluble functional food components have been shown to improve with processing techniques that increase surface area, such as size-reduction, example, cutting and chopping, as well as heat treatment that breaks down protein and carbohydrate matrix [128, 129]. Also, the brewing of tea leaves, whether black or green, releases 69-85% of their bioactive flavonoids within 3-5 min in hot water [129].

In contrast, drying pre-treatment before boiling was found to reduce cooking time thereby leading to less leaching of anti-oxidants in edible Irish seaweed *Homanthalia elongata*. In terms of extract, drying followed by boiling of *Homanthalia elongata* had the most significant effect on phytochemicals as its total phenolic content increased by 174%. However, this processing treatment reduced its anti-microbial activity compared to extracts from fresh samples [130]. Table 3 below gives a summary of the effect of some processing techniques on the anti-oxidant content of some foods.

Table 3 Effect of processing on antioxidant content of some foods compared to nonprocessed foods[b]

S/N	Food	Type of processing	Effect (%)
1.	Apple	Peeling	(−) 33–66%
2.	Carrots	Steaming	(+) 291%
3.	Carrots	Boiling	(+) 121–159%
4.	Cucumbers	Peeling	(−) 50%
5.	Asparagus	Steaming	(+) 205%
6.	Broccoli	Steaming	(+) 122–654%
7.	Green cabbage	Steaming	(+) 448%
8.	Red cabbage	Steaming	(+) 270%
9.	Green pepper	Steaming	(+) 467%
10.	Red pepper	Steaming	(+) 180%
11.	Potatoes	Steaming	(+) 105–242%
12.	Tomatoes	Steaming	(+) 112–164%
13.	Spinach	Boiling	(+) 114–184%
14.	Sweet potatoes	Steaming	(+) 413

[b]Hadvorsen et al. [131]

8 Conclusion

It is a widely held view that the consumption of a healthy diet can help prevent non-communicable degenerative diseases. This is as a result of the presence of non-nutritive or secondary metabolites known as functional components especially in plant based foods. Similarly, there is also growing evidence that functional components including phytochemicals and bioactives play an integral role in the link between food, optimal health and well-being. Epidemiological data support this preventive and protective effects of food against chronic degenerative pathologies such as cancer, diabetes, and cardiovascular diseases as a result of their functional components. Such functional components include dietary fibers, fucoidans, carotenoids, phenolics, phytosterols, tocopherols/trienols, organo-sulfur compounds, prebiotics and probiotics.

Increasing scientific evidence indicate that insufficient functional components in diets through the low consumption of foods such as fruits, vegetables, nuts, whole grains, spices is among the risk factors which contribute to increased incidence of noncommunicable "silent killer" diseases in the world today. Some properties which link functional components to potential health benefits can be classified into antioxidation, anti-cancerous, anti-diabetic, anti-inflammatory, cardiovascular, anti-microbial, immunomodulatory and anti-hypertensive, anti-coagulant and anti-thrombotic activity of blood plasma, anti-virus, reduction of blood lipids including cholesterol, anti-complementary properties, activity against hepatopathy, uropathy and renalpathy, protective effects on gastric organ and gastric mucosa.

Thus, the idea of food these days has obviously gone beyond mere provision of biochemical building blocks necessary to refresh and nourish life in the form of primary nutrients to cells of an organism. While products intended to cure diseases could be classified as medicine, a healthy diet consisting of appropriate food types with functional components can serve as a scientific alternative to reduce the risk of developing certain diseases. Obviously, functional components of food will play an important role in health maintenance in the future as a result of their medicinal properties. However, their bioavailability and levels required in humans are critical factors necessary to optimize their potential benefits. Hence, in this era when the role of a healthy diet in preventing non-communicable diseases is well accepted, the borderline between food and medicine is becoming very thin.

Acknowledgements The author expresses grateful acknowledgement to Dr. Elena Barascu of Department of Food Engineering, Valahia University, Targoviste, Dambovita, Romania and Prof. Lavinia Buruleanu, the Editor-in-Chief, Annals Food Science and Technology for granting kind permission to reuse the original article, Current developments on β-glucans as functional components of food: a review published in volume 14, issue 2, and pages 217–229 of its 2013 edition in this work. It is further acknowledged that the journal article, Functional components and medicinal properties of food: a review which was first published in the Journal of Food Science and Technology, volume 52, issue 5, pages 2522–2529 in 2015 also contributed to this book chapter project.

References

1. Lapedes DN (1977) Encyclopedia of food, agriculture and nutrition. McGraw Hill, New York
2. Bazzano LA (2005) Dietary intake of fruit and vegetables and risk of diabetes mellitus and cardiovascular diseases [electronic resource]. In: Background paper of the joint FAO/WHO workshop on fruit and vegetables for health. World Health Organization, Kobe
3. Agudo A (2004) Measuring intake of fruit and vegetables. Background paper the joint FAO/WHO workshop on fruit and vegetables for health, 1–3 Sept 2004, Kobe.
4. Murano PS (2003) Phytochemicals and phytonutrients. In: Understanding food science & technology. Wadsworth, Bemont
5. Swanson JE (2003). Bioactive food components. Encyclopedia of Food and Culture. http://www.enotes.com/bioactive-food-components-reference/bioactive-food-components. Accessed 18 Nov 2012
6. Srividya AR, Nagasamy V, Vishnuvarthan VJ (2010) Nutraceutical as medicine: a review. Pharmanest 1(2):132–145
7. Shibamoto T, Kanazawa K, Shahidi, F (2008) Functional food and health, ACS Symposium. ISBN: 978-0-8412-6982-8
8. Wildman REC (2001) Handbook of nutraceuticals and functional foods (1st ed.). CRC Series. Boca Raton, Florida. ISBN: 0-8493-8734-5
9. Pravst I (2012) Functional foods in Europe: a focus on health claims. In: Valdez B (ed), Scientific, health and social aspects of the food industry. http://www.intechopen.com/books/scientific-health-and-social-aspects-of-the-food-industry/functional-foods-ineurope-a-focus-on-health-claims. Accessed 25 Jan 2013
10. Zanotti I, Dall'Asta M, Mena P, Mele L, Bruni R, Ray S, Rio DD (2015) Atheroprotective effects of (poly)phenols: a focus on cell cholesterol metabolism. Food Funct 6:13–31
11. Pang G, Xie J, Chen Q, Hu Z (2012) How functional foods play critical roles in human health. Food Sci Hum Wellness 1:26–60. https://doi.org/10.1016/j.fshw.2012.10.001
12. Kasote DM, Katyare SS, Hegde MV, Bae H (2015) Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci 11(8):982–991
13. Korkina LG (2007) Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. Cell Mol Biol 53:15–25
14. Patra B, Schluttenhofer C, Wu Y, Pattanaik S, Yuan L (2013) Transcriptional regulation of secondary metabolite biosynthesis in plants. Biochim Biophys Acta 1829:1236–1247
15. Bohn T, McDougall GJ, Alegria A, Alminger M, Aura A, Brito C, Cilla A, El SN, Arrigoni E, Karakay S, Martinez-Cuesta MC (2015) Santos CN. Mind the gap – deficits in our knowledge of aspects impacting the bioavailability of phytochemicals and their metabolites– a position paper focusing on carotenoids and polyphenols Mol Nutr Food Res 59:1307–1323. https://doi.org/10.1002/mnfr.201400745
16. Kang KS, Yamab N, Wen Y, Fukui M, Zhu BT (2013) Beneficial effects of natural phenolics on levodopa methylation and oxidative neuro-degeneration. Brain Res 25:1–14
17. Tresserra-Rimbau A, Medina-Remon A, Perez-Jimenez J, Martínez-Gonzalez MA, Covas MI, Corella D, Salas-Salvadó J, Gómez-Gracia E, Lapetra J, Arós F, Fiol M, Ros E, Serra-Majem L, Pinto X, Munoz MA, Saez GT, Ruiz-Gutierrez V, Warnberg J, Estruch R, Lamuela-Raventos RM (2013) Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: the PREDIMED study. Nutr Metab Cardiovasc Dis 23:953–959
18. Giampieri F, Tamara Y, Forbes-Hernandez GM, José M, Alvarez-Suarez S, Afrin Bompadre S, Quiles JL, Mezzett B, Battino M (2015) Strawberry as a health promoter: an evidence based review. Food Funct 6:1386–1398
19. Sheu SC, Lai MH (2012) Composition analysis and immuno-modulatory effect of okra (*Abelmoschus esculentus* L.) extract. Food Chem 134:1906–1911
20. Abuajah CI, Ogbonna AC, Osuji CM (2015) Functional components and medicinal properties of food: a review. J Food Sci Technol 52(5):2522–2529. https://doi.org/10.1007/s13197-014-1396-5

21. Akramien D, Kondrotas A, Didziapetriene J, Kevelaitis E (2007) Effects of β-glucans on the immune system. Medicina (Kaunas) 43(8):597–606
22. Chan GCF, Chan WK, Sze DMY (2009) The effects of β-glucan on human immune and cancer cells. J Haematol Oncol 2:25. https://doi.org/10.1186/1756-8722-2-25
23. Havrlentova M, Petrulakova Z, Burgarova A, Gago F, Hlinkova A, Sturdik E (2011) Cereal β-glucans and their significance for the preparation of functional foods – a review. Czech. J Food Sci 29(1):1–14
24. AACC (2001) The definition of dietary fibre. Cereal Foods World 46:112–129
25. Andlauer W, Furst P (2002) Nutraceuticals: a piece of history, present status and outlook. Food Res Int 35:171–176
26. Charalampopoulos D, Wang R, Pandella SS, Webb C (2002) Application of cereals and cereal components in functional food: a review. Int J Food Microbiol 79:131–141
27. Ahmad A, Anjum MF, Zahoo T, Nawaz H, Din A (2009) Physicochemical and functional properties of barley b-glucan as affected by different extraction procedures. Int J Food Sci Technol 44:181–187. https://doi.org/10.1111/j.13652621.2008.01721.x
28. Duss R, Nyberg L (2004) Oat soluble fibres (β-glucans) as a source for healthy snack and breakfast foods. Cereal Foods World 49(6):320–325
29. Li B, Lu F, Wei X, Zhao R (2008) Fucoidan: structures and bioactivity. Molecules 13:1671–1695. https://doi.org/10.3390/molecules13081671
30. Becker DJ, Lowe JB (2003) Fucose: biosynthesis and biological function in mammals. Glycobiol 13(7):41R–53R. https://doi.org/10.1093/glycob/cwg054
31. MSKCC (2011) Fucoidan. Memorial Sloan-Kettering Cancer Center. http://www.mskcc.org/cancer-care/herb/fucoidan. Accessed 18 Oct 2013.
32. Izydorczyk MS, Dexter JE (2008) Barley β-glucan and arabinoxylans: molecular structure, physicochemical properties and uses in food products – a review. Food Res Int 41(9):850–868
33. Du B, Lin CY, Bian Xu BJ (2015) An insight into anti-inflammatory effects of fungal beta-glucan. Trends Food Science Technol 41:49–59
34. Brown GD, Gordon S (2001) Immune recognition: a new receptor for betaglucans. Nature 413:36–37
35. Brown GD (2006) Dectin-1: a signalling non-TLR pattern-recognition receptor. Nat Rev Immunol 6:33–43
36. Novak M, Vetvicka V (2008) Beta-glucans, history, and the present: immunomodulatory aspects and mechanisms of action. J Immunotoxicol 5:47–57
37. Babal, K, Gionta, RA (2010) Seafood sense. https://www.google.com/search?q=readhowyouwant&safe=strict&gws_rd=ssl. Accessed 18 Oct 2011.
38. WineBrenner J (2007) Fucose or fucoids are the fourth essential sugar. Nutr health. Newsletter 2(5):1–3
39. Ali R, Athar M, Abdullah US, Abibi A, Qayyum M (2009) Nutraceuticals as natural healers: emerging vvidences. Afr J Biotechnol 8(6):891–898
40. Parker RS (2000) Phytochemicals: carotenoids. In: Francis FJ (ed), Wiley encyclopedia of food science and technology (2nd ed., vol. 3), Wiley, New York.
41. Rio DD, Rodriguez-Mateos A, Spencer JPE, Togrolini M, Borges G, Crozier A (2013) Dietary (poly)phenolics in human halth: structure, availability and evidence of protective effects against chronic diseases. Antiox Redox Signal 18(14):1819–1892. https://doi.org/10.1089/ars.2012.4581
42. Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56(11):317–333
43. Kumar S (2011) Free radicals and antioxidants: human and food system. Adv Appl Sci Res 2(1):129–135
44. Boothe DM (1978) Nutraceuticals in veterinary medicine. Part- I: definition and regulations. Comp Cont Ed 19(11):1248–1255
45. Gropalakrishnan L, Doruja K, Kumar DS (2016) Moringa Oleifera:a review on nutritive importance and its medicinal application. Food Science Hum Wellness 5:49–56

46. Shimizu H, Ross RK, Bernstein L, Pike MC, Henderson BE (1990) Serum estrogen-levels in postmenopausal womenomparison of American whites and Japanese in Japan. Brit. J Cancer 62(4):51–53
47. Cassidy A, Bingham S, Setchell KDR (1994) Biological effects of isoflavones present in soy in premenopausal women: implications for the prevention of breast cancer. American. J Clin Nutr 60(3):30–40
48. Cassidy A, Bingham S, Setchell KDR (1995) Biological effects of isoflavones in young women: importance of the chemical composition of soya products. Brit. J Nutr 74(5):87–90
49. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW (1998) Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr 68(1):325S–379S
50. Setchell KDR, Cassidy A (1999) Dietary isoflavones: biological effects and relevance to human health. J Nutr 129(7):58S–67S
51. Mukhtar H, Ahmad N (2000) Tea polyphenols: prevention of cancer and optimizing health. Am J Clin Nutr 71(1):698S–702S
52. Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P (1994) Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proc Natl Acad Sci 91(3):147–150
53. Yang CS, Chhabra SK, Hong JY, Smith T (2001) Mechanisms of inhibition of chemical toxicity and carcinogenesis by diallyl sulfide (DAS) and related compounds from garlic. J Nutr 131(1):41S–45S
54. Brennan LA, Morris GM, Wasson GR, Hannigan BM, Barnett YA (2000) The effect of vitamin C or vitamin E supplementation on basal and H_2O_2-induced DNA damage in human lymphocytes. Brit J Nutr 84:195–202
55. Dureja HD, Kaushik KV (2003) Development of nutraceuticals. Ind J Pharmacol 35:363–372
56. Crandell K, Duren S (2007) Nutraceuticals: what are they and their works? J Biotechnol 34(3):29–36
57. Hardy G, Hardy I, McElory B (2002) Nutraceuticals, pharmaceutical viewpoint: current opinion. Clin Metab Care 5:671–677
58. Dzanis DA (1998) Nutraceuticals: food or drug? J Nutr 42(2):430–431
59. Diplock AT, Aggett PJ, Ashwell M, Borner F, Fern EB, Robertford MB (1999) Scientific concepts of functional foods in Europe: concesdocument. Brit J Nutr S1:520–527
60. Chang J (2000) Medicinal herbs: drug or dietary supplements? Biochem Pharmacol 59:211–219
61. Halt M (1998) Moulds and mycotoxin in herb tea and medicinal plants. Eur J Epidemiol 14:269–274
62. Rockwood JL, Anderson BG, Casamatta DA (2015) Potential uses of *Moringa oleifera* and anexamination of antibiotic efficacy conferred by *M. oleifera* seed and leaf extracts using crude extraction techniques to underserved indigenous populations. Int J Phytotherap Res 3:61–71
63. Iwe MO (2006) Current trends in processed foods consumption – emphasis on prebiotics and probiotics. A technical paper presented at the quarterly meeting of the south-east chapter of NIFST at MOUA, Umudike, Abia state, Nigeria on 11 March 2006
64. Jegtvig S (2012) Prebiotics and probiotics. http://nutrition.about.com/od/therapeuticnutrition1/p/proprebiotics.htm Accessed 28 May 2012
65. Kovacs, B (2012). Probiotics. http://www.medicinenet.com/probiotics/page4.htm. Accessed 27 May 2010
66. Chow (2002) Probiotics and prebiotics: a brief overview. J Ren Nutr 12(2):76–86
67. Asmahan A (2010) Beneficial role of lactic acid bacteria in food preservation and human health: a review. Res J Microbiol 5:1213–1221
68. Kohen R, Nyska A (2002) Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol 30:620–650
69. Aqil F, Munagala R, Jeyabalan J, Vadhanam MV (2013) Bioavailability of phytochemicals and its enhancement by drug delivery systems. Cancer Lett 334(1):133–141. https://doi.org/10.1016/j.canlet.2013.02.032

70. Hegde MV, Patil S, Bhalerao S (2013) A philosophy for integration of ayurveda with modern medicine: a biochemist's perspective. Current Sci 95:721–722
71. Howlett J (2008) Functional foods: from science to health and claims. ILSI Europe, Brussels
72. Alois J, Svjetlana M (2012) Anti-inflammatory properties of culinary herbs and spices that ameliorate the effects of metabolic syndrome. Maturitas 71:227–239
73. Taylor JRN, Belton PS, Beta T, Duodu KG (2013) Increasing the utilization of sorghum, millets and pseudocereals: development in the science of their phenolic phytochemicals, biofortification and protein functionality. J Cereal Sci 59:257–275. https://doi.org/10.1016/j.jcs.2013.10.009
74. Doughari HJ (2012) Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In: Rao V (ed) Phytochemicals – a global perspective of their role in nutrition and health. http://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-in-nutrition-andhealth/phytochemicals-extraction-methods-basic-structures-and-mode-of-action-as-potentialchemotherapeutic-. Accessed 26 Dec 2015
75. Pham-Huy LA, He H, Pham-Huy C (2008) Free radicals, antioxidants in disease and health. Int J Biomed Sci 42:89–96
76. Shinde A, Ganu J, Naik P (2012) Effect of free radicals and antioxidants on oxidative stress: a review. J Dent Allied Sci 1:63–66
77. Fearon IM, Faux SP (2009) Oxidative stress and cardiovascular disease: novel tools give (free) radical insight. J Mol Cell Cardiol 47:372–381
78. Chandrasekra A, Shahidi F (2011) Inhibitory activities of soluble and bound millet seed phenolics on free radicals and reactive oxygen species. J Agric Food Chem 59:428–436
79. Pasko P, Zagrodski P, Barton H, Chlopika J, Gorinstein S (2010a) Effect of quinoa seed (*Chenopodium quinoa*) in diet on some biochemical parameters and essential elements in blood of high fructose-fed rats. Plant Foods Hum Nutr 65:333–338
80. Moraes EA, Natal DIG, Quieroz VAV, Schaffert RE, Cecon PR, de Paula SO, dos Anjos Benjamin L, Ribeiro SMR, Martino HSD (2012) Sorghum genotype may reduce low-grade inflammatory response and oxidative stress and maintains jejunum morphology of rats fed a hyperlipidic diet. Food Res Int 49:553–559
81. Lee CC, Shen SR, Lai YJ, SC W (2013) Rutin and quercetin, bioactive compounds from tartary buckwheat, prevent liver inflammatory injury. Food Func 4:794–802
82. Halliwell B (1991) Drug antioxidant effects. Drugs 42:569–605
83. Nordgren M, Fransen M (2014) Peroxisomal metabolism and oxidative stress. Biochimie 98:56–62
84. Awika JM, Yang L, Browning JD, Faraj A (2009) Comparative antioxidant, anti-proliferrative and phase II enzyme inducing potential of sorghum (*Sorghum bicolor*) varieties. LWT – Food Sci Technol 42:1041–1046
85. Yang L, Browning JD, Awika JM (2009) Sorghum 3-deoxyanthocyanins possess strong phase II enzyme inducer activity and cancer cell growth inhibition properties. J Agric Food Chem 57:1797–1804
86. Cao W, Chen WJ, Suo ZR, Yao YP (2008) Protective effects of ethanolic extracts of buckwheat groats on DNA damage caused by hydroxyl radicals. Food Res Int 41:924–929
87. Suganyadevi P, Saravanakumar KM, Mohandas S (2012) DNA damage protecting activity and free radical scavenging activity of anthocyanins from red sorghum (*Sorghum bicolor*) bran. Biotechnol Res Int 2012:1–9. https://doi.org/10.1155/2012/258787
88. Soriano Sancho RA, Pastore GM (2012) Evaluation of the effects of anthocyanin in type 2 diabetes. Food Res Int 46:378–386
89. Kim JS, Hyun TK, Kim MJ (2011) The inhibitory effects of ethanol extracts from sorghum, foxtail millet on α-glucosidase and α-amylase activities. Food Chem 124:1647–1651
90. Kunyanga CN, Imungi JK, Okoh MW, Biesalski HK (2012) Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients. LWT – Food Sci Technol 45:269–276

91. Hedge PS, Rjasekeran NS, Chandra TS (2005) Effects of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced rats. Nutr Res 25: 1109–1120
92. Issa AY, Volate SR, Wargovich MJ (2006) The role of phytochemicals in inhibition of cancer and inflammation: new directions and perspectives. J Food Comp Anal 19:405–419
93. Serhan CN, Savill J (2005) Resolution of inflammation: the beginning programs the end. Nat Immunol 6:1191–1197
94. Eguchi K, Manabe I, Oishi-Tanaka Y, Ohsugi M, Kono N, Ogata F, Yagi N, Ohto U, Kimoto M, Miyake K, Tobe K, Arai H, Kadowaki T, Nagai R (2012) Saturated fatty acid and TLR signaling linking β cell dysfunction and islet inflammation. Cell Metab 15:518–533
95. Luft VC, Schmidt MI, Pankow JS, Couper D, Ballantyne CM, Young JH, Duncan BB (2013) Chronic inflammation role in the obesity-diabetes association: a case-cohort study. Diabetol Metab Syndr 5:31
96. Baniyash M, Sade-Feldman M, Kanterman J (2014) Chronic inflammation and cancer: suppressing the suppressors. Cancer Immunol Immunother 63:11–20
97. Reyes M, Quintanilla C, Burrows R, Blanco E, Cifuentes M, Gahagan S (2015) Obesity is associated with acute inflammation in a sample of adolescents. Pediatr Diab 16:109–116
98. Chai EZ, Siveen KS, Shanmugam MK, Arfuso F, Sethi G (2015) Analysis of the intricate relationship between chronic inflammation and cancer. Biochem J 468:1–15
99. Burdette A, Garner PL, Mayer EP, Hargrove JL, Hartle DK, Greenspan P (2001) Anti-inflammatory activity of select sorghum (*Sorghum bicolor*) brans. J Med Food 13:879–887
100. Rengstrom J, Strom K, Moldeus P, Nilsson J (1993) Analysis of lipoprotein diene formation in human serum exposed to copper. Free Rad Res Comm 19:267–278
101. Baba S, Osakabe N, Kato Y, Natsume M, Yasuda A, Kido T, Kukuda K, Muto Y, Kondo K (2007) Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. Am J Clin Nutr 85:709–717
102. Lin LY, Peng CC, Yang YL, Peng RY (2008) Optimization of bioactive compounds in buckwheat sprouts and their effects on blood cholesterol in hamsters. J Agric Food Chem 56:1216–1223
103. Pasko P, Borton H, Zagrodski P, Izewska A, Krosniak M, Gawlik M, Gorinstein S (2010b) Effect of diet supplemented with quinoa seeds on oxidative status in plasma and selected tissues of high fructose-fed rats. Plant Foods Hum Nutr 65:146–151
104. Letelier ME, Rodriguez-Rojas C, Sanchez-Jofre S, Aracena-Parks P (2011) Surfactant and antioxidant properties of an extract from *Chenopodium quinoa* wild seed coats. J Cereal Sci 53:239–243
105. Kooshki A, Hoseni BL (2014) Phytochemicals and hypertension. Shiraz E-Med J 15(1): e19738
106. de Paula TP, Steemburgo T, de Almeida JC, Dall'Alba V, Martinez JA, Martinez-Gonzalez MA (2004) Fruit and vegetable consumtion is inversely associated with blood pressure in a Mediterranean population with a high vegetable-fat intake: the Seguimiento Universidad Navarra (SUN) study. Brit J Nutr 92(2):311–319
107. Al Disi SS, Anwar MA, Eid AH (2016) Anti-hypertensive herbs and their mechanisms of action: part I. Front Pharmacol 6(323):1–24
108. Business Dictionary (2012) www.businessdictionary.com. Accessed 18 Apr 2017
109. St Claire L, Watkins CJ, Billinghurst B (1996) Differences in meanings of health: an exploratory study of general practitioners and their patients. Fam Pract 13:511–516
110. Stoewen DL (2015) Health and wellness. Can Vet J 59(9):983–984
111. Bazzano LA, Serdula MK, Liu S (2003) Dietary intake of fruits and vegetables and risk of cardiovascular disease. Current Atherosclerosis Report 5:492–499
112. Awad AB, Bradford PG (2005) Nutrition and cancer prevention. CRC, Taylor and Francis Group, Boca Raton

113. Aggarwal BB, Shishodia S (2006) Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 71:1397–1421
114. Choi S, Friso S (2006) Nutrient-gene interactions in cancer. Taylor and Francis Group, Boca Raton
115. Francis MS, Wolf-Watz H, Forsberg A (2002) Regulation of type III secretion systems. Curr Opinion Microbiol 5(2):166–172
116. Cassidy A, Mukamal KJ, Liu L, Franz M, Eliassen AH, Rimm EB (2013) High anthocyanin intake is associated with a reduced risk of myocardial infarction in young and middle-aged women. Circulation 127:188–196
117. Alvarez-Suarez JM, Giampieri F, Tulipani S, Casoli T, Di Stefano G, González-Paramás AM, Santos-Buelga C, Busco F, Quiles JL, Cordero MD, Bompadre S, Mezzetti B, Battino M (2014) One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. Journal of Nutr Biochem 25:289–294
118. Chaudhuri S, Banerjee A, Basu K, Sengupta B, Sengupta PK (2007) Interaction of flavonoids with red blood cell membrane lipids and proteins: antioxidant and antihemolytic effects. Int J Biol Macromol 41:42–48
119. Tulipani S, Alvarez-Suarez JM, Busco F, Bompadre S, Quiles JL, Mezzetti B, Battino M (2011) Strawberry consumption improves plasma antioxidant status and erythrocyte resistance to oxidative hemolysis in humans. Food Chem 128:180–186
120. Abuajah CI, Ogbonna AC, Umoren PE (2013) Current developments on β-glucans as functional components of food: a review. Annals. Food Technol 14(2):217–229
121. FDA (1997) Food labelling: health claims; oats and coronary heart disease. U.S. Food and Drug Administration rules and regulations. Fed Regist 62:3584–3601
122. EFSA (2011a) Scientific opinion on the substantiation of health claims related to beta-glucans from oats and B\barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852), reduction of post-prandial glycaemic responses (ID 821, 824), and digestive function (ID 850) pursuant to Article 13(1) of regulation (EC) No 1924/2006. EFSA J 9(6):2207. https://doi.org/10.2903/j.efsa.2011.2207
123. EFSA (2011b) Scientific opinion on the substantiation of health claims related to oat and barley grain fibre and increase in faecal bulk (ID 819, 822) pursuant to Article 13(1) of regulation (EC) No 1924/2006. EFSA J 9(6):2249. https://doi.org/10.2903/j.efsa.2011.2249
124. JHIC (2004) Oat beta-glucan claim. UK Joint Health Claims Initiative, Surrey. http://www.jhci.co.uk/. Accessed 25 Apr 2011
125. Maheshwari G, Sowrirajan S, Joseph B (2017) Extraction and isolation of β-glucn from grain sources-a review. J Food Sci 00 (00):1–11
126. Howard LA, Jeffery EH, Wallig MA, Klein BP (1997) Retention of phytochemicals in fresh and processed broccoli. J Food Sci 62(10):98–104
127. Song K, Milner JA (2001) The influence of heating on the anticancer properties of garlic. J Nutr 131(10):54S–57S
128. Stahl W, Sies H (1992) Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. J Nutr 122(2):161–161
129. Trevisanato SI, Kim YI (2000) Tea and health. Nutr Rev 58:10
130. Cox S, Abass-Ghannam N, Gupta S (2011) Effects of processing conditions on phytochemical constituents of edible Irish seaweed *Homanthalia elongata*. J Food Process Preserv. www.arrow.dit.ie/schfseh/99 Accessed 28 Sept 2013
131. Hadvorsen BL, Carlsen MH, Phillips KM, Bohn SK, Holte K, Jacobs DR Jr, Blomohoff R (2006) Content of redox active compounds (antioxidants) in foods consumed in the United States. Am J Clin Nutr 84(1):95–135

Development of Functional Dairy Foods

46

Natália Martins, Maria Beatriz P. P. Oliveira, and
Isabel C. F. R. Ferreira

Contents

Abstract

There has been a growing interest on functional foods, markedly recognized as being able to provide additional benefits on health promotion, wellbeing maintenance, and disease prevention. Based on this scenario, food industries have been increasingly focused in developing added-value foodstuffs, being dairy foods one of the most currently used food products for functional purposes. Different

N. Martins
Mountain Research Centre (CIMO), Polytechnique Institute of Bragança, Bragança, Portugal

REQUIMTE/LAQV, Faculty of Pharmacy, University of Porto, Porto, Portugal
e-mail: natas_cruz213@hotmail.com

M. B. P. P. Oliveira
REQUIMTE/LAQV, Faculty of Pharmacy, University of Porto, Porto, Portugal
e-mail: beatoliv@ff.up.pt

I. C. F. R. Ferreira (✉)
Mountain Research Centre (CIMO), Polytechnique Institute of Bragança, Bragança, Portugal

Instituto Politécnico de Bragança, Bragança, Portugal
e-mail: iferreira@ipb.pt

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_35

extraction and encapsulation technologies have been used to obtain target food bioactive ingredients and to ensure an effective functionalization of dairy products, respectively. Probiotics, prebiotics, mushrooms, and plant food bioactive extracts comprise the most commonly used food ingredients to produce functional dairy foods, mostly fermented milk, yogurt, and cheese. In fact, dynamic and promissory biological effects have been documented for these functional dairy foods, among them antioxidant, cardioprotective, antihypertensive, immunomodulatory, antimicrobial, antidiabetic, anti-inflammatory, neuromodulatory, and even bone protection. However, besides the impact of health benefits on consumers' acceptance and subsequent consumption of functional dairy foods, other factors, such as consumers' familiarity with new products and functional ingredients used on their formulation, consumers' knowledge and awareness about the credibility of shared health effects, and finally the organoleptic and sensory evaluation of the developed functional dairy foods, have also a determinant role. Anyway, consumers are considered self-contributors for this promising food innovation. Thus, the concept of functional dairy foods may represent an upcoming multiniche market and sustainable trend to be exploited.

Keywords
Functional foods · Dairy products functionalization · Food ingredients · Extraction/encapsulation technologies · Consumers' acceptance

1 Introduction

Consumers' perception about the real importance of a balanced diet and healthy lifestyle on disease prevention, health maintenance, and longevity promotion has driven the development of multiple and increasingly deepen studies [1, 2]. Foods, food ingredients, and even bioactive molecules with health-promoting abilities present a large demand by worldwide consumers [3]. In fact, biotechnological and agro-food industries have been increasingly focused on the development of highly specific methodological procedures to incorporate certain bioactive molecules on daily products, making them more safe, effective, bioavailable, and even able to confer certain additional biological attributes [4, 5]. Despite the inexistence of a general consensus, these food products are commonly referred as functional foods, i.e., "*foods that when included in the normal diet display one or more target functions in the human body, being able to improve the health status and/or to reduce the likelihood of some disorders occurrence*" [6]. Thus, functional foods make part of daily diet, both as natural, whole, or unmodified foods; however, they may also be used and/or incorporated as food constituents, added and/or removed by technological or biotechnological procedures that modulate their bioavailability, focusing on the improvement of their biological effects [6, 7]. Several food groups, such as meat and fish products, ice cream, cheese, yogurt, and other dairy products present very interesting chemical characteristics and nutritional composition that

triggers the focus of researchers to the incorporation and application of bioactive molecules in those food matrices [3].

Dairy products are amongst the most commonly tested and selected food groups toward the incorporation of food ingredients for functional purposes. Milk, cream, yogurt, kefir, powdered milk, condensed milk, ricotta, butter, casein, fermented dairy drinks, infant milk formula, colostrum, cheese, and ice creams are the most common traditional dairy products considered as functional foods [8, 9]. All these products have on their chemical composition recognized ingredients that provide, besides the nutritional benefits, a considerable improvement on human health and well-being [8]. Numerous nutrients are present on dairy food products, being vitamins, minerals, and proteins the most abundant ones. However, some preparations present active ingredients with low availability and/or nutritional deficits of these constituents, being milk fortified with calcium and vitamin-D (fortified product) one of the most representative examples of this situation [10, 11]. Moreover, the incorporation of milk supplements and milk-based food products, such as probiotic bacteria and prebiotic polysaccharides, omega-3 fatty acids, and other compounds, is becoming increasingly common (enriched product) [10, 11]. Moreover, there are some situations in which some deleterious components need to be removed, reduced, or replaced by another substance with beneficial effects, for example, fibers as fat releasers in ice cream products (altered products) [10, 11]. Curiously, functional dairy foods are virtually found in all these types of functional food categories, despite they are not homogeneously scattered over all segments of the growing market.

In this sense, the present chapter aims to provide an overview on functional dairy foods, emphasizing the role of biotechnological and agro-industrial prospects in their development, describing the most commonly used plant food bioactives on functional dairy foods formulation, and lastly their bioactive and health-promoting abilities.

2 Dairy Food Products: An Overview

Innovations in the agro-food sector are mostly focused on the improvement of the currently available food products, always taking into account some specific criteria, such as the quality, safety, and even distinct characteristics related with bioactive and health promoter effects conferred by these matrices [12–15]. Besides, the information about nutrients, ingredients, and food additives should also be prioritized [12, 14]. Specifically, the food industry has been the target of intensive and impressive innovative processes along with different parts of the food chain, being the most evident ones classified in five distinct sectors: (1) new food ingredients and materials; (2) innovation in fresh foods; (3) innovation in food quality; (4) new food process techniques; and (5) new packaging methods [12]. All these innovative aspects result from consumers' demand that trigger the search and development of highly effective products, techniques, and services by food science and technology researchers in articulation with the food industry (Fig. 1). Within the scope of food

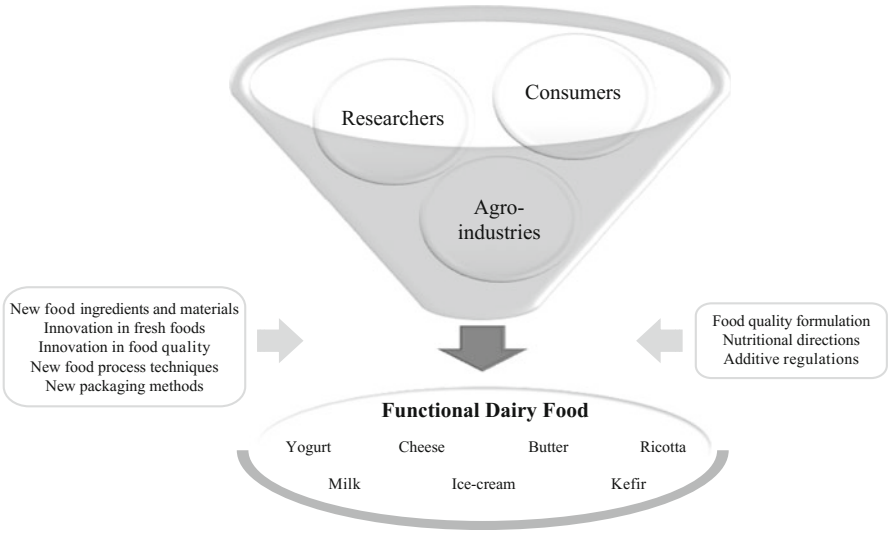


Fig. 1 Main actors and factors involved in the development of functional dairy foods

science and technology, the market of dairy products has gained a renowned niche of demand and recognition by consumers, besides of their increasing research interest and innovation procedures.

Milk is one of the most widely consumed dairy product, being used over centuries as part of daily diet [15, 16]. Despite a growing evidence showing that their unlimited ingestion is associated with several disorders, including some forms of cancer, asthma, diabetes, obesity, cardiovascular diseases, and even osteoporosis, more recent clinical data have demonstrated that calcium supplementation helps to promote bone health besides other benefits [9, 15, 17]. Nevertheless, there are some data that still remains inconclusive concerning to the linkage between high dietary calcium intake from milk and prevention of osteoporosis and bone loss [16]. On the other hand, and considering that many people are lactose-intolerant or even allergic to milk, the interest was progressively driven to milk fermented products, such as yogurt and cheese [16, 18, 19]. Fermented dairy products contain live beneficial microorganisms that can digest lactose and other nontolerable milk constituents, exert cholesterol-lowering effects, and also contribute to cancer risk reduction [15]. These promising modulatory effects are mainly reached through action of those microorganisms, namely probiotic bacteria, that significantly enhances the health-promoting abilities of dairy products, contributing for a longer and healthier lifetime [9, 16]. Not least important to highlight is that the benefits of probiotic bacteria may be achieved both directly and/or indirectly, being all these aspects carefully described in a specific section of this chapter.

Considering the recent findings on this field, milk and other dairy products, and more specifically fermented dairy products, may have a crucial and determinant functional role in contributing to health and well-being of worldwide population.

This aspect has driven an increasing interest of researches in developing functional foods based on milk and other dairy products, which are known by humanity since medieval times due to their health benefits. More recently, the biologically active constituents have been increasingly studied and their benefits reported, remaining, however, the interest to deepen knowledge on this field, in order to promote their strengthening and valorization.

3 Functional Foods: Innovative Trends on Dairy Products

Functional foods have been the target of an intense investigation by researchers, highly demanded by consumers as also with increasingly strict regulatory processes applied to agro-industries. Despite dairy products present a broad history of use, in the last years a high demand by consumers for enhanced-dairy foods with additional health-promoting abilities has been observed [20, 21]. The large evidence that foods in general, and specifically dairy foods with balanced-enriched nutritional composition, may confer additional health benefits has stimulated the development of novel formulations of dairy products containing active ingredients [9, 18, 22]. Until now, prebiotics, probiotic bacteria, vitamins and minerals, flavoring agents, plants, fruits, and even its derived extracts are amongst the most frequently used food ingredients in the design of functional dairy food formulations (Fig. 2). In fact, the development and formulation of functional dairy foods are on the top of the current studies, being yogurt, followed by cheese, butter, milk through different preparations (powdered,

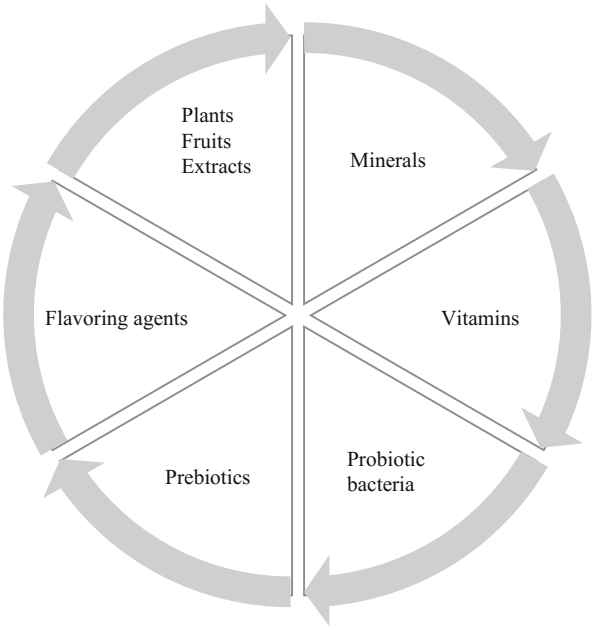


Fig. 2 Most commonly used ingredients in the formulation of functional dairy foods

processed, condensed, and even milk-based foods), ice cream, kefir, and ricotta the most widely investigated toward the incorporation of the previous mentioned ingredients for different functional purposes (Fig. 1). This information is briefly summarized in Table 1 and described in detail in the following paragraphs.

Studies with probiotics are on the top of dairy products' investigations, including the use of microorganisms, such as *Bifidobacterium, Lactobacillus, Streptococcus*, and *Saccharomyces*, individually or in cocultures [4, 14, 18]. Very interesting results have been reported related with food quality characteristics, effectiveness, and acceptance by consumers [4, 18]. There is an increasing evidence related with the effective biological potential and health benefits of probiotic bacteria, namely on the prevention of gastrointestinal disorders, microbiota modulation, selective inhibition of opportunistic microorganisms, on the metabolism of toxic substances, as anti-carcinogenic/antimutagenic agents, and even on the reduction of cholesterol levels and of lactose intolerance [3, 4, 7]. This last one has received a pivotal attention by food industries, due to the increasing rates of lactose intolerance, which directly interfere with food selection by worldwide consumers [4]. Different levels of lactose intolerance require specific dietary recommendations, including to avoid dairy products and derivatives [16]. Therefore, the incorporation of probiotic bacteria in various matrices of dairy products to reduce lactose intolerance is very important. Yogurts and cheeses are amongst the most investigated dairy products toward the incorporation of probiotic bacteria for functional purposes [4].

The incorporation of vitamins and minerals on dairy foods is not less common, both in bioavailable, stable, and soluble forms to enrich the nutritional value as also to extend the shelf life of dairy products [17]. Calcium, iron, and vitamins C, D, and

Table 1 Types of food ingredients commonly incorporated in the formulation of functional dairy foods

Types of food ingredients	Detailed food ingredients	Dairy products incorporation
Prebiotics	Milk proteins, lactose, saccharose, lactulose, fructo-, glyco- and galactooligosaccharides, arabinose, galactose, inulin, raffinose, mannose, lactulose, staquiose, xylooligosaccharides, isomaltulose (palatinose), isomaltooligosaccharides, and soy oligosaccharides	Cheese, yogurt
Probiotics	Species: *Bifidobacterium, Lactobacillus, Streptococcus* and *Saccharomyces*	Cheese, yogurt
Vitamins and minerals	Calcium, iron, vitamins C, D, B2, B9, and B12	Milk, cheese, yogurt
Flavoring agents	Polyphenols, flavonoids, carotenoids	Milk, cheese, yogurt
Plants, fruits, and its derived extracts	Chamomile, fennel, honey, mushrooms, chestnut, lemon balm, rosemary, basil	Cheese, yogurt
Others	Omega-3 fatty acids are linoleic acid (LA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA); β-carotene	Milk, yogurt

B12 are among the most frequent ones [9]. Other B vitamins, such as folate and riboflavin, have been also used; some of them are even synthesized from various nonvitamin precursors through action of certain bacteria in plant and dairy foods [14]. Although several years ago synthetic formulas were the most common, presently the incorporation of natural ingredients rich on these nutrients are on the top of agro-industrial selection and consumers' preference [3]. A similar scenario is observed for the addition of flavors and aromas, in which essences, plant fruits and/or plant fruit extracts, and even honey have been increasingly used, while artificial flavoring agents become secondary in the formulation of dairy products [2, 23, 24]. The addition of plants and fruits and/or its derived extracts seems to present modulatory effects on gut microbiota, i.e., favors the growth of beneficial bacteria and limits the growth of opportunistic microorganisms [14]. Still, these active ingredients are also able to contribute for the improvement of the dairy products shelf life at large extent, being therefore currently used for different purposes [25].

Plants and fruits are *per* definition, composed by a rich and complex pool of chemical molecules, most of them already characterized and properly identified as having important bioactive effects at different levels [26]. Within the different classes of bioactive molecules, phenolic compounds have received a special attention, due to their well-known antioxidant properties [26]. Nevertheless, most of these bioactive molecules present inactive forms, being transformed into active forms by metabolic processes; in this field lactic acid bacteria (*Lactobacillus* and *Streptococcus*) display a very important role, favoring the conversion of inactive phenolic compounds into their biologically active forms, via expression of glycosyl hydrolase, esterase, decarboxylase, and phenolic acid reductase enzymes [14]. These biochemical reactions lead to the formation of several biologically active molecules, namely pyranoanthocyanidins and 3-desoxypyranoanthocyanidins that display determinant and regulator effects as health promoters and disease preventers [14]. Once again, probiotic bacteria seem to exert multiple contributing effects when combined with other food ingredients in dairy functional products' formulation. However, besides plant extracts, several other ingredients, such as milk proteins, inulin, oligosaccharides, and lactulose, have been incorporated for prebiotic purposes, i.e., to promote the growth of *Bifidobacterium* and other probiotic bacteria, to ensure gut health, to keep harmful bacteria under control, and to enhance immune system [9, 27]. Besides prebiotic effects, these active ingredients also show other important functions, such as improvement of minerals and vitamins absorption, decrease of triglycerides and cholesterol levels, and due to their high contents in dietary fiber, improvement of fecal transit time and stool weight, crucial to prevent constipation [9, 22]. Not least important to point out is that the above referenced health conditions are amongst the most common disorders observed in modern society.

The latest studies performed on this area have investigated different parameters related with the general quality and acceptability of the incorporation of honey to produce functional yogurts [2], mushrooms on functional cheese formulations [23], and even phenolic extracts derived from different plant parts on dairy functional

yogurts and cheese formulation [25, 28–36]. Nevertheless, further studies are necessary to evaluate the feasibility, efficacy, effectiveness, and bioavailability of bioactive ingredients used to functionalize dairy products, and not least important to evaluate the consumers' acceptance.

4 Functional Dairy Foods: Agro-Industrial and Biotechnological Prospects

A balanced and healthy diet is a key determining factor for overall quality of life, which has been increasingly evident for worldwide consumers [6]. In fact, the perception about the real importance of some specific foods and even food ingredients in the daily diet is increasingly common, up to a point that the consumers' demand of healthy and functional foods reached exponential levels [13, 14]. Being consumers directly involved on food selection, development, and evaluation of its overall quality and acceptability, the food industry is increasingly focused on the formulation of foods with abilities to improve the health and well-being of consumers, at the same time that favor digestive system (which is considered a key factor for overall quality of life), besides other physiological and metabolic effects [9, 11]. Therefore, the development of functional dairy foods provides a great opportunity to contribute to the improvement of food quality and consumers' health and well-being.

Among the wide variety of food ingredients, there are three main groups that have become increasingly common in functional foods formulation, such as: probiotics (live bacteria), prebiotics (compounds as fibers), and antioxidants [27, 37]. The latest group of antioxidants mainly includes vitamins, minerals, plants and fruits, and even its derivatives (Table 1). Most of the food ingredients currently incorporated in functional dairy foods formulation naturally occurs on these dairy matrices, such as bioactive peptides, probiotic bacteria, antioxidants, vitamins, specific proteins, oligosaccharides, organic acids, highly absorbable calcium, conjugated linoleic acid, and other biologically active components [9, 37]. Notwithstanding, and despite their natural occurrence, a limited content of bioactive compounds is present, which can compromise the effective value of these products [31]. Therefore, the incorporation of these active ingredients in the functionalization of milk and dairy products is of the utmost importance. Using these food ingredients, new functional dairy products may be formulated, through modification of the traditional formulas, adding, eliminating, or even substituting some constituents, and even though the addition of some wholesome compounds.

For example, Caleja et al. [31] observed that the incorporation of antioxidants from natural origin (from chamomile and fennel extracts) in yogurts not only improved their biological activity but also satisfied consumer demands, in comparison with the synthetic additive, potassium sorbate [31]. Also important to highlight is that the yogurt functionalization did not alter significantly its nutritional profile, external appearance, pH, and even fatty acids content, being therefore suitable for upcoming use both in food industry and in the dairy sector, where synthetic additives

are commonly used [31]. On the other hand, Heleno et al. [36] performed an experiment using *Agaricus bisporus* extracts obtained by ultrasound-assisted extraction, and ergosterol, to incorporate in dairy beverages at concentrations mimicking those that commercially occur in phytosterol-added yogurts. The authors observed that samples incorporated with the extract and with ergosterol showed a higher antioxidant capacity at same time that protected yogurt from oxidation, improving therefore their shelf life, without modifying the nutritional value in comparison with the original product [36]. Similar findings were reported by Carocho et al. [34] using chestnut flowers, lemon balm, and its respective aqueous extracts (decoction) to incorporate into "Serra da Estrela" cheese, aiming not only to assess their ability to maintain its nutritional value but mainly to promote additional characteristics toward new dairy foodstuffs formulation [34]. Caleja et al. [25] and Carocho et al. [34] incorporated, respectively, fennel in cottage cheese [38] and basil in "Serra da Estrela" cheese [35], aiming to assess their functional and preserving abilities. The authors found that both samples did not alter significantly the nutritional value of cheeses, at same time that improved their antioxidant properties, preserved the contents in unsaturated fatty acids and proteins, as also exerted a prominent functionalizing and preservative effect.

Concerning the incorporation of prebiotics and probiotics in functional dairy products formulation, multiple findings have reported that, by one hand the incorporation of prebiotics not only conferred protection but also improved the viability of probiotic bacteria, presupposing therefore there action as symbiotic ingredients [39]. On the other hand, it was also shown that the incorporation of stingless bee honey in goat yogurt containing *Lactobacillus acidophilus* positively affected several characteristics, among them the color, syneresis, viscosity, sensory acceptance, and even purchase intention [2]. But besides to these aspects, probiotic bacteria also exert other highly valuable biological effects, such as being responsible for reinforcing natural intestinal flora, decreasing serum triglycerides, cholesterol, transaminase, and total bilirubin levels, exerting immunomodulatory effects, protecting against gastrointestinal pathogens, contributing for lactose metabolism, infantile diarrhea, besides reducing the incidence of urogenital and respiratory diseases and preventing the occurrence of some cancers [3, 40].

It is clearly evident that these food ingredients may provide considerable nutritional and sensory attributes of quality and acceptability, despite having a great market potential to be exploited considering the functional properties. In this sense, and considering that the top research areas in the Food Science and Technology comprises the extraction and characterization of new natural ingredients with biological effects for further incorporation into functional foods formulation, it is very important to use proper extraction methodologies and even effective technologies in functional foods preparation. Therefore, there is not only interest in ensuring a proper extraction of the bioactive constituents, but also the use of effective encapsulation methodologies toward the preservation of all the characteristics presents in the developed functional food until the physiological site of action is reached.

Considering the existence of a wide variety of bioactive molecules with suitable characteristics for upcoming use as food ingredients on functional foods

formulation, it is mandatory to select the most appropriate extraction methodology aiming to achieve the highest extraction yield, without affecting both the chemical composition and even the final bioavailability and subsequent biological activity. The quality of the bioactive compounds is closely dependent on the suitability of the extraction process and the proper selection of separation steps, which will also affect the subsequent identification and characterization of those biomolecules [3, 4]. In this sense, the selected extraction technique will also need to convert bioactive compounds into more suitable forms both for detection and subsequent characterization, but also to provide a strong and reproducible method to be used in other molecules belonging to the same group [3, 4]. There are a wide variety of extraction techniques, but the most commonly used are briefly described in Table 2, namely Soxhlet extraction (SoE), Ultrasound-assisted extraction (UAE), Supercritical-fluid extraction (SFE), Accelerated solvent extraction (ASE), and finally Shake extraction (ShE).

Table 2 Most commonly used extraction technologies in functional foods formulation

Extraction techniques	
Soxhlet extraction (SoE)	Commonly used to extract lipophilic compounds (hexane or petroleum ether as solvents), but can be also used to extract polar compounds, such as some specific phenolic compounds that are effectively extracted using methanol. More recently has been also applied to extract volatile compounds. *Bioactive molecules*: carotenoids, phenolic compounds, sterols, and aromatic compounds.
Ultrasound-assisted extraction (UAE)	Based on ultrasonic vibrations, being a fast and simple method, allowing the extraction of multiple samples at same time. The solvent extraction used (mainly methanol, acetone, water, and ethyl acetate) displays a crucial role, but also sample size, pH, temperature, and pressure determines extraction quality and yield. *Bioactive molecules*: carotenoids, polysaccharides, proteins, phenolic compounds, aromatic compounds, and sterols.
Supercritical fluid extraction (SFE)	Currently considered a "green" methodology, this technique is used on the extraction of multiple nutraceutical ingredients, usually performed using carbon dioxide under high pressure. This technique allows the use of low temperatures, presents a high selectivity, needs low solvent volume, small samples, short extraction times, and great automation procedures. *Bioactive molecules*: carotenoids, sterols, tocopherols, and phenolic compounds.
Accelerated solvent extraction (ASE)	This technique is carried out under high pressure and temperatures, presenting, however, some advantages in relation to the previous ones, such as enable the extraction of fresh samples, short extraction times, higher automation procedures, and good extraction rates. *Bioactive molecules*: vitamins, phenolic compounds, and carotenoids.
Shake extraction (ShE)	This technique needs the use of a shaking device, allowing more effective extractions at short times. The main advantage is to increase the surface where solvent extraction interacts with the sample material. *Bioactive molecules*: phenolic compounds.

Encapsulation technique is a complex process that includes the creation of a barrier, more or less complex, that acts covering bioactive components inhibiting the occurrence of chemical interactions, protecting against environmental factors (i.e., temperature, pH, enzymes, and oxygen), and even allowing the progressive release of the active components under certain conditions [3, 4].

There are three main groups of encapsulation techniques, i.e., microencapsulation, nanoencapsulation, and even emulsions, as briefly described in Table 3. The selection of a specific encapsulation procedure mainly depends on two distinct characteristics: the type of core material and characteristics of the final product where the technique will be applied. Moreover, the material of capsule wall, size, shape, and structure of capsule particles also determines the stability of the bioactive molecules during production, storage, and even releasing during consumption [3, 4]. However, the encapsulation technologies mainly aspire in ensuring a proper stabilization of the bioactive compounds, warranting that they reach consumers in appropriate levels, maintaining their bioavailability, avoiding the formation of harmful and unpleasant compounds and even to mask some undesirable sensorial attributes characteristic from some bioactive substances [3, 4].

5 Mushrooms and Plant-Food Bioactives: Key Factors on Functional Dairy Products Formulation

The world of plant-food bioactives has received a pivotal attention in the last years, being the assessment of their pharmacological effects and related biotechnological applications an exponential niche of market to be exploited. Regarding particularly their biotechnological applications, the sector of food industry and specifically the subsector of development of new food ingredients and materials are under dynamic innovation and overall exploitation. Inside this subsector, the area of functional foods' formulation is of largest demand not only by consumers but also of the utmost interest of researchers and industries (Fig. 1). Moreover, being the group of dairy products one of the most widely recognized food categories for functional purposes, considering the presence of promissory molecules with multiple benefits, a pivotal attention and intensive research work has been carried out on this field. On the other hand, and despite probiotics and prebiotics have been on the top of the latest studies on this area, a great attention has been given to plants, fruits, and its derived extracts on fortification of dairy food products and subsequent functionalization. As briefly described in Table 1, different plant, fruits, derived extracts, and even other food constituents have been used to functionalize dairy products. Yogurt and cheese are the most commonly selected to be investigated. Also interesting to point out is that these food ingredients are not only used to improve the nutritional value, health-promoting effects, and to confer additional benefits to dairy food products but also to improve their shelf life and even to remove some unpleasant and even nonbeneficial, nontolerated, and unaccepted characteristics by consumers.

Concerning the use of plant food bioactives in the development of functional dairy foods, chamomile and fennel have been incorporated both in yogurt [31] and

Table 3 Most commonly used encapsulation technologies in functional foods formulation

Encapsulation technologies	
Microencapsulation	
Spray drying	This is the most commonly used preparation, destined to prepare dry and stable food additives and flavors. It is based on the injection of a liquid suspension of the bioactive compound at the top of a vessel, drying the droplet into a fine powder particle concomitantly with hot air. Then, liquid droplet solidified and entraps the bioactive molecule. The technique is economic, flexible, and suitable to be used in a continuous operation, besides to provide a high protection to short exposure periods to acids, humidity, and oxygen.
Freeze drying	Commonly known as lyophilization or cryodesiccation. This technique is great to be used in a wide variety of heat-sensitive ingredients. It consists in homogenize core materials from the initial solution and then originates colyophilizes, i.e., irregular particles. This technique is suitable to encapsulate water-soluble essences, natural aromas, and even drugs.
Coacervation	This technique is based on the phase separation of hydrocolloids present in an initial solution and further deposition of the newly formed coacervates around the suspended or emulsified bioactive molecule. It is considered an expensive method, being particularly suitable to be used in high-value and sensitive functional ingredients (i.e., polyphenols).
Liposome entrapment	This technique consists in the formation of a lipid membranous system that encapsulates a hydrophilic space, leading to the formation of a suitable place to encapsulate water-soluble, lipid-soluble, and amphiphilic functional ingredients. With this technique, it is possible to control the release rate and exact point of incorporated materials delivery. Encapsulated materials through this technique are protected from stomach digestion, so ensuring their subsequent bioavailability and bioactivity in the next section of the gastrointestinal tract.
Cocrystallization	This technique consists in the modification of the sucrose structure, leading to the formation of an irregular agglomerated crystal. Bioactive ingredient is therefore incorporated into the porous matrix of the newly formed microsized crystals. This technique improves the solubility, homogeneity, dispersibility, hydration, anticaking, stability, and flowability of the encapsulated functional ingredients. It also allows the conversion of liquid materials into powders, which facilitates their subsequent application in pharmaceutical industry.
Yeast-encapsulation	This technique is based on the use of yeast cells (*Saccharomyces cerevisiae*) as the encapsulating material, being also usually applied to essential oils, flavors, and polyphenols. The target ingredients can pass across cell wall and remains inside the cells, being also ensured the control of the diffusion of the incorporated functional ingredients. Finally, this technique is also embroiled with green chemistry principles, once no additives besides water, yeast, and target functional ingredients are used. It is also a low-cost technique that allows the processing of high volumes of bioactive ingredients.
Cold gelation	This is a recent method, consisting from an alternative way to develop protein microparticles in food industry. No organic solvents are necessary, being encapsulation achieved under mild conditions, minimizing the destruction of sensitive nutraceutical compounds. Moreover, it is also possible to denature, dissociate, and even aggregate under different conditions of pH, ionic strength, and temperature.

(*continued*)

Table 3 (continued)

Encapsulation technologies
Nanoencapsulation
This technique is more complex that microencapsulation, mainly because of the intricate morphology of the capsule and core material, and the demand in controlling the release rate of the nanoencapsulates. It involves the incorporation, absorption, or dispersion of the bioactive compounds and subsequent formation of the functional ingredients encapsulated in small vesicles (<100 nm). Nanoparticles ensure a greater surface area, increase the solubility and enhance the bioavailability of the target ingredients, due to their subcellular size and improved controlled release. They can also easily penetrate tissues through fine capillaries, crossing epithelial lining fenestration, being therefore efficiently taken up by cells and allowing and efficient delivery of the active compounds in the target sites of body.
Emulsions
In a broad sense, an emulsion is a mixture of at least two immiscible liquids, usually oil and water, where one of the liquids is dispersed as small spherical droplets in the other. Therefore, emulsions are turbid, with small droplets sizes ranging from 0.2 mm to 10 mm, may remaining stable for a considerable time-period, despite being classified as thermodynamically unstable systems. Emulsions may be also classified into two distinct groups: microemulsions and nanoemulsions. Microemulsions are systems consisting from a mixture of water, hydrocarbons and amphiphilic compounds, forming kinetically and thermodynamically stable compounds, transparent, homogeneous, and isotropic solutions, ranging particle sizes from 5 nm to 100 nm. Nanoemulsions consists from fine dispersed emulsions and submicron emulsions, that in contrary of microemulsions, are not thermodynamically stable. They are produced by microfluidization or micelle formation techniques, exhibiting poor solubility and low bioavailability.

cheese [25, 30, 38] samples for subsequent analytical and methodological studies. Lemon balm [33, 34], rosemary [32], chestnut [33, 34], and basil [35] were incorporated in cheese samples, while honey [2], wild blackberry [41], and seaweed extracts [42] were used to functionalize yogurt samples. More recently, dairy beverages were tentatively functionalized with pure ergosterol and mycosterols from *Agaricus bisporus* extracts [36], as an alternative to phytosterol-based beverages; and also incorporated antioxidant extracts from two mushrooms species, namely *Suillus luteus* (L.: Fries) and *Coprinopsis atramentaria* (Bull.), acting as food ingredients in the functionalization of cottage cheese [23]. Interestingly, in all the above described experiments, prominent results were achieved both in terms of protection against oxidation, improving consequently the shelf life of the functionalized dairy products, as also in terms of nutritional value, stronger antioxidant activity, moisture and sensory attributes, such as external color, syneresis, and viscosity [31, 32, 34]. Moreover, organoleptic characteristics also display a crucial role in overall sensory acceptance and purchase intention, which was achieved though the functionalization of these dairy products that provide beneficial characteristics both for consumers (contributing for healthier lifestyles) and producer industries (commercialization of added-value products) [2, 33]. From the point of view of consumers, two additional aspects should be also considered. The first one is related with the healthier substitution of artificial preservatives from natural ones, improving consequently the acceptability, quality, and security of the final product

[11]. In fact, modern consumers are increasingly focused on the consumption of naturally-derived and safer products and formulated with natural food ingredients. The second one is related with the health-promoting abilities attributed to the consumption of these functional dairy foods; it has been increasingly documented and widely recognized that daily diet exerts a determinant role in health maintenance and disease prevention [8, 22].

The last aspect that is not least important is related with the high interest by food industry in always searching for new products, being this goal markedly driven by the production of cheaper food products, but healthier for consumers also looking for longer storage periods [7, 14, 33]. Therefore, consumers' acceptance and subsequent selection of a specific functional dairy product is markedly determined by a host of factors. The first one is related with the primary health concerns, as already highlighted, the consumers' familiarity with the new functional dairy food and with the functional food ingredients used on their formulation (i.e., nature of the carrier product, delivery of health effects, etc.) [11, 43]. On the other hand, consumers are awareness about the health effects of multiple substances and food ingredients, being thus essential to provide specific and credible information about the latest documented studies on the area. Finally but also determinant is the overall organoleptic and sensory evaluation carried out by consumers about the new product. In fact, different surveys have been applied to consumers, being clearly evident the key conditions for acceptance, such as taste, product quality, price, convenience, and even the reliability of health claims [11, 43, 44]. Therefore, during the development of a functional dairy food, all these aspects should be taken into consideration, besides to ensure their added value in terms of biological activity.

6 Bioactive Effects of Functional Dairy Foods

There is a widespread knowledge about the real benefits of functional dairy foods at the level of health promotion, well-being maintenance, and longevity [27, 44]. By themselves dairy products can confer several biological effects due to the naturally occurrence of biologically active constituents on its chemical composition, such as bioactive proteins, lipids, oligosaccharides, immunoglobulins, enzymes, hormones, antimicrobial peptides, cytokines, growth factors, among others [37]. Nevertheless, these constituents exist in limiting quantities, being therefore extremely usefulness their incorporation in dairy products functionalization [31]. Furthermore, it has been increasingly clear that some fermented dairy foods promote human health not so much directly attributable to the starting materials, but instead from the outcomes of fermentation, thus conferring additional properties beyond basic nutrition [14].

In fact, the formulation of dairy food products able to promote health and well-being is one of the main key research priorities by food industries, that has mostly favored the selection and subsequent consumption of foods enriched with physiologically active components, such as probiotics, prebiotics, and more recently plant food bioactives [3, 14, 37]. One of the main and most widely recognized biological effects of functional dairy products are their recognized modulatory activities, through the action of probiotic

Table 4 Biological activity of functional dairy foods

Bioactive effect	Detailed activity	Functional dairy food
Cardiovascular disease	Improvement of total cholesterol, non-HDL-C, and LDL concentrations	Fermented milk
Hypertension	Antihypertensive peptides acting as angiotensin-converting-enzyme (ACE) inhibitors	Fermented milk, Cheese, Yogurt
Immunomodulatory	Stimulation of the natural immunity, modulation of the production of cytokines, inhibition of carcinogenic effects, gut fermentation, metabolites	Yogurt, Cheese, Kefir
Infection control	Peptides exerting antimicrobial effects, reduction of the incidence of fever and constipation	Fermented milk
Inflammatory bowel syndrome	Reduction of symptoms of flatulence, abdominal pain, cramps, and stomach rumbling	Fermented milk, yogurt
Mood and brain activity	Modulation of the activity of brain regions responsible for controlling central processing of emotion and sensation	Fermented milk
Muscle soreness	Suppression of muscle soreness	Fermented milk
Obesity	Improvement of body fat percentage, waist circumference and waist-to-hip ratio, lean body mass	Yogurt, Fermented milk
Osteoporosis	Increase of bone mineral density and short-term changes in turnover	Kefir
Type-2 diabetes	Decrease of insulin resistance and increase of insulin sensitivity, improving glucose tolerance, and metabolism	Yogurt, Fermented milk

bacteria, also existent in fermented milk products [14, 37]. Besides other aspects, they exert an important role in gut fermentation metabolites, and therefore contributes to local and systemic beneficial effects in humans, not only at a level of digestion, absorption, and metabolism but also elimination, once favor a healthy bowel movement [14, 15]. More interestingly is that the functional effect of dairy products may be achieved directly through the action of probiotic bacteria or even indirectly because of their metabolic activity, i.e., microbial metabolites, such as vitamins, proteins, peptides, oligosaccharides, organic acids, and bacteriocins [14, 37]. In fact, the modulatory effects conferred by probiotic bacteria are both related with the stimulation of the natural immunity as also modulation of the production of cytokines, antimicrobial peptides, and inhibition of the carcinogenicity of multiple substances [45]. Another pronounced bioactive effect of functional dairy products is their ability to minimize the incidence of hypertension [14, 46]. In fact, dairy products, such as milk and cheese, contain significant amounts of antihypertensive peptides, including lactopeptides isoleucine-proline-proline and valine-proline-proline, that mainly acts as antihypertensive angiotensin-converting-enzyme (ACE) inhibitors [14, 46, 47]. On the other hand, whey and casein fractions of yoghurt have also shown strong antihypertensive effects being documented by several studies their role in curtail several syndromes among which in controlling blood pressure [46]. Other studies have even revealed that other peptides from dairy fermented products also exert antithrombotic, satiety, opioid,

immunomodulatory, antimutagenic, osteogenic, and antioxidative effects [14, 48]. Additionally, interesting reports have revealed a positive association between fermented dairy foods' consumption and weight maintenance and body fat reduction, besides their ability to reduce the risk of type 2 diabetes and other metabolic disorders, and overall morbidity and mortality, mainly from yogurt consumption. The effects on glucose metabolism seems to be mainly related with the improvement of glucose metabolism and insulin sensitivity, and reduction of muscle soreness [14, 46]. The positive effects on inflammatory bowel diseases and other immune-related pathologies, including arthritis, sclerosis, fibromyalgia, migraine, and depression have been also increasingly evident, as also their modulatory effects at a level of brain and mood [14, 49], still, however, being essential to deepen knowledge on this field. All the above described biological effects are briefly summarized in the Table 4.

7 Concluding Remarks

The world of functional foods development has led to one of the most promising and dynamic development on a specific segment of food industry. The increasing consumer awareness and demands for healthy products, in association with the latest advances on the area of food science and technology are the most important factors supporting the new revolution on food science and innovation. Nevertheless, consumers' acceptance and preferences are not homogenous, being observed a large distribution and differences on functional foods acceptance. Specifically, the development and commercialization of functional dairy products is not easy, it involves complex, expensive, and specific requirements. Besides the consumers' demand, during the development of a dairy functional product, the technical conditions as also legislation conditions should also be considered. Special requirements stablished to apply during the development and marketing of functional dairy foods should be met, as also their economic potential; health impact, acceptance, and overall interest should not be forgotten. Particularly, consumers' acceptance has been considered a key success factor on market orientation, brand new product introduction, and consumer-led product development, besides to be successful in negotiating market opportunities. Therefore, by purchasing functional dairy foods, consumers may have the overall health benefits of functional foods, besides to achieve a positive and valuable impression of themselves. In fact, they are self-contributors for this achievement, once they interfere both on selection, formulation, evaluation, and overall qualification of these products. Besides to the highlighted aspects, consumers may follow a balanced diet and acquire a healthy lifestyle, which differ from the conventionally stablished healthy diet. Finally and not least important is that the concept of functional dairy foods may represent a sustainable trend in a multiniche market to be exploited.

Acknowledgments The authors are grateful to Foundation for Science and Technology (FCT, Portugal) for financial support to CIMO (UID/AGR/00690/2013) and REQUIMTE (UID/QUI/50006/2013 - POCI/01/0145/ FEDER/007265) research centres.

References

1. Gulseven O, Wohlgenant M (2014) Demand for functional and nutritional enhancements in specialty milk products. Appetite 81:284–294
2. Machado TADG, Oliveira MEG, Campos MIF, Assis POA, Souza EL, Madruga MS, Pacheco MTB, Pintado MME, RCRE Q (2017) Impact of honey on quality characteristics of goat yogurt containing probiotic *Lactobacillus acidophilus*. Food Sci Technol 80: 221–229
3. Silva BV d, Barreira JCM, Oliveira MBPP (2016) Natural phytochemicals and probiotics as bioactive ingredients for functional foods: extraction, biochemistry and protected-delivery technologies. Trends Food Sci Technol 50:144–158
4. Dias DR, Botrel DA, Fernandes RVDB, Borges SV (2017) Encapsulation as a tool for bioprocessing of functional foods. Curr Opin Food Sci 13:31–37
5. Ferrão LL, Silva EB, Silva HLA et al (2016) Strategies to develop healthier processed cheeses: reduction of sodium and fat contents and use of prebiotics. Food Res Int 86:93–102
6. Ferreira ICFR, Morales P, Barros L (2017) Wild plants, mushrooms and nuts: functional food properties and applications, 1st edn. Wiley, UK
7. Illanes A, Guerrero C (2016) Functional foods and feeds: probiotics, prebiotics, and synbiotics. In: Lactose-derived prebiotics: a process perspective. Elsevier, Amsterdam. https://doi.org/10.1016/B978-0-12-802724-0.00002-0
8. Playne MJ, Bennett LE, Smithers GW (2003) Functional dairy foods and ingredients. Aust J Dairy Technol 58:242–264
9. Santillán-Urquiza E, Ruiz-Espinosa H, Angulo-Molina A, Ruiz JFV, Méndez-Rojas MA (2017) Applications of nanomaterials in functional fortified dairy products: benefits and implicatios for human health. In: Nutrient delivery. Acadamiic press, New York. https://doi.org/10.1016/B978-0-12-804304-2/00008-1
10. Kaur N, Singh DP (2017) Deciphering the consumer behaviour facets of functional foods: a literature review. Appetite 112:167–187
11. Siró I, Kápolna E, Kápolna B, Lugasi A (2008) Functional food. Product development, marketing and consumer acceptance – a review. Appetite 51:456–467
12. Bigliardi B, Galati F (2013) Innovation trends in the food industry: the case of functional foods. Trends Food Sci Technol 31:118–129
13. Samoggia A (2016) Healthy food: determinants of price knowledge of functional dairy products. J Food Prod Mark 22:905–929
14. Marco ML, Heeney D, Binda S et al (2017) Health benefits of fermented foods: microbiota and beyond. Curr Opin Biotechnol 44:94–102
15. Murray MT, Pizzorno J (2005) The encyclopedia of healing foods. Atria Books, New York
16. Murray MT, Pizzorno J (2012) The encyclopedia of natural medicine. Atria Books, New York
17. Bagchi D (2006) Nutraceuticals and functional foods regulations in the United States and around the world. Toxicology 221(1):1–3. https://doi.org/10.1016/j.tox.2006.01.001
18. Bakr SA (2015) The potential applications of probiotics on dairy and non-dairy foods focusing on viability during storage. Biocatal Agric Biotechnol 4:423–431
19. Berner LA, O'Donnell JA (1998) Functional foods and health claims legislation: applications to dairy foods. Int Dairy J 8:355–362
20. Litwin NS, Bradley BHR, Miller GD (2015) Dairy proteins in nutrition and food science: functional ingredients in the current global marketplace. J Food Sci 80:A1
21. Maynard LJ, Franklin ST (2002) Functional foods as a value-added strategy: the commercial potential of "cancer-fighting" dairy products. Rev Agric Econ 25:316–331
22. Kandylis P, Pissaridi K, Bekatorou A, Kanellaki M, Koutinas AA (2016) Dairy and non-dairy probiotic beverages. Curr Opin Food Sci 7:58–63
23. Ribeiro A, Ruphuy G, Lopes JC, Dias MM, Barros L, Barreiro F, Ferreira ICFR (2015) Spray-drying microencapsulation of synergistic antioxidant mushroom extracts and their use as functional food ingredients. Food Chem 188:612–618

24. Dias MI, Barros L, Fernandes IP, Ruphuy G, Oliveira MBPP, Santos-Buelga C, Barreiro MF, Ferreira ICFR (2015) A bioactive formulation based on Fragaria vesca L. vegetative parts: chemical characterisation and application in k-carrageenan gelatin. J Funct Foods 16:243–255
25. Caleja C, Barros L, Antonio AL, Ciric A, Barreira JCM, Sokovic M, Oliveira MBPP, Santos-Buelga C, Ferreira ICFR (2015) Development of a functional dairy food: exploring bioactive and preservation effects of chamomile (Matricaria recutita L.) J Funct Foods 16:114–124
26. Martins N, Barros L, Ferreira ICFR (2016) In vivo antioxidant activity of phenolic compounds: facts and gaps. Trends Food Sci Technol 48:1–12
27. Morais EC (2016) Prebiotic addition in dairy products: processing and health benefits. In: Probiotics, prebiotics, synbiotics: bioactive foods in health promotion. Elsevier, Amsterdam. https://doi.org/10.1016/B978-0-12-802189-7.00003-4
28. Martins A, Barros L, Carvalho AM, Santos-Buelga C, Fernandes IP, Barreiro F, Ferreira ICFR (2014) Phenolic extracts of Rubus ulmifolius Schott flowers: characterization, microencapsulation and incorporation into yogurts as nutraceutical sources. Food Funct 5:1091–1100
29. Caleja C, Barros L, Antonio AL, Ciric A, Soković M, Oliveira MBPP, Santos-Buelga C, Ferreira ICFR (2015) Foeniculum vulgare Mill. As natural conservation enhancer and health promoter by incorporation in cottage cheese. J Funct Foods 12:428–438
30. Caleja C, Ribeiro A, Barros L, Barreira JCM, Antonio AL, Oliveira MBPP, Barreiro MF, Ferreira ICFR (2016) Cottage cheeses functionalized with fennel and chamomile extracts: comparative performance between free and microencapsulated forms. Food Chem 199:720–726
31. Caleja C, Barros L, Antonio AL, Carocho M, Oliveira MBPP, Ferreira ICFR (2016) Fortification of yogurts with different antioxidant preservatives: a comparative study between natural and synthetic additives. Food Chem 210:262–268
32. Ribeiro A, Caleja C, Barros L, Santos-Buelga C, Barreiro MF, Ferreira ICFR (2016) Rosemary extracts in functional foods: extraction, chemical characterization and incorporation of free and microencapsulated forms in cottage cheese. Food Funct 7:2185–2196
33. Carocho M, Barreira JCM, Antonio AL, Bento A, Morales P, Ferreira ICFR (2015) The incorporation of plant materials in "Serra da Estrela" cheese improves antioxidant activity without changing the fatty acid profile and visual appearance. Eur J Lipid Sci Technol 117:1607–1614
34. Carocho M, Barreira JCM, Bento A, Fernández-Ruiz V, Morales P, Ferreira ICFR (2016) Chestnut and lemon balm based ingredients as natural preserving agents of the nutritional profile in matured "Serra da Estrela" cheese. Food Chem 204:185–193
35. Carocho M, Barros L, Barreira JCM, Calhelha RC, Soković M, Fernández-Ruiz V, Buelga CS, Morales P, Ferreira ICFR (2016) Basil as functional and preserving ingredient in "Serra da Estrela" cheese. Food Chem 207:51–59
36. Heleno SA, Rudke AR, Calhelha RC, Carocho M, Barros L, Gonçalves OH, Barreiro MF, Ferreira ICFR (2017) Development of dairy beverages functionalized with pure ergosterol and mycosterol extracts: an alternative to phytosterol-based beverages. Food Funct 8:103–110
37. Bhat ZF, Bhat H (2011) Milk and dairy products as functional foods: a review. Int J Dairy Sci 6:1–12
38. Caleja C, Barros L, Antonio AL, Ciric A, Soković M, Oliveira MBPP, Santos-Buelga C, Ferreira ICFR (2015) Foeniculum vulgare Mill. as natural conservation enhancer and health promoter by incorporation in cottage cheese. J Funct Foods 12:428–438
39. Desmond C, Corcoran BM, Coakley M, Fitzgerald GF, Ross RP, Stanton C (2005) Development of dairy-based functional foods containing probiotics and prebiotics. Aust J Dairy Technol 60:121–126
40. Prisco A, Mauriello G (2016) Probiotication of foods: a focus on microencapsulation tool. Trends Food Sci Technol 48:27–39
41. Martins A, Barros L, Carvalho AM, Santos-Buelga C, Fernandes IP, Barreiro F, Ferreira ICFR (2014) Phenolic extracts of Rubus ulmifolius Schott flowers: characterization, microencapsulation and incorporation into yogurts as nutraceutical sources. Food Funct 5:1091

42. O'Sullivan AM, O'Grady MN, O'Callaghan YC, Smyth TJ, O'Brien NM, Kerry JP (2016) Seaweed extracts as potential functional ingredients in yogurt. Innovative Food Sci Emerg Technol 37:293–299
43. Rahnama H, Rajabpour S (2017) Factors for consumer choice of dairy products in Iran. Appetite 111:46–55
44. Nolan-Clark DJ, Neale EP, Probst YC, Charlton KE, Tapsell LC (2011) Consumers' salient beliefs regarding dairy products in the functional food era: a qualitative study using concepts from the theory of planned behaviour. BMC Public Health 11:1–8
45. Pang G, Xie J, Chen Q, Hu Z (2012) How functional foods play critical roles in human health. Food Sci Human Wellness 1:26–60
46. Khan MI, Anjum FM, Sohaib M, Sameen A (2013) Tackling metabolic syndrome by functional foods. Rev Endocr Metab Disord 14:287–297
47. Beltrán-Barrientos LM, Hernández-Mendoza A, Torres-Llanez MJ, González-Córdova AF, Vallejo-Córdoba B (2016) Fermented milk as antihypertensive functional food. J Dairy Sci 99:4099–4110
48. Castro-Gómez P, Rodríguez-Alcalá LM, Monteiro KM, Ruiz ALTG, Carvalho JE, Fontecha J (2016) Antiproliferative activity of buttermilk lipid fractions isolated using food grade and non-food grade solvents on human cancer cell lines. Food Chem 212:695–702
49. Bertazzo A, Ragazzi E, Visioli F (2016) Evolution of tryptophan and its foremost metabolites' concentrations in milk and fermented dairy products. PharmaNutrition 4:62–67

Nutrients and Nutraceuticals from Seafood 47

V. Venugopal

Contents

V. Venugopal (✉)
Former Scientific Officer Food Technology Division, Bhabha Atomic Research Center, Mumbai, India

Visiting faculty, Department of Food Science and Technology, Kerala University of Fisheries and Ocean Sciences (KUFOS), Kochi, Kerala, India

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_36

Abstract

Seafood comprising of finfish and shellfish significantly contribute to world food security. Seafood species are nutritious since they are rich in proteins and other nutrients including peptides, essential amino acids, long-chain omega-3 polyunsaturated fatty acids, carotenoids, vitamins including vitamin B_{12}, and minerals such as calcium, copper, zinc, sodium, potassium, selenium, iodine, and others. Commercial fish processing generates about 30 million metric tons of discards consisting of shell, head, bones, intestines, fin, skin, etc. These discards are rich in several nutraceuticals and biologically active compounds, which include oils containing omega-3 PUFA; carotenoids such as astaxanthin and β-carotene; proteins including myosin, collagen, and gelatin; enzymes; essential amino acids and peptides; polysaccharides and their derivatives including chitin, chitosan, glucosamine, and glycosaminoglycans; and mineral-based compounds. These compounds, depending on their nature, can have varying physiological functions including antioxidant, anti-inflammatory, anti-allergic, antitumor, anti-obesity, anticoagulant, antimicrobial, immunomodulatory, and other activities, which are valuable in healthcare. Marine biotechnology offers several techniques to isolate these nutraceuticals from seafood and their processing discards. This article briefly surveys nutrients and nutraceutical contents of seafood and their potential benefits in human nutrition and healthcare and current commercial status.

Keywords

Seafood · Processing discards · Nutrients · Nutraceuticals · Health benefits · Marine biotechnology

Abbreviations

AAS	Amino acid score
CHD	Coronary heart disease
CS	Chondroitin sulfate
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
EAA	Essential amino acids
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration, USA
FSA	Food Standard Agency, UK
MUFA	Monounsaturated fatty acid
NMFS	National Marine Fisheries Service, USA
PER	Protein efficiency ratio
RDA	Recommended Dietary Allowance
ROS	Reactive oxygen species
SFA	Saturated fatty acid
USDA	United States Department of Agriculture
WHO	World Health Organization

1 Introduction

Seafood, in a broader perspective, comprises of both finfish and shellfish items from marine, estuarine, brackish, and freshwater habitats and forms a sizeable component of the total world food production. According to the State of World Fisheries and Aquaculture, published by the FAO, in the year 2014, an amount of 167.2 million metric tons (MMT) of seafood was globally available, supplying about 20 kg of fish per capita [1]. The major finfish species include anchovies, mackerel, tuna, herring, cod, whiting, and others, while shellfish include two major groups, crustaceans and mollusks, based on their structural features. Shrimp, lobster, crayfish, crab, and krill belong to crustaceans, while mollusks are composed of bivalves (consisting of mussels, oysters, clams, and scallops), cephalopods (squid, cuttlefish, and octopus), and gastropods (abalone, sea snail, cockle, and whelks). Increasing consumer demand has resulted in aquaculture of 73.8 MMT of preferred seafood items in 2014, consisting of 49.8 MMT of finfish, 16.1 MMT of mollusks, and 6.9 MMT of crustaceans [1]. Commercial fishing operations often result in large volumes of bycatch having poor consumer appeal, which is mostly used as landfill, fertilizer, or animal feed. Commercial seafood processing leads to huge volume of discards weighing as high as 50% of total landings, consisting of shell, head, bones, intestines, fin, skin, and others [2, 3]. High-value seafood, bycatch, and processing discards are all rich in many nutrients including peptides; essential amino acids; long-chain omega-3 polyunsaturated fatty acids (PUFAs); carotenoids; vitamins A, B_3, B_6, B_{12}, and D; and various minerals such as calcium, copper, zinc, sodium, potassium, selenium, iodine, and others [4]. Besides, they contain many nutraceuticals, which may be nitrogen, lipid, polysaccharide, or mineral based. These compounds may be proteins including collagen and gelatin; protein hydrolyzates; peptides having interesting bioactivities; lipids rich in long-chain PUFA; hydrocarbons such as squalene; carotenoids; polysaccharides such as chitin, chitosan, glycosaminoglycans, and their derivatives; and mineral-based products such as bone powders. These, depending upon their nature, have interesting bioactivities offering potentials for development of functional foods and uses as natural food additives, medicinal drugs, and encapsulation as well as carrier materials for nutraceuticals [4–7]. With changing consumer interests toward natural bioactive compounds for healthcare and rapid developments in biotechnology, there is a vast scope for secondary processing of seafood and their processing discards for isolation of nutraceuticals. Such an attempt also helps total utilization of seafood for human consumption favoring minimizing seafood-associated environmental problems. This article discusses various nutrients, nutraceuticals, and other bioactive compounds present in both edible portions and processing discards of seafood products. At the onset, a brief discussion on nutraceuticals and functional foods is provided.

2 Nutraceuticals and Functional Foods

The term *nutraceutical* is a contraction form that links both the words nutrition and pharmaceutical, coined in 1989 by Dr. Stephen L. DeFelice, founder and chairman of the Foundation for Innovation in Medicine (FIM), Crawford, New Jersey. It is

defined as any substance that may be considered a food or part of a food which provides medical or health benefits including the prevention and treatment of disease and includes isolated nutrients and compounds that are used as dietary supplements [8–10]. The term *functional food* was coined in Japan in the mid-1980s and is defined as "foods for specified health uses." These foods contain ingredients that can address diseases such as hypertension, allergy, etc. [11]. A food can be regarded as functional if it is satisfactorily demonstrated to benefit one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved health or well-being or to a reduction in the risk of disease [12]. Plaza et al. [13] emphasized three important characteristics of a functional food, namely: (i) the functional effect is different from that of normal nutrition, (ii) the functional effect will be demonstrated satisfactorily, and (iii) the benefit can result in an improvement of physiological function or in a reduction of risk of developing a pathological process. It is now well recognized that dietary habit is one of the most important determinants of chronic diseases. This has led to an increased interest of the consumers toward natural bioactive compounds as nutraceuticals. Fish and shellfish are well recognized to possess appreciable nutraceutical potential to protect human health. This aspect will be broadly covered in this chapter. At the beginning, nutrient contents and nutritional value of seafood are considered.

3 Nutrients from Seafood

The percentage of edible lean tissue in aquatic species is appreciably greater than that in beef, pork, or poultry. About 50–60% of the weight of fish is constituted by its muscle. The proximate composition of fish muscle gives a general idea on its nutrient contents. The proximate composition of fish muscle varies greatly among species, their age, sex, habitats, and season. Information on proximate composition of fish and shellfish are provided by various databases. These include the global database of FAO/INFOODS [14], the USDA [15], the US National Marine Fisheries Service [16], the Department of Health UK [17], and FSANZ [18]. Besides, several authors have also discussed proximate compositions and nutrients in various fish and shellfish [19–24]. Information is also available on the website, SELFNutritionData [25]. The muscle contains water (52–82%), proteins (16–21%), lipids (0.5–2.3%), ash (1.2–1.5%), and a small proportion (about 0.5%) of carbohydrates. The various nutrients present in seafood are briefly discussed below.

3.1 Proteins

Seafood items are rich in Proteins. More than 50% of the world's seafood catch come from developing countries. Therefore, people from these countries derive much of their protein requirements from fishery products [1]. In the year 2014 an amount of 146.3 MMT of seafood was used as human food, giving a per capita seafood consumption of 20.1 kg, which contributed to about 20% of total average per capita

intake of animal protein [1]. Proteins in finfish are distributed in both light and dark muscle. Demersal fish such as cod and haddock have low percentage of dark muscle, which is present under the skin on both sides of the body. On the other hand, in pelagic fish, such as herring and mackerel, the proportions of dark muscle are high, with more vitamins and fats, providing energy for their rapid movement. Light muscle is more abundant in demersal fish. There are three main fractions of muscle proteins in fish and shellfish, namely, myofibrillar (structural proteins) consisting of myosin, actin, and others; sarcoplasmic (soluble proteins) consisting of myoglobin, hemoglobin, globulins, albumins, and various enzymes; and stroma (connective tissue) proteins consisting mainly of collagen, elastin, and gelatin. These fractions constitute 70–80%, 20–30%, and 3% of total muscle proteins, respectively. Muscle proteins are conveniently characterized by their solubility properties. Sarcoplasmic proteins are defined as those soluble when the muscle is extracted with water or solutions of low ionic strength, physiological or less. Myofibrillar proteins are soluble at elevated ionic strengths (> 0.3). Myofibrillar proteins correspond to 65–75% (w/w) of the total protein in fish and shellfish muscle [4, 22]. These proteins consist mainly of myosin (which account for 65–78%) and also actin (F and G types), tropomyosin, M protein, α-actinin, β-actinin, C protein, as well as troponins I and C, which are involved in muscle contraction. The myosin molecules resemble a thread (2 nm x 160 nm) with two globular heads (19 mm long) attached at one end to a tail portion. Fish myosins, compared to myosins from terrestrial animals, are more unstable. A novel protein, paramyosin (molecular weight 200 to 250 kDa), is also found at varying levels in invertebrate myofibrils, but not in vertebrate myofibrils. The protein is involved in catch contraction of bivalves [22]. Fish meat, unlike bovine meat, contains much less stroma proteins, which are the least soluble. When heated, fish muscle connective tissue proteins dissolve more readily and soften, contributing to softness of the cooked meat, in comparison with the tough connective tissue of land animals [24].

The contents of proteins vary from 18% to 23% depending on the seafood items, on the habitats (marine water, freshwater, or brackish water), and on the depth, i.e., pelagic (fast-moving species on surface waters such as tuna, herring, mackerel, sprat, anchovy, and sardine) or demersal (slow-moving species in deep waters such as cod) [21, 24, 26]. Shellfish, in general, contain slightly more proteins than finfish. The reported average protein contents (g%, raw meat) of various shellfish are shrimp, 17.0–22.1; scallop, 14.8–17.7; squid, 13.2–19.6; crab, 15.0–18.4; lobster, 18.2–19.2; krill, 12.0–13.0; clam, 9.0–13.0; mussel, 12.6–13.0; cuttlefish, 16.6–17.3; and oyster, 8.9–14.3 [16–22]. Myofibrillar proteins are the major fractions of the foot and mantle of the clam, constituting 31 to 40% [26]. The claw meat and hepatopancreas of the green crab (*C. mediterraneus*) had protein levels of 17.8–18.2% and 13–14%, respectively [27]. The raw meat of brown shrimp (*Crangon crangon*) from the Black Sea has a protein content of about 18.5% [28]. The white shrimp has higher protein contents than black tiger shrimp, the former having higher amounts of stromal proteins with greater pepsin-soluble collagen [29]. Marine snail meat and hepatopancreas are important sources of protein [30]. The edible portion of common octopus (*O. vulgaris*) has appreciable level of proteins

[31]. Protein contents of some raw shellfish species from Indian waters vary from 14.0 to 21.6% [32].

An adequate diet must contain an appropriate level of nutritive protein to support long-term health. Fish muscle proteins are considered nutritionally equivalent or slightly superior to meat proteins due to their lower collagen contents and higher sensitivity to proteolytic digestion [33–35]. The nutritive value of a protein is determined by its primary structure, amino acid composition, contents of essential amino acids, susceptibility to enzymatic digestion, and chemical changes during processing such as thermal treatment. Most seafood proteins show a digestibility above 90% associated with release of copious proportions of essential amino acids (EAAs) (lysine, methionine, threonine, tryptophan, isoleucine, leucine, phenylalanine, and valine), suggesting high nutritive value of the proteins [35, 36]. Enzymatic digestion of proteins in the human digestive system gives rise to many peptides and amino acids, which can be absorbed in the intestine in the form of single amino acids and oligopeptides [25, 33, 34]. Animal feeding experiments are used to determine nutritive value of proteins. These include the nitrogen balance method based on protein digestibility, determination of protein efficiency ratio (PER, weight gained per g of protein consumed), net protein utilization (NPU, ratio of amino acid converted to protein to the ratio of amino acids intake), and biological value (a measure of absorption and utilization of protein by the living organism). These studies have suggested the high nutritive value of fish proteins [37]. It is generally accepted that the relative content of EAAs is the major factor determining the nutritional value of a food protein. Most animal proteins have a satisfactory essential amino acid (EAA) pattern required to satisfy amino acid requirements. The amino acid score (AAS) of a protein is indicative of its nutritional quality; an AAS score of 100 means high protein quality. Proteins of seafood including shellfish, cooked under moist heat, have AAS scores as high as 100 indicating their high nutritional quality [25, 38]. The protein digestibility-corrected amino acid score (PDCAAS) is based on the amino acid content of food protein, its digestibility, and its ability to supply EAAs according to requirement [19, 25]. The PDCAAS of shrimp is 1, indicating its good protein quality [38]. Shellfish proteins have PER values that are slightly above that of casein, the major milk protein [22, 24].

Dietary fish proteins, rich in arginine, glycine, and taurine, may have ability for improved resolution of inflammation in the muscle, attributed to their ability to decrease the production of major pro-inflammatory cytokines (TNF-α, IL-6) and to limit the accumulation of pro-inflammatory macrophages at the site of injury. Cod protein promotes growth and regeneration of the skeletal muscle after trauma, partly because of the improved resolution of inflammation [39]. Krill meat concentrate has 78% protein and 8% fat on dry weight basis. The protein has PDCAAS and PER equal to casein and hence is a promising protein source for human consumption [40]. Spray-dried protein powder from threadfin bream *(Nemipterus japonicus)* has a PER value of 3.52 ± 0.27 and trypsin and pepsin digestibility up to 80% [41]. In view of their nutritional properties, seafood proteins are able to enhance the nutritive value of proteins from plants, legumes, grains, nuts, seeds, and vegetables, which may be deficient in one or more of the essential amino acids [42, 43]. Fish meat and also

other commonly consumed protein foods are also significant sources of nutrients such as calcium, vitamin D, potassium, iron, and folate [35, 44].

3.2 Amino Acids

Seafood items are important sources of both EAAs and nonessential amino acids. The predominant EAAs in seafood are lysine and leucine, and within the nonessential, aspartic and glutamic acids are more abundant. The nonprotein nitrogen fraction of fish and shellfish muscle, which is usually higher than that of terrestrial animals, contains amino acids in addition to small peptides, creatin, creatinine, trimethylamine oxide, and nucleotides. Glycine, taurine, alanine, and lysine are generally present in relatively higher amounts. Certain nonessential amino acids, such as aspartic acid, glycine, and glutamic acid, necessary for growth and maintenance in humans, are present in seafood, as revealed by the amino acid compositions of 60 commercially important fish and shellfish [45].The whole body tissues of Atlantic halibut, yellowtail flounder, and Japanese flounder contain all the EAAs [46]. The freshwater fish, carp, has high content of free amino acids, irrespective of the area of breeding, the dominant amino acids being histidine, methionine, cysteine, phenylalanine, tyrosine, lysine, and threonine [24].

Crustaceans have more amino acid contents than finfish. The amino acids alanine, glutamic acid, and glycine are mostly responsible for the flavor of cooked shellfish. Alanine and glycine contribute to sweet tastes and glutamic acid to the "umami" taste of crustaceans [47]. Shrimp has good contents of glycine (up to 1% of the fresh muscle), alanine, and proline [16]. The major EAAs of shrimp are arginine, lysine, leucine, and methionine, while the nonessential amino acids are glutamic acid, aspartic acid, proline, and glycine [48]. Glutamic acid and glycine contents were greater in black tiger shrimp meat, while white shrimp had good levels of hydroxyproline. Arginine, leucine, isoleucine, and proline were predominant in both shrimps [29]. The Norway lobster has threonine, leucine, valine, lysine, and arginine, each at levels of about 40 mg% of raw meat. It has also glycine, alanine, and glutamic acid at 58, 57, and 31 mg%, respectively. Besides, the lobster also contained the dipeptide anserine at 53 mg% of raw meat [49]. Edible tissues of the marine snail are good sources of EAAs, particularly aspartic acid [30]. Green crab meat is balanced in EAA contents, except for tryptophan [50]. Fish sauces are products that contain significant amounts of peptides and amino acids [52].

Cooking, in general, enhances the digestibility of fish proteins. Mild cooking causes little loss of protein with only a slight loss in available lysine, whereas drastic heating can significantly reduce the protein quality. Boiling has little effect in the composition of shellfish. Moisture, protein, fat, ash, and carbohydrate contents of the cooked horse mackerel ranged from 56 to 61%, 21 to 24%, 13 to 20%, 1.7 to 2.5%, and 1 to 4%, respectively. The changes in amino acids in the fish were significant after frying, grilling, and steaming. Cooking increased the contents of essential and nonessential amino acids compared to raw fish. Amino acid contents of grilled horse mackerel were higher than those found in fried and steamed fish [52]. Literature on

the influence of processing on nutritional value of seafood has been reviewed by Venugopal [22].

Taurine (2-aminoethanesulfonic acid) contents were relatively high in flatfish and ray and low in silver pomfret, yellow croaker, and baby croaker. Although humans synthesize taurine from methionine and cysteine, aging lowers production of taurine in the body, necessitating supplementation of the amino acid. Shellfish and other seafood are good sources of taurine; its contents in the muscle of mussel, oyster, cuttlefish, and squid vary between 7.5 and 12.0% of total amino acids [32]. Taurine acts beneficially on glucose metabolism and also lowers blood pressure by reducing cholesterol absorption [43, 53, 54]. Processed fishery products may be supplemented with taurine, the added taurine retaining in the product without adversely affecting the sensory acceptability [55].

3.3 Lipids

The contents of lipids in fishery products vary depending on the species, season, geographic location, age, gender, and diet. Feed has a strong influence on the lipid contents of fishery products including cultured items [20]. The lipid content can vary from 4% to more than 30% in Atlantic mackerel and from 2 to 25% in Atlantic herring, depending on the season. Species living in temperate waters have more lipid contents in their flesh than leaner tropical fish [24]. Contrary to terrestrial animals, which deposit their lipids in the adipose tissue, fish have lipids in the liver, muscle, and perivisceral and subcutaneous tissues. Lipid content in dark muscle is much higher than in white muscles. Pelagic fish, which contain more dark muscle, have more fat than the demersal species such as cod, which have higher proportions of white meat. Depending on the fat content, fish are classified as lean (less than 2% fat), low-fat (2–4% fat, such as cod and hake), medium-fat (4–8% fat), and fatty fish (more than 8% fat, such as mackerel and herring).

The fatty acid composition of seafood is different from red meat, vegetable, and dairy products and also showing marked variability within and between species. The fatty acids are present as triglycerides, which are prone to hydrolysis by lipases. The phospholipids of tropical fish are more saturated than those from cold water. The major phospholipids include phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin. In contrast to red meat, marine fish lipids have appreciable proportions of n-3 (omega-3) long-chain PUFA, particularly eicosapentaenoic acid (EPA) ($C_{20:5w3}$ *cis*-5,8,11,14,17) and docosahexaenoic acid (DHA) ($C_{22:6w3,}$ *cis*-4,7,10,13,16,19). Fish obtain these from PUFA-rich phytoplankton used as feed by the animal. Fatty fish such as herring and mackerel contain more omega-3 PUFAs (particularly EPA and DHA) than leaner species. Saturated fatty acid (SFA) content is comparatively less. Freshwater fish generally contain lower proportion of n-3 PUFA than marine fish [47]. Marine steroids are composed of cholesterol, which is present at 20 to 50 mg per 100 g raw meat of finfish and 250 to 650 mg per 100 g in roe [21].

Shellfish items have low crude lipid contents, generally up to 2% (w/w) of their raw muscle. The contents of EPA and DHA in shellfish usually range between 300 and 500 mg% of raw muscle; their contents are generally lower than those of oily finfish such as Atlantic mackerel and sardine [19, 21]. Shellfish lipids, like those of finfish, are rich in PUFA generally exhibiting a ratio of n-3 PUFA to n-6 PUFA above 1.0 [22, 56]. The Mediterranean giant red shrimp (*Aristaeomorpha foliacea*) has good levels of n-3 PUFA, particularly EPA and DHA [57]. The lipids of the shrimp spp. (*P. longirostris* and *A. antennatus*) and Norway lobster (*N. norvegicus*) contain 42–48% PUFA, 26–35% MUFA, and 23–27% SFA [48]. The crude fat (1%, w/w) of brown shrimp (*C. crangon* L) meat consisted of 33% SFA, 22% MUFA, and 29% n-3 PUFA. The SFA and MUFA fractions have 21% palmitic acid and 14% oleic acid, respectively. The PUFA consisted of EPA and DHA at 41% and 32%, respectively [28]. The n-3 PUFAs of white shrimp (*P. vannamei*) and black tiger shrimp (*P.monodon*) are 42 to 44% of crude lipids. PUFAs of the white and black shrimps have DHA-to-EPA ratios of 1.05 and 2.15, respectively [29]. Ozogul et al. [58] reported that the common cuttlefish (*S. officinalis*), European squid (*L. vulgaris*), common octopus (*O. vulgaris*), and musky octopus (*E. moschata*) had PUFA, SFA, and MUFA contents of 43.6–56.5%, 28.2–35.3%, and 4.4–9.5%, respectively. The fatty acids of the common octopus (*O. vulgaris*) consisted of 58.6% of n-3 PUFA (composed of 20.1% EPA and 26.3% DHA), 25.9% SFA, and 15.4% MUFA [31]. The mussel (*M. coruscus*) has higher contents of PUFA than SFA and MUFA, with DHA and EPA comprised of 12–18% and 10.8–14.6% of total fatty acids, respectively [59]. Crustaceans, bivalves, and cephalopods may contain total sterols at 150–250 mg%, w. wt. [16, 19, 28].

The changes that take place in fats during heat processing greatly depend on the fatty acid composition. Unsaturated lipids are very susceptible to oxidation. Enzymes such as lipoxygenase, peroxidase, and microsomal enzymes can potentially initiate lipid peroxidation producing hydroperoxides, which decompose to a wide range of compounds. These compounds, which include aldehydes, ketones, alcohols, small carboxylic acids, and alkanes, give rise to very broad odor spectrum and also yellowish discoloration to the product. These substances are formed slowly at normal frying temperatures in pure fats, but their formation is catalyzed by traces of metals such as iron and copper present in the fish. The common dietary antioxidants vitamin E (tocopherol), vitamin C, polyphenols including flavonoids, and carotenoids are able to control lipid oxidation (see also Sect. 3.4**)**.

The human diet contains long-chain PUFA belonging to the n-6 and n-3 families. Major dietary n-6 PUFAs include linoleic, $C_{18:2w6}$ (18:2); γ-linolenic, $C_{18:3w6}$ (18:3); and arachidonic, $C_{20:4w6}$ (20:4) acids, whereas major dietary omega-3 PUFAs include α-linolenic, $C_{18:3w3}$ (18:3), EPA, and DHA. Linoleic, α-linolenic, and γ-linolenic acids are present in large quantities in foods of plant origin. Arachidonic acid originates from muscle and organ meats or may be synthesized within the body from linoleic acid. In comparison with n-6 PUFA, n-3 PUFA can have significant health benefits. The cardioprotective role of n-3 PUFA has been determined by several studies [60, 61]. Other benefits include their antidepressive, antiaging, and antiarthritic effects, among others [61] (see also discussion on Sect. 4.2.1). The mode

of action of EPA and DHA is attributed to their ability to give rise to a class of pharmacologically important groups of compounds, such as prostaglandins, prostacyclins, thromboxanes, and leukotrienes (collectively called eicosanoids). Both n-3 and n-6 PUFAs are precursors of eicosanoids. Whereas eicosanoids derived from n-6 PUFA, such as arachidonic acid, have pro-inflammatory functions, eicosanoids derived from n-3 PUFA have anti-inflammatory properties. The n-3 PUFA-derived eicosanoids antagonize the formation of inflammatory eicosanoids such as prostaglandin E_2, derived from arachidonic acid and other n-6 PUFAs. The n-3 PUFAs impart their anti-inflammatory effects via reduction of nuclear factor-κB activation. This transcription factor is a potent inducer of pro-inflammatory cytokine production. Through these actions, the n-3 PUFAs alter cell and tissue functions that favor disease prevention and maintenance of health [61, 62].

3.4 Carotenoids

Carotenoids are a group of pigments that contribute to the yellow, orange, and red color of aquatic organisms. Carotenoids exist in mollusks (oyster and scallop), crustaceans (shrimp, lobster, crayfish, and crab), and finfish (salmon, trout, sea bream, and tuna). They are also present in their processed products influencing consumer acceptability. Animals, including humans, do not synthesize carotenoids de novo and rely upon diet as the source of these compounds. Fish and shellfish accumulate carotenoids in their body tissues from carotenoid-rich plants, which are used as their feeds. The carotenoids may be either hydrocarbons or xanthophylls (the oxygenated derivatives) and include astacene, astaxanthin, canthaxanthin, cryptoxanthin, fucoxanthin, lutein, neoxanthin, violaxanthin, zeaxanthin, alloxanthin, and β-carotene [4, 63, 64]. Astaxanthin, perhaps, is the important pigment present in fishery products. The red-orange color of salmonid fish is due to the presence of astaxanthin. The carotenoids β-carotene and β-cryptoxanthin ingested by the animals may be converted to different compounds, including astaxanthin and vitamin A. In shellfish carotenoid contents vary depending on body parts; they are high in their carapace, followed by the head, while their meat has minimum contents. For example, the total carotenoid contents (mg g^{-1}) of raw meat, head, and carapace of shallow water shrimp (*P. monodon*) are 17.4, 58.4, and 86.6, respectively. Similar pattern is observed in carotenoid contents in the body parts of shallow water shrimps (*M. dobsoni*, *P. stylifera*, and *P. indicus*), deep-sea shrimps, *S. indica*, and A. *alcocki* [64]. Carotenoid content in raw body tissues of scallop ranged from 7 to 60 μg g^{-1}. The pigment contents varied in this order: gonad > mantle > adductor > gill [65].

Antioxidants, defined as substances capable of delaying, retarding, or preventing the development of rancidity or other flavor deteriorations, can inhibit or retard oxidation either by scavenging the free radicals that initiate oxidation or by breaking the oxidative chain reactions. The mechanisms also involve binding of metal ions, scavenging of oxygen, converting hydroperoxides to non-radical species, deactivating singlet oxygen, and thereby suppressing the generation of free radicals and reducing the rate of oxidation. Autooxidation of unsaturated lipids leads to

formation of reactive oxygen species (ROS), which include peroxy radicals ($ROO^{\cdot}$), superoxide anion (O_2^{-}), hydroxyl radical (HO^{-}), and alkoxy radical (RO^{-}), among others. Non-radical derivatives are hydrogen peroxide (H_2O_2), ozone (O_3), and singlet oxygen ($^1O_2$). The hydroxyl radical is the most reactive ROS, followed by singlet oxygen. Reactions of ROS with food components destroy nutrients; change the functionalities of proteins, lipids, and carbohydrates; and lead to the formation of undesirable volatile compounds and carcinogens. Carotenoids are natural antioxidants, which are able to quench singlet oxygen and act as in vivo scavengers of ROS. The antioxidant activities of carotenoids have been attributed to the presence of conjugated double bonds in their structures [66, 67]. The singlet oxygen scavenging ability is the highest for β-carotene, followed by tocopherol, riboflavin, vitamin D, and ascorbic acid [66]. Free radical stress can cause tissue damage and result in an inflammatory response. Adequate intakes of carotenoids and also other antioxidants such as vitamin A, vitamin C, and vitamin E may help prevent free radical-induced adverse oxidative processes in living systems. Carotenoids, in general, also possess anti-inflammatory properties, presumably due to their effects on intracellular signaling cascades, thereby inhibiting production of inflammatory cytokines [67, 68]. Astaxanthin, the major shellfish carotenoid, has the ability to protect body tissues from oxidative damage by UV light. The antioxidant activity of astaxanthin is higher than that of β-carotene, lutein, lycopene, α-tocopherol, and canthaxanthin. It also has anti-inflammatory and cardioprotective properties [69]. The potential health benefits of astaxanthin also include its anticancer, antiaging, and immunostimulating activities. The carotenoid also possesses antidiabetic properties, controls cataracts, and inactivates gram-negative bacteria such as *Helicobacter pylori*, responsible for chronic gastritis [63, 68, 69]. Canthaxanthin and β-carotene protect macrophage receptors from ROS, while β-carotene protects neutrophils, a major class of white blood cells, which use ROS to kill phagocytized bacteria [70].

3.5 Vitamins

Vitamins and minerals are essential for normal physiological functions. Their contents in fishery products vary with the species, age, season, sexual maturation, and geographic area. Fatty fish are excellent source of fat-soluble vitamins A, D, and E, which are more concentrated in their liver, viscera, and eyes but in less amounts in their flesh [21]. The flesh of lean fish contains about 25 to 50 IU of vitamin A, while in fatty fish such as mackerel and herring, the content ranges between 100 and 1500 IU. Vitamins A and D are also present in copious levels in the liver of lean species like cod. In freshwater fish, vitamin A exists mainly in the form of 3,4-dehydroretinol. Considerable amount of vitamin A, as retinol, is found in shark and oysters, as well as in oily fish such as small sardines, herring, and horse mackerel. Fish liver oil is an excellent source of vitamin A. Shrimp, blue mussel, oyster, and scallop are good sources of vitamin A [15–17, 21, 23].

Vitamin D mainly occurs in nature as ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3). Fish obtain their requirements of vitamin D_3 through phyto- and

zooplanktons used as food. Fatty fish like horse mackerel or herring have a higher content of vitamin D than lean species like flounder or marine trout. Contrary to the general belief, there was no significant correlation between contents of fat and vitamin D. Pelagic fish with high fat content provides excellent dietetic sources of vitamin D_3. Shrimp recorded about 0.06 μg vitamin D_3 per 100 g [71]. Fish liver and oils are also rich sources of vitamin D_3 containing up to 300 μg of the vitamin per kg oil [24]. The anticancer properties of vitamin D have been pointed out recently [72]. Vitamin E comprises of four tocopherols, α-, β-, γ-, and δ-tocopherol, all of them are present in seafood and aquacultured products, particularly fatty fish items [73]. Through its potent antioxidant activity, the vitamin quenches free radicals formed by oxidative processes, thereby controlling inflammatory processes; heating processes, boiling, grilling, and frying, caused a decrease in tocopherol contents in fishery products [74]. Fish flesh seems to be considerably rich in the antihemorrhagic vitamin K [24].

Water-soluble vitamins are distributed along the whole fish, with niacin, thiamine, riboflavin, pyridoxine, and B_{12} being the most representative in aquatic organisms [4, 24]. The average content of thiamin varies from 4 to 400 μg per 100 g meat. However, much of thiamin is destroyed by heat and oxygen or is lost in cooking water or when exposed to ionizing radiation. Modest amounts of riboflavin are present particularly in the dark flesh of some species like canned herring, mackerel, and pilchard. Ideal sources of pyridoxine (vitamin B_6) are salmon and tuna and, to some extent, shellfish. Niacin, like thiamine, forms part of a coenzyme essential in the production of energy. The vitamin ranges from 0.9 mg to 3.1 mg per 100 g raw fish flesh. Vitamin B_{12} (cobalamin) is found in significant quantities in fish, particularly fatty fish. In general, seafood items contain 0.89 to 42 μg of vitamin B_{12} per serving (3 oz). Anchovies, clams, herring, oysters, pilchard, and sardines have vitamin B_{12} ranging from 25 to 40 μg per 100 g raw meat. Shellfish species contain all the vitamins, particularly vitamin B_{12}. Oyster, mussels, and short-necked clam have a content of 46.3, 15.71, and 87.0 μg per 100 g, respectively. Contents of vitamin B_{12} are generally higher in muscle tissues of crab and lobster [14]. Certain fish like salmon, sardine, trout, and tuna have a B_{12} content varying from 3.8 to 8.9 μg 100 g^{-1}; however, mollusks and clams have an average of 98 μg 100 g^{-1} of B_{12} [75]. The flesh of Atlantic salmon, European hake, and sardine has folic acid concentrations of 10, 27, and 24 μg 100 g^{-1}, respectively [76].

Vitamins are considered the most susceptible to loss during heat treatment; the magnitude of loss depends on the specific vitamins and the conditions employed. Water-soluble vitamins may be lost due to leaching. A combination of oxygen, light, and heat causes a greater loss of vitamins than any one of these factors individually. Folate and vitamin B_6 are susceptible to destruction due to oxidation. Riboflavin is reasonably stable during cooking but is sensitive to light as it decomposes on exposure to ultraviolet rays. The fat-stable vitamin A and also carotenes are relatively stable at normal cooking temperatures, but the high temperatures used in frying can produce oxidative losses and isomerization of the carotenes, with significant losses of biological activity. Vitamin E is slowly destroyed during frying and is decomposed by light.

3.6 Minerals

Several minerals are essential for life. The process of bone formation, for example, requires adequate and constant supply of nutrients such as calcium, magnesium, vitamin D, vitamin K, and fluoride, besides proteins. The ash contents up to 2.0% (w/w) of seafood carry macroelements such as calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), and phosphate (Pi) and microelements such as zinc (Zn), copper (Cu), chromium (Cr), manganese (Mn), selenium (Se), fluorine (F), and iodine (I), which are important for human nutrition [15, 16, 19, 35]. Fe, Zn, Cu, and Mn concentrations are generally higher in the liver than in muscle tissues, either being wild or farmed fish. The minimum contents (μg g^{-1} edible fish flesh) of Na, K, Mn, Ca, Cu, Pi, and I are 250, 250, 100,100, 1.1, 250, and 0.1, respectively [21]. Fish is a good source of Zn and Cu that ranged from 7 to 34 μg g^{-1} in wild sea bass and from 3.7 to 76.2 μg g^{-1} in the farmed ones [21]. An average serving of fish can grossly satisfy the total human requirements for essential microelements [21, 24].

Most shellfish are good sources of Na, K, Pi, Fe, Zn, Se, and Cu. Most fresh marine fish may be considered moderately low-sodium foods delivering approximately 140 mg sodium per serving. However, the sodium content of most processed fish and seafood products (frozen, canned, smoked, cured) can be substantially high, ranging from 3 to 9 mg g^{-1}. Seafood is a source of calcium, its contents varying from 60 to 1200 μ per g^{-1} depending upon the species. The iron content of edible tissue may vary as 90 μg g^{-1} in cod, flounder, and pollock and 90–200 μg g^{-1} in carp, catfish, salmon, and trout. Copper, which is necessary for normal blood formation and maintenance of blood vessels, tendons, and bones, is sufficiently available from shellfish. Mollusks and crustaceans contain appreciable levels of Cu and Zn [19]. Oysters are rich in zinc, iron, and copper [21]. Mg, Ca, and Fe are in appreciable levels in white and black shrimps [29]. The contents of Ca, Mg, Pi, and Na in blue crab vary in claw, breast meat, and hepatopancreas [77]. The edible portions of clam are rich in Na, K, Ca, Mg, Fe, Zn, and Cu [26]. The contents (mg 100 g^{-1}) of iron, zinc, and calcium in 55 samples of fresh fishery products from Bangladesh ranged from 0.34 to 19, 0.6 to 4.7, and 8.6 to 1000, respectively. Shrimp had maximum Cu and I contents of 12 and 1.2 μg g^{-1}, respectively [71]. Fish and shellfish are rich sources of iodine, being highest in oysters, followed by clams, lobster, shrimp, and crab followed by ocean fish. Oceanic fish has 0.3 to 3.0 μg g^{-1}iodine and freshwater fish, 0.02 μg to 0.04 μg g^{-1} [20].

3.7 Evaluation of the Nutritional Value of Fishery Products

It is interesting to evaluate the nutritive value of fishery products against the US 2015–2020 *Dietary Guidelines*, which provides consumers of different age and sex guidance to choose a healthy eating pattern to prevent diet-related chronic diseases. The *Guidelines* are designed to meet the Recommended Dietary Allowances (RDA) and the Adequate Intakes for Essential Nutrients set by the Institute of Medicine

(IOM), US National Academies [78]. The *Guidelines* recommend daily intake of the following amounts of nutrients by males aged 19–30, namely:

Protein: 56 g
Total fat: 20–35 g (saturated fat, <10% of total fat)
Macro-minerals (in mg): Na, 2300; Ca, 1000; K, 4700; Pi, 700; and Mg, 400
Micro-minerals (in mg except vitamin D and vitamin B_{12}): Fe, 18; Zn, 11; Mn, 2.3; Cu, 0.9; and Se, 0.055
Vitamins (in mg except vitamin B_{12} which is in μg and vitamin D, which is in IU): A, 900; B_1, 1.2; B_2, 1.3; B_6, 1.3; B_{12}, 2.4 μg; niacin, 16; D, 600 IU; E, 15; K, 0.1; and folate 400
Calories: 2400 to 3000

The daily nutritional goals for other age and sex groups are also given in the Guidelines [78]. Seafood including shellfish can significantly satisfy requirements of almost all of the nutrients, mentioned above. Dayal et al. [38] calculated percent direct value (%DV) (representing ability to satisfy dietary requirement of each nutrient as per recommended values) for various nutrients present in seafood. They reported %DV values of 75 for total EPA and DHA contents and a value of 70 for the essential amino acids methionine, tryptophan, and lysine present in tiger shrimp (*P. monodon*) [38]. Consumption of 100 g of most shellfish can meet at least 50% requirement for proteins by the adult [23]. Compared with suggested amino acid requirements by the FAO/WHO, the hydrolyzates of the little *Loligo* squid (*U. chinensis*) have high nutritional value and are a potential nutritious supplement used in various food products [79]. Selenium has a %DV as high as 110, suggesting that the shrimp fully satisfied the dietary requirement for this mineral [38]. Shellfish and other seafood provide good measures of vitamin B_{12} and vitamin D. A 100 g serving of shellfish, except squid, scallop, and crayfish, can provide appreciable amounts of the vitamin B_{12} necessary to satisfy its dietary requirement. The giant red shrimp, as well as Norway lobster, is a valuable source of nutrients, including proteins and antioxidants, among others, for the human diet [48]. The muscle and gonads of female crab (*C. pagurus*) have favorable n-3 to n-6 PUFA ratios and a well-balanced essential amino acid composition [80, 81]. American and European lobsters have nutritive values compatible with nutritional foods [82]. The mussel *P. viridis* has balanced ratios of essential to nonessential amino acids and also of *n*-3 to *n*-6 PUFA contents [83]. Furthermore, the consumption of mollusks can make an important contribution to the daily dietary intake requirement of Se, Cu, and Zn [84]. The presence of appreciable levels of PUFA (including EPA and DHA), vitamins, minerals, and amino acids qualifies the oyster (*C. madrasensis*) as a potential "health" food. The shellfish has also atherogenic and thrombogenicity indices together with a good hypocholesterolemic to hypercholesterolemic ratio, pointing out its health benefits [85]. The blue crab could be used as a dietary supplement to balance human nutrition [77]. Nutritional claims have also been made with respect to common octopus [31] and Asian hard clam [26]. The nutritional value of seafood has also been pointed out by many studies [19, 23, 35]. The Second International

Conference on Nutrition (ICN2), held in Rome in November 2014, confirmed the importance of seafood including shellfish as a source of nutrition and health [1].

4 Nutraceuticals and Bioactive Compounds from Seafood Discard

Industrial processing of seafood generates voluminous discards such as scales, shells, frames, backbones, viscera, head, liver, skin, belly flaps, dark muscle, roe, and others. Processing discards of crustaceans are composed of the cephalothorax, carapace, and tail; shell discards of shrimp, krill, and crab constitute as high as 50% of the shellfish. Increased aquaculture production of popular shrimp and other shellfish items has led to a significant amount of discards. Depending upon finfish, discards may range from 25 to 50% of the raw material. The backbone accounts for about 15% of the wet weight of fish such as Atlantic cod. In addition, approximately 50,000 tons of scale is produced from the current global fish processing operations [3]. Currently most of these discards are used as landfill or converted into fertilizer or used for animal feed and ensilage. For example, the Norwegian fisheries annually produce more than 615,000 tons of discards; most of it is converted into fish silage and fish meal as animal feed [52]. Furthermore, commercial fishing operations results in a large proportion of bycatch, which has poor consumer value due to inherent problems related to unattractive color, flavor, texture, small size, and high fat content. Most of these underutilized fish belong to the abundantly available pelagic species and are considered as bycatch [86]. Secondary processing of seafood discards can significantly reduce the environmental hazards associated with the industry, besides yielding valuable ingredients for diverse applications.

The important ingredients that can be recovered from seafood discards include proteins including collagen and gelatin and their hydrolyzates, peptides, enzymes, oil, carotenoids, bone calcium, chitin, chitosan, and other compounds, many of which have nutraceutical potentials [2, 36, 87–89]. During the recent years, there is increased consumer interest toward nutraceuticals and natural bioactive compounds as functional ingredients to prevent, alleviate, or treat diverse diseases [88]. An example may be cited. The processing of the Black Sea anchovy gives rise to approximately 32% (w/w) discards consisting of the head, frame, and viscera. These have protein and fat contents ranging from 12 to 17% and 10 to 23.9%, respectively. The discards are also rich sources of lysine and leucine, constituting 6–7% and 5–6% of total amino acids, respectively, PUFA (about 32–40% of total fatty acids, with n-3 fatty acids comprising about 27–34% of total fatty acids), and also various minerals. The anchovy discards are raw material for protein powder, protein hydrolyzates, fish oils, and mineral supplements [90].

Interests in seafood- and other marine-derived compounds stem from the fact that most aquatic organisms have inbuilt mechanisms to survive hostile oceanic environments such as varying degrees of salinity, pressure, temperature, and illuminations. Most marine organisms produce several secondary metabolites, which although are not directly involved in central physiological functions, yet contribute

to their survival. They synthesize novel compounds with interesting bioactivities, which facilitate them to adapt to these conditions. Seafood species, for example, in the extremely low temperatures of the polar region possess enzymes having novel features relating to isozyme distribution, substrate binding, amino acid sequence, low activation energies, high-specific activities, and thermal sensitivities [91, 92]. Besides, most marine organisms also produce several secondary metabolites such as sulfated polysaccharides, peptides, alkaloids, terpenoids, and others, which although are not directly involved in central physiological functions, yet contribute to their survival [4]. These novel compounds may possess a wide spectrum of bioactivities encompassing anticancer, anti-inflammatory, antiviral, antimicrobial, antioxidative, antihypertensive, anti-atherosclerotic and anticoagulant, immunomodulatory, analgesic, antidiabetic, appetite-suppressing, neuroprotective, and other interesting functions. Therefore, during the recent years, the search for nutraceuticals and other medicinal compounds from seafood and other marine organisms such as sponges, tunicates, bryozoans, mollusks, and sea slugs and other organisms has intensified for the potential control of various diseases such as cardiovascular disease (CVD), diabetes, osteoporosis, arthritis, Alzheimer's disease, and others [5, 88, 91]. Recent studies have indicated that specific compounds from fish such as cod, anchovy, eel, and shellfish like mollusks (mussel, oyster, clams, and abalone) as well as sea cucumbers may possess in vivo/in vitro anticancer/antitumor activities, supporting health benefits of these organisms. Some of the compounds can also function as hypotensive agents, cardioactive substances, muscle relaxants, antibiotics, and antiviral agents [4, 92]. Nguyen [93] observed that about 50,000 tons of lobster processing discard by-products can be valuable sources of bioactive compounds having potential applications in nutraceutical, pharmaceutical, and medicine, besides water treatment, agriculture, and other industries. Table 1 shows major nutraceuticals and bioactive components available from seafood.

The remaining part of this chapter is intended to briefly discuss various nutraceuticals and other bioactive compounds from seafood processing discards and underutilized fish. For the convenience of discussion, these compounds are grouped as those of nitrogenous origin, lipid-based compounds, polysaccharides and their derivatives, and mineral-based compounds [6].

4.1 Nutraceuticals of Nitrogenous Origin

4.1.1 Proteins

Seafood proteins, besides notable nutritive properties, as discussed above, also have excellent functional properties, which include solubility, viscosity, water-holding capacity, gelation, adhesion, elasticity, foaming, and oil emulsification capacities, which make them interesting food ingredients [4]. These properties emanate from their compositional and structural features, which include hydrophilic character, hydrodynamic size and shape, and ability for hydrogen bonding and ionic hydration, among others [4]. Seafood processing discards are therefore valuable sources of functionally active proteins. Techniques for isolation of these

Table 1 Major nutraceuticals and bioactive components from seafood

Finfish
Bioactive peptides
Biological calcium
Carotenoids
Enzymes including cold-adapted enzymes
Glycosaminoglycans including chondroitin sulfate, dermatan sulfate, and hyaluronic acid
Long-chain omega-3 polyunsaturated fatty acids (PUFAs)
Phosphopeptide from fish bone
Protein hormones such as calcitonin
Protein isolates including collagen and gelatin
Squalene and squalamine
Shellfish (crustaceans and mollusks)
Bioactive peptides
Carotenoids
Chitin, chitosan, and chitosan derivatives
Enzymes
Glucosamine
Long-chain omega-3 polyunsaturated fatty acids (PUFAs)
Mussel polysaccharides, lipids, and other products
Protein isolates including collagen and gelatin

proteins involve extraction by dilute acids, bases, or isoelectric solubilization/precipitation [94, 95]. Filleting operations of large fish such as hake, seer, and others give rise to frames that carry large amounts of meat portions. Mechanical deboning helps to recover meat from the frames. Thorough washing of the recovered meat gives surimi, which because of its excellent gel-forming properties, is an ideal raw material for the development of restructured imitation seafood [94]. Generally fish protein isolates maintain their properties for about 6 months when stored at 5 °C but loses them rapidly at 30 °C. Deterioration during storage is prevented by lowering the moisture content of the product and by eliminating oxygen from the package [96]. The protein isolate can be used as an ingredient to enhance protein contents of food products and also as a nutraceutical. Fortification of extruded corn snacks with up to 18% fish protein powder and 17% omega-3 fish oil did not influence the sensory attributes of the products when served within 12 weeks of production [96]. Soluble powders recovered from processing discards of Alaska pollock had protein contents ranging from 65% to 79% and nitrogen solubility value as high as 86%. Emulsion capacity (mL of oil emulsified per mg^{-1} protein) and emulsion stability of the pollock protein powders ranged from 29% to 35% and 65% to 78%, respectively, with a maximum fat-adsorption values of 10.6 mL of oil g^{-1} protein. The protein powder was also a good source of K, P, Mg, and also amino acids. Emulsions made with soluble protein powders from pollock exhibited viscoelastic characteristics, suggesting its use as a food fortificant and functional additive [97]. The nutraceutical potential of protein isolates has also

been indicated. For instance, the protein isolate from striped bass (*Morone saxatilis*) has a positive effect in control of CVD [95].

Preparation of fish protein hydrolyzates (FPHs) is a plausible method for utilization of proteins from seafood discards. Currently hydrolyzed fish proteins are used as condiments and sauces in the East Asian countries. FPHs have potential as a functional ingredient and protein supplement for developing formulated ready-to-eat products, which can help increase in protein consumption by the general public for health benefits [98]. In the year 2014, a landing of 7.7 MMT of tuna was recorded [1]. A sizeable portion of tuna is used for canning, which generates as much as 70% solid discards from the fish, consisting of muscle (after loins are taken), viscera, gills, dark flesh, head, bone, and skin. Proteins from these discards can be recovered as FPH for use as food supplement to enhance functional effects such as whipping, gelling, and texturing properties [99]. FPH has also been recognized as potential nutraceutical essentially due to the activity of antioxidant peptides present in the product [99] (see Sect. 4.1.2.). Both in vitro and in vivo studies have shown that certain peptide fractions in FPHs may stimulate the non-specific immune defense system [51]. Preliminary studies have indicated that expression levels of tumor necrosis factor (TNF-α) had a tendency to be lower after the addition of FPH. Furthermore, the combination of FPH and omega-3 fatty acids synergistically decreased expression levels of TNF-α, compared to individual effects of omega-3 fatty acids or FPH [100]. Animal feeding study conducted at Norway suggested the effect of fish protein hydrolyzate as a cardioprotective nutrient. The fish protein treatment reduced plasma cholesterol level and altered the fatty acid composition in liver, plasma, and triglycerol-rich lipoproteins in obese Zucker rats. The ratio of HDL cholesterol to total cholesterol was greater in the animals, compared with casein-fed animals [101]. Although not a nutraceutical, mussels possess specialized proteinous glue, collectively referred to as mussel adhesive proteins (MAPs), which adhere well to surfaces despite the presence of water. The protein is rich in the amino acid, L-3,4-dihydroxyphenylalanine (DOPA). MAPs may be used for surgical tissue adhesion or as components of muco-adhesive drug delivery systems. Another protein, green fluorescent protein (GFP), isolated from the jellyfish in 1962 has interesting applications in biotechnology and cell biology [4].

Frames discarded from fish filleting operations, fins, scales, air bladder, and fish heads are also good sources of collagen. Collagen and its partially hydrolyzed form, gelatin, contain more than 80% nonpolar amino acids such as glycine, alanine, valine, and proline and hence are different in their amino acid contents, in comparison with fish muscle proteins. Collagen-based biomaterials are extensively used as food additives and in ophthalmology, dermatology, drug delivery, cosmetics, and other industries [102, 103]. Fish collagen has lower denaturation temperatures than porcine collagens. Therefore, collagen from fish offal can be a better alternative to mammalian collagen for use in foods, cosmetics, and medicine. Collagen hydrolyzates are beneficial for the treatment of osteoarthritis and other joint disorders. Orally administered collagen hydrolyzate is absorbed intestinally and is accumulated in the cartilage. Ingestion of collagen hydrolyzate stimulates synthesis of macromolecules by chondrocytes in extracellular matrix [104]. Collagens can be easily converted into

gelatin by hot water treatment. Gelatin has found extensive application in the pharmaceutical industry. It is also being used as a matrix molecule for nanoparticle-based drug delivery applications. Most of the current production of gelatin is from porcine and bovine hides, bones, and hooves. The incidents of mad cow disease have made adverse impact on the use of mammalian gelatins. Fish gelatins could be plausible substitutes to mammalian gelatins, since they have properties such as bloom strength (the gel strength equivalent referred to in the industry), viscosity, and solubility comparable with those of mammalian gelatins [102, 103].

Fishery discards such as viscera, liver, and head are sources of proteases, lipases, transglutaminases, amidases, lipases, phospholipase, chitinases, alginate lyases, β-1,3-glucanase and carrageenases, and other enzymes. These can have potential applications in diverse fields such as food processing, pharmaceuticals, textiles, and protein engineering. Enzymes from fish and shellfish from cold habitats are particularly useful since they can function effectively at lower temperatures, thereby saving energy and protecting the food products [105, 106]. For example, pepsin from the gastric mucosa of polar cod has high specific activity at low temperature, and a fish trypsin exhibits high salt stability [107]. Cold-active proteases are useful for controlled protein digestions and extraction of carotenoproteins, which are known to be thermolabile. Transglutaminase is an enzyme that catalyzes cross-linking of glutamine and lysine in proteins through acyl-transfer reaction involving the γ-carboxamide group of peptide-bound glutamine residues and the ε-amino group of lysine residues. The enzyme from cold-adapted organisms can modify texture of protein foods at low temperatures [107, 108]. A number of seafood enzymes can replace conventional seafood processing operations such as isolation and modification of fish proteins and marine oils, production of bioactive peptides, acceleration of traditional fermentation, peeling and deveining of shellfish, scaling of finfish, removal of membranes from fish roe, extraction of flavors, shelf life extension, texture modification, removal of off-odors, and quality control either directly or as components of biosensors; non-fishery applications include use of trypsin from the stomach of tuna for milk clotting, cheese ripening, meat tenderization, and other processes [109]. Table 2 indicates various enzymes from fishery sources and their potential applications.

4.1.2 Bioactive Peptides

Bioactive peptides are protein fragments that have various physiological functionalities in the human body following consumption. These peptides are derived through enzymatic hydrolysis of proteins including food proteins including seafood muscle and the stromal proteins, collagen and gelatin [97, 110]. Enzymatic hydrolysis of fish processing discards under controlled conditions gives rise to peptides, which are separated by ultrafiltration or other suitable techniques [98]. Peptides from marine by-products have high nutraceutical potentials to address important public health issues like obesity, stress, hypertension, and others. These arise from their interesting bioactivities, which are dependent on their amino acid composition and sequences, and include their antimicrobial, antiviral, antitumor, antioxidative, antihypertensive, cardioprotective, anti-amnesiac, immunomodulatory, analgesic,

Table 2 Enzymes from fishery sources and their potential applications

Enzymes	Fishery sources	Characteristics and potential applications
Proteases including chymotrypsin and Cathepsin D	Carp, capelin, carp, Herring, Atlantic Cod, rainbow trout, Scallop, dogfish, squid, prawn, sardine	Milk clotting activity at alkaline pH. Degrade proteins including Myofibrillar proteins. Preparation of FPH
Collagenases	Carp, catfish, cod, Crab species, pacific Rock fish, bivalves	Comparable to mammalian metalloproteinases
Chymosin (rennin)	Carp, seal	Optimal pH 2.0–3.5, possesses high milk clotting activity
Lipases	Atlantic cod, seal, Salmon, sardine, Indian mackerel, Red sea bream	Production of omega-3-enriched triglycerides, flavor enhancement
Transglutaminases	Various fishery products	Comparable to mammalian metalloproteinases
Chitinases	Shellfish, squid Liver, octopus saliva	Optimal pH 2.0–3.5, possesses high milk clotting activity
β-1,3-glucanase, β-galactosidase	Abalone, scallop, Tilapia, sea cucumber	Production of omega-3-enriched triglycerides, flavor enhancement
Lysozyme	Arctic scallop shell, crab shell	Can produce low molecular weight antibacterial chitosans in coordination with cellulase and chitinase
Catalase, Glutathione Peroxidase	Marine mussel and other organisms	Antioxidants
Carbohydrases such as β-galactosidase	Finfish such as tilapia	Deacetylation of chitin

Adapted from Ref. [109]

antidiabetic, antiaging, appetite-suppressing, and neuroprotective activities [7, 70, 98, 110–113]. A major bio-function of many peptides is in the control of CVD. High blood pressure is one of the relevant independent risk factors for CVD. Angiotensin I-converting enzyme (ACE) plays a critical role in the regulation of blood pressure and is a multifunctional enzyme, which promotes conversion of angiotensin I to the potent vasoconstrictor angiotensin II. Inhibition of ACE is considered to be a useful therapeutic approach in the treatment of hypertension. A number of seafood-derived peptides can inhibit angiotensin I-converting enzyme (ACE) (EC3.4.15.1). ACE-inhibitory peptides have been produced from salmon, oyster, squid, sea urchin,

shrimp, snow crab, seahorse, jelly fish, Alaska pollock, bigeye tuna muscle, sea cucumber, sea bream, yellowfin sole, and others [112, 113]. A number of peptides from fishery sources are capable of scavenging free radicals and reactive oxygen species, thereby preventing oxidative damage [110, 112]. Fish and shellfish are also good sources of antimicrobial peptides, which are described in the hemolymph of spider crab, oyster, American lobster, shrimp, and green sea urchin [110]. A novel anticancer peptide from the shellfish *C. gigas* exhibited cytotoxic activity, inducing death of prostate, breast, and lung cancer cells [114]. Table 3 gives peptides from various fishery sources and their bioactivities.

4.2 Lipid-Based Compounds

4.2.1 Fish Oils

Processing discards, particularly the liver of fish such as albacore, cod, salmon, shark, haddock, and tuna, are good sources of oil and rich in omega-3 PUFA and vitamins A and D. The oil of fish species, such as Atlantic mackerel, shark, anchovies, menhaden, and Atlantic sardine, can have up to 35% omega-3 fatty acids, with EPA and DHA at around 10% of the oil. The European fish species such as capelin, herring, sand eel, and sprat are intermediate in oil contents, ranging between 18% and 25%. The demersal fish such as cod and halibut have a lower oil content of 15% to 20% [115–117]. Oil from whole fish or their discards are traditionally recovered by the wet reduction process. It involves steaming of the whole fish or processing discards and pressing of the cooked material to separate the solid portion as press cake for use as fish meal. The oil and water released during the pressing stage are pumped through screens and decanters to remove suspended solids and then centrifuged to separate the oil, which is then filtered and stabilized with antioxidants for storage [118]. Extraction of oil from various fish such as cod, herring, salmon, and other fish has been discussed [4]. The traditional cooking process for oil extraction has been replaced by isoelectric precipitation of oil-bound proteins [119], enzymatic treatment [120], or supercritical extraction using CO_2 [121]. Principal fatty acids in some fish oils are shown in Table 4.

The liver of shark is 22 to 30% of body weight, and the oil content in the liver may be as high as 90% of its weight. Recovery of oil from shark consists of natural decomposition of the liver, acid ensilage in the presence of formic acid, alkali digestion, and steam rendering (90 °C for 30 min) [4]. Shark oils could be attractive sources of n-3 fatty acids, specifically DHA, with content between 13% and 18% [116]. The oil of *Echinorhinus brucus* (bramble shark) has DHA (18%), EPA (16%), palmitic acid (15%), oleic acid (12%), stearic acid (8%), and squalene (38.5%), besides fat-soluble vitamins such as vitamin A, 17 mg%; vitamin D, 15 mg%; and vitamin K, 11.5 mg%. The oil showed high in vitro cytotoxic effect against the human neuroblastoma cell line (SHSY-5Y) [123]. Oil from the liver of black shark (*Labeo chrysophekadion*), Mako shark (*Isurus oxyrinchus*), and hammerhead shark (*Sphyrna* spp.) is also rich in vitamins A and D [4]. Salmon oil is an excellent source of EPA (18%) and DHA (12%) and is commercially available [7]. Oil of Antarctic

Table 3 Bioactive peptides from some fishery sources and their hydrolyzates (FPHs)

Fish/Shellfish	Sequence and other characteristics	Functions/activities
Alaska pollock	Gly-Pro-Leu	ACE inhibitory
Wakame	Tyr-Asn-Lys-Leu	ACE inhibitory
Bigeye tuna frame	Gly-Asp-Leu-Gly-Lys-Thr-Thr-Thr-Val-Ser-Asn-Trp-Ser-Pro-Pro-Lys-Try-Lys-Asp-Thr-Pro	ACE inhibitory
Shrimp (protease)	Le-Phe-Val-Pro-Ala-Phe	ACE inhibitory
Shrimp	Peptide[a] (molecular size, <10, 10 to 30, >30 kDa)	Anticancer
Wakame (papain)	Tyr-Asn-Lys-Leu	–
Oyster	Cys, Leu, Glu, Asp, Phe, Tyr, Ile, Gly	Antimicrobial
American lobster	Gln-Tyr-Gly-Asn-Leu-Leu-Ser-Leu-Leu-Asn-Gly-Tyr-Arg	Antimicrobial
Jumbo squid	Gelatin peptide[a]	Antioxidant
Blue mussel (fermentation)	Glu-Ala-Asp-Ile-Asp-Gly-Asp-Gly-Gln-Val-Asn-Tyr-Glu-Glu-Phe-Val-Ala-Met-Met-Thr-Ser-Lys	ACE inhibitory, anticoagulant, antioxidant
Scallop	γ-glutamyl-valyl-glycine	Flavoring agent
Spider crab	Proline-arginine-rich peptide[a]	Antimicrobial
Snow crab (protamex)	Cationic peptide[a]	Anticancer
Oyster	Peptide[a]	Anticancer, immune-stimulant
Clam (thermolysin)	Peptide[a]	ACE inhibitory
Clam (protamex)	Various peptides[a]	Hypocholesterolemic effect
Oyster (thermolysin)	Peptide[a]	Antioxidant
Oyster (subtilisin)	Pro–Val-Met-Gly-Asp and Glu-His-Gly-Val peptides	Antioxidant
Squid skin collagen	Peptide[a]	ACE inhibitory
Krill	Various peptides[a]	ACE inhibitory
Naturally present peptides in raw muscle		
Crab, shrimp	Crustin or crustin-like, callinectin, tachyplesin	Antimicrobial
Crayfish	Astacidin[a]	Antimicrobial
Lobster	Crustin or crustin-like[a]	Antimicrobial
Mussel	Tyr-Pro-Pro-Ala-Lys	Antioxidant
Mussel	Mytilin, mytimycin, myticin, pernin[a]	Antimicrobial
Shrimp	Penaeidin, crustin-like peptide	Antimicrobial, antioxidant
Scallop	γ-glutamyl-valyl-glycine	Flavoring agent

Summarized from various sources
[a]Sequence not reported
Enzymes used for FPH given in parentheses

Table 4 Principal fatty acids in some fish oils

Fatty acid	Capelin	Atlantic mackerel	Atlantic sardine	Anchovies
Myristic (14.0)	7	8	8	9
Palmitic (16:0)	10	14	18	19
Palmitoleic (16:1)	10	7	10	9
Oleic (18:1)	14	13	13	13
Eicosanoid (20:1)	17	12	4	5
Cetoleic	14	15	3	2
EPA (20:5)	8	7	18	17
DHA (22:6)	6	8	9	9

Values are % of total lipids
Source: Adapted from Ref. [122]

krill (*E. suberba*) contains omega-3 fatty acids, phospholipids and also natural pigments, and vitamins. However, since the oil content is only about 3% of the body weight, a large amount of krill needs to be processed for oil recovery [124]. The hydrolyzate of squid processing by-products had phospholipids fraction (45.6% of oil) which contained EPA and DHA at 16.9% and 29.2%, respectively. Peptides isolated from the soluble fraction exhibited angiotensin I-converting enzyme (ACE) inhibitory activity. The presence of both omega-3 fatty acids and ACE inhibitory peptides suggested the nutraceutical potential of the squid hydrolyzate [125].

Appreciable amounts of PUFA particularly EPA and DHA in fish oils denote significant therapeutic value of the oil. Moderate consumption of fish oil has shown to decrease the risk of major cardiovascular events including coronary heart disease (CHD) and atrial fibrillation [60, 126–128]. Shark oil has been an age-old remedy for various diseases as well as a source of strength and virility [116]. The favorable nutritional composition of bramble shark oil makes it a nutritional supplement [123]. While fish is the preferred source of omega-3 PUFAs, for those who do not consume fish, omega-3 PUFA supplementation is a feasible alternative [126]. This also holds good for consumers of Western-type diet, which is generally deficient in n-3 fatty acids [12]. It has been suggested that the daily intake of combined EPA and DHA should be at least 500 mg for individuals without underlying overt cardiovascular disease and at least 800 to 1000 mg for individuals with known coronary heart disease [128]. DHA is crucial for development of the brain and the central nervous system in infants and also to suppress neuro-inflammation and oxidative stress [70]. Studies indicate that long-chain n-3 PUFA can also have anticancer, anti-depressive, antiaging, and antiarthritic effects as well as neurodevelopment in children and can address a number of chronic diseases, including a spectrum of liver fat-related conditions [61, 62, 129–133]. Regular consumption of whole milk fortified with omega-3 along with oleic acid, minerals, and vitamins has been reported to reduce cell adhesion besides having anti-inflammatory effects in healthy children [131]. The mode of action of the PUFAs, particularly EPA and DHA, is attributed to their ability to give rise to a class of pharmacologically important groups of compounds, collectively called eicosanoids, as discussed in Sect. 3.3. In view of their therapeutic

benefits, many international and national health organizations recommend daily intakes of EPA and DHA in the range of 250 to 500 mg for general health and higher amounts for people diagnosed with CVD [126, 128]. Professional bodies such as the American Heart Association, Department of Health UK, and Food Safety Authority (FSA), among others, suggest daily total intake of EPA and DHA varying from 250 to 1000 mg/day [4, 35]. Microencapsulation technologies (spray-drying, spray-chilling, extrusion, co-crystallization, freeze-drying, and others) provide superior process to make odorless capsules containing the PUFA in gelatin or other capsules [4]. In view of the sensitivity of PUFA to oxidation, it is advisable that those who consume omega-3 may take adequate amounts of antioxidant nutrients, especially vitamin E, vitamin C, and selenium.

4.2.2 Squalene, Squalane, and Related Compounds

Squalene is a hydrocarbon ($C_{30}H_{50}$), having structures 2,6,10,15,19,23-Hexamethyltetracosa-2,6,10,14,18,22-hexaene. Livers of deep-sea shark species contain high contents of squalene and also other hydrocarbons like pristine, presumably for buoyancy, since they lack swim bladders. Liver oils of deep-sea sharks, mainly *Centrophorus* spp., found under depths in the Pacific, North Atlantic, and Indian Oceans contain about 85–90% unsaponifiable matter, which is essentially squalene [117]. The liver oil of shovelnose dogfish contains 60% hydrocarbons [4]. The liver oil of *Echinorhinus brucus* (bramble shark) has 39% squalene [123]. Squalene is a skin rejuvenating agent and possesses antilipidemic and membrane-stabilizing properties. Both squalene and its hydrogenated product, squalane, are considered to possess huge potential in nutraceutical, pharmaceutical, and cosmeceutical industries [134]. Pristane (C_{19} H_{40}) (2,6,10,14-tetramethylpentadecane), a natural saturated terpenoid alkane, and squalamine, an amino sterol antibiotic, are also found in shark liver oil. Squalamine has remarkable anti-angiogenic, antitubercular, and antiviral properties [4].

Other lipid-based bioactive compounds have been identified, particularly from mussels. A lipid extract of hard-shelled mussel (*Mytilus coruscus*) possesses strong anti-inflammatory activity and has the potential to treat rheumatoid arthritis [135]. The immune-strengthening properties of lipid extracts of the New Zealand green-lipped mussel (*Perna canaliculus*) have made the shellfish an alternative to conventional drugs in the treatment of rheumatic diseases like osteoarthritis, joint pain, and also atopic asthma [136, 137] .

4.2.3 Carotenoids

Shells of shrimp, prawn, krill, crab, and lobster contain astaxanthin ranging from 40 to 200 mg kg^{-1} dry weight, which is present as free or bound to proteins and/or chitin [63, 64]. Other shellfish carotenoids include canthaxanthin present in crayfish, mytiloxanthin in mussel, and mactraxanthin and fucoxanthin present in clams [2, 63, 64, 138]. The main pigment in the muscle tissues of the Yesso scallop, one of the important farmed scallops in China, was identified as pectenolone (3,3′-dihydroxy-β, β-caroten-4-one) [139]. The process of extraction of carotenoids from seafood discards involves initial digestion with proteolytic enzymes such as pepsin, trypsin,

or alcalase, followed by drying of the shell discard and recovery of the carotene pigments by organic solvents such as acetone, hexane, or isopropyl alcohol. If vegetable oil such as soybean oil is used for extraction, the isolate is pigmented oil. In recent years supercritical CO_2 has been successfully used for extraction of the pigments [64]. While the extracted carotenoids are used in aquaculture to impart color to the farmed fish and shellfish, in recent years there has been awareness on their bio-functions in human health. These include their antioxidant activities, their role as provitamins, and their anticancer activities, among others [63, 64, 66]. There is an immense scope to make use of these pigments for prevention of various diseases related to oxidative stress and resulting tissue damage, as discussed earlier (see Sect. 3.4).

4.3 Polysaccharide-Based Compounds

Polysaccharides are emerging ingredients against many chronic diseases. These macromolecules are biodegradable and characterized by water- and fat-binding capacities, gelation, viscosity, and other functional properties, which offer them various biomedical and food applications. Functions of polysaccharides in food and medicine encompass adhesive action, coating, emulsification, encapsulation, film formation, foam stabilization, and as swelling and thickening agents. Polysaccharides are amenable to chemical modifications, providing derivatives having enhanced and specific functions. While agricultural products are the traditional sources, marine polysaccharides are gaining importance because of recognition of their interesting therapeutic and other functions. These facilitate medicinal applications of marine polysaccharides and their derivatives in drug delivery, tissue regeneration, wound healing, dental implants, blood plasma expanders, vaccines, non-viral gene delivery, and many others [140, 141]. The major polysaccharide-based compounds from seafood and their various applications are briefly discussed below:

4.3.1 Chitin, Chitosan, and their Derivatives

Chitin is the second most abundant natural biopolymer derived from exoskeletons of crustaceans and also from cell walls of fungi and insects [142]. In lobster, crab, krill, shrimp, and prawn, chitin forms the outer protection coatings of the animal through a covalently bound network with proteins. The dry shell discards of crab, shrimp, and lobster may contain chitin up to 70% on dry weight basis, while its contents in dry squid skeleton pen and deproteinized shell of krill may be at a lower value of about 40%. Although not a nutraceutical, chitin offers various applications essentially through its deacetylated form, chitosan, as well as its numerous derivatives in medicine, food, and many other areas. The literature is voluminous on the topic and only a very brief discussion will be attempted here.

The process of isolation of chitin from crustacean discards consists essentially of three steps: demineralization of dried and pulverized shell discards usually by dilute hydrochloric acid, deproteinization by dilute alkali, and finally decoloration by sun

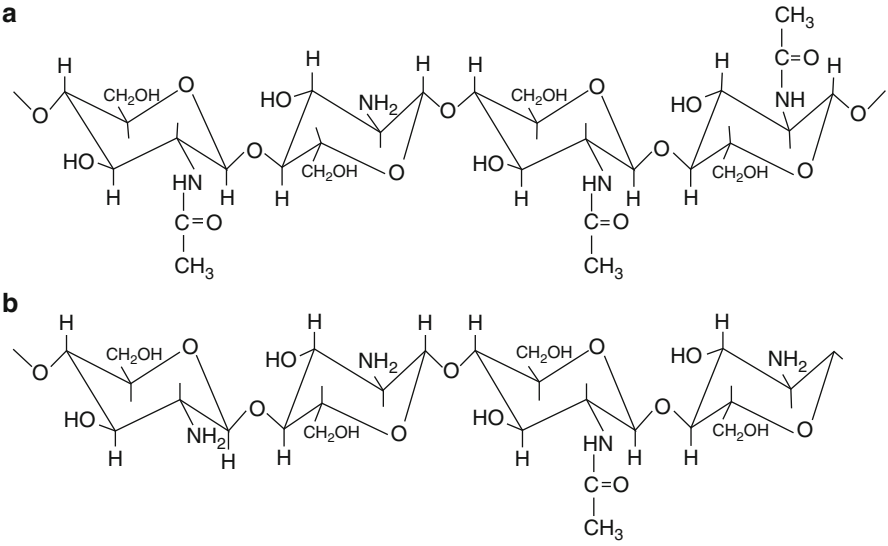


Fig. 1 Structure of chitin (**a**) and chitosan (partially deacetylated) (**b**)

drying, followed by washing and drying. Demineralization needs to be done under controlled conditions to prevent deacetylation of chitin. Proteins which exist as caroteno-protein complexes may be separated by trypsin treatment in the presence of ethylenediaminetetraacetic acid (EDTA). Decolorization can be performed by solvent extraction employing acetone or ethanol [142, 143]. An integrated process for isolating bioactive molecules including protein, chitin, carotenoids, and glycosaminoglycans from Pacific white shrimp (*Litopenaeus vannamei*) processing waste has been reported. Autolysis of 1 kg of the shrimp head followed by lyophilization of the autolyzate gave about 120 g of protein hydrolyzate. The recoveries of chitin and carotenoids were 25 ± 2 mg g^{-1} and 195 g^{-1} wet processing waste [144].

Chitin is a cationic polymer formed by units of N-acetyl-D-glucosamine, joined by ß-linkages. The structure of chitin is ß-(1–4)-N-acetyl-D-glucosamine, which is ß-(1–4)-N-acetyl-2-amino-2deoxy-D-glucose (Fig. 1a). It may be also regarded as a derivative of cellulose, in which, the C-2 hydroxyl group is substituted by an acetyl amino group. Chitin occurs in three polymorphic forms, the most common α-chitin and also β- and γ-chitins, which differ in their arrangement of the molecular chains. Chitin structure has an extensive intermolecular hydrogen bonding associated with exclusion of water, leading to its stability. Chitin is a very light, white or yellowish, powdery/flaky product. It is insoluble in water as well as almost all common organic solvents and acidic and basic aqueous solutions. Chitin swells in cold alkali when some deacetylation takes place. Chain lengths of chitin and degree of acetylation differ according to sources and recovery conditions. Chemical derivatives of chitin include carboxymethyl chitin, hydroxyethyl chitin, ethyl chitin, chitin sulfate, glycol chitin, and glucosylated chitin, which have varied chemical and functional properties [142].

Chitin upon deacetylation yields chitosan, which is a collective name representing chitin deacetylated to various degrees. Generally chitosan is produced by deacetylation of chitin using 30–60% (w/v) sodium or potassium hydroxide at 80–140 °C followed by drying to get chitosan flakes. Chitosan with a variable degree of deacetylation ranging from 60 to 80% was recovered at 17 ± 4 mg g^{-1} head discard (wet weight) of white shrimp (*L. vannamei*) [144]. Chitosan has also been extracted by various enzymes such as lysozyme, neutral protease, and chitin deacetylase. The average molecular weight of chitosan obtained using the enzymatic method is 268 kDa [145]. Higher extraction temperatures enhance deacetylation and also result in fragmentation of the chitosan. Chitosan polymers are thought to contain more than 5000 repeating acetylglucosamine and glucosamine units. The molecular weights of chitosan range from 300 to over 1000 kDa and its degree of deacetylation from 30% to 95%. Both molecular weight and degree of deacetylation have influence on the functionality of chitosan. A minimum deacetylation of 70% is required for chitosan to be acceptable for various purposes.

The structure of chitosan is β-(1–4)-linked-D-glucosamine, i.e., poly [β-(1–4)-linked-2-amino-2-deoxy-D-glucose] (Fig. 1b). Chitosan is a cationic polyelectrolyte white solid, insoluble in pure water, but unlike chitin, it is soluble in weakly acidic aqueous media. Chitosan derivatives in the form of acetate, ascorbate, lactate, and malate are water-soluble. Chitosan is soluble in aqueous acid and crystallizable from aqueous alkaline solution. The value of pKa for the positively charged ammonium group of chitosan is about 6.2. When the pH is raised to about 6.5, chitosan precipitates in a gel form. The net cationic charge as well as presence of multiple reactive functional groups in the molecule makes chitosan a valuable compound for practical uses.

Chitosan can be easily converted to various derivatives for diverse applications, as shown in Fig. 2. These derivatives, depending on their chemical nature, have interesting characteristics such as biocompatibility, biodegradability to harmless products, nontoxicity, physiological inertness, and antimicrobial and gel-forming properties, among others. Chitosan derivatives, depending on their chemical nature, can have bioactivities such as antioxidant, anti-inflammatory, anti-allergic, antitumor, anti-obesity, antidiabetic, anticoagulant, antiviral, immunomodulatory, cardioprotective, and others. These properties make chitosan and its derivatives valuable compounds in several fields including food, nutrition, medicine, agriculture, biotechnology, and material science [146–150]. Due to its cationic nature, chitosan binds dietary fat in the stomach, leading to decreased intestinal absorption of fat and cholesterol, thereby reducing low-density lipoproteins in the blood and liver. For dietary purpose, chitosan needs to be introduced through food and should be soluble at acidic pH. Chitosan can be a food preservative due to its antimicrobial activity against a wide variety of pathogenic and spoilage-causing microorganisms, including fungi and gram-positive and gram-negative bacteria. Chitosan films can be used as a packaging material for the preservation of foods [145]. Potential medicinal applications of chitosan and its derivatives include drug delivery, hemodialysis membranes, artificial skin, hemostatic agents, hemoperfusion columns, wound healing and blood

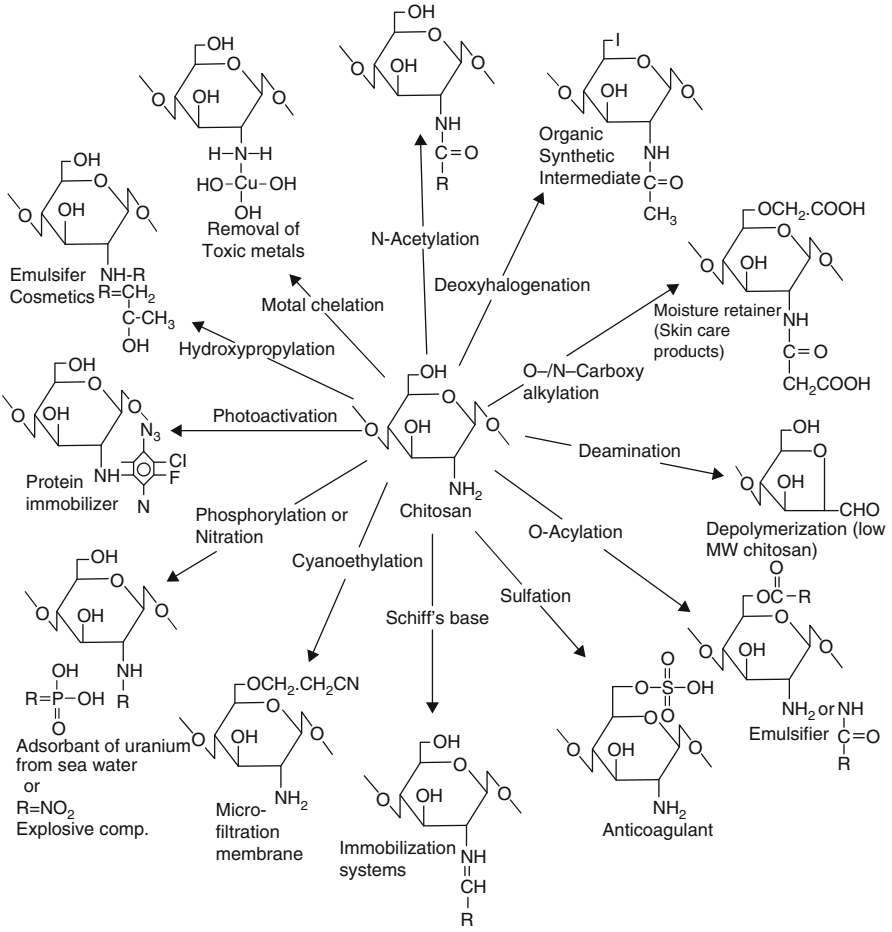


Fig. 2 Various chemical derivatives of chitosan and their uses (Source: Ref. 142 with permission from Taylor & Francis)

coagulation, and also gene therapy. The property of chitosan to form gel at slightly acid pH provides antacid and anti-ulcer activities [4, 140].

Chitosan and also other polysaccharides such as hyaluronic acid among others have received attention in the fields of regenerative medicine and tissue engineering, as ideal materials for artificial extracellular matrices [151]. Various physical forms of chitosan include nanoparticles, nanofibrils, microspheres, composite gels, fibers, films, and bandages. Chitosan-carrageenan composite nanoparticles have shown promise as carriers of therapeutic macromolecules. Nanocomposite films of chitosan and other marine hydrocolloids such as agar, alginate, and carrageenan can be a good carrier for nutraceuticals including omega-3 PUFA in the future [4]. Chitosan-based hydrogels and wound healing bandages have found commercial acceptance. Chitosan-based bone graft substitutes are biocompatible and biodegradable, with

structural similarity to the bone, having excellent mechanical strength. Derivatives of chitin and chitosan have applications as cosmetic ingredients. These include O-carboxymethyl chitin and O-hydroxypropyl chitin, carboxymethyl chitosan, chitosan acetate, adipate, glycolate, hydrolyzed chitosan, pyrrolidone carboxylic acid (PCA), hydroxyl methyl, hydroxyl propyl, formate, ascorbate, and salicylate of chitosan, among others [4, 103, 152]. Methods of manufacture of chitosan derivatives and biomedical applications have been recently discussed [153]. A number of these products are presently commercially available [91, 151, 153–155]. Salient biomedical applications of chitosan are pointed out in Table 5.

The biodegradation of chitosan by chitinases leads to formation of nontoxic oligosaccharides of variable length [149, 156]. While these oligosaccharides are not digested by gastrointestinal enzymes, they modify the viscosity and freezing point of foods, affect emulsification and gel formation, possess bacteriostatic properties, and act as humectants. Chitosan oligosaccharides are used as antioxidant, antitumor agent, and antimicrobial agents. They stimulate growth of lactobacilli and other probiotic organisms. Low molecular weight chitosans (25–50 kDa) suppress *H. pylori* and also have been recognized to protect normal cells from apoptosis [156].

4.3.2 Glucosamine

Glucosamine is the end product of hydrolysis of chitosan. It is an amino sugar that is naturally produced in humans and a key building block in the synthesis of glycosaminoglycans. Glucosamine is a recognized nutraceutical for joint pain relief. It promotes synthesis of cartilage proteoglycans and synovial production of hyaluronic

Table 5 Salient applications of chitosan in biomedical applications

Applications	Salient property
Wound healing, burn therapy	Forms tough, water-absorbent, biocompatible films that promote tissue growth
Hemodialysis membranes	Chitosan-cellulose blended membranes have improved dialysis properties in artificial kidney due to improved permeability
Drug delivery	Inexpensive carrier encapsulating nutraceuticals and drugs. It also helps transdermal delivery of drugs
Removal of toxins	Chitosan-encapsulated activated charcoal has potential to remove toxins
Hemoperfusion	Chitosan and its oligomers can satisfy the requirements of specificity and blood compatibility
Artificial scaffolds	Chitosan along with chondroitin sulfate supports proteoglycan production in cartilages
Anticholesterol drug	Reduces lipid absorption and hence reduces cholesterol
Dental bioadhesive biodegradable sutures	Collagen-chitosan membrane has potential for treatment of periodontal defects in dentistry
Composite films and nanoparticles with other polysaccharides	Various processes in tissue engineering for drug delivery, skin recovery, scaffolds, and others

Adapted from various references

acid (HA), which has anti-inflammatory and analgesic properties. Glucosamine along with chondroitin sulfate (see Sect. 4.3.3) is a highly effective treatment for arthritis and osteoporosis [148, 149].

4.3.3 Glycosaminoglycans

Glycosaminoglycans (GAGs) are heteropolysaccharides defined by a repeating disaccharide unit without branched chains, in which one of the two monosaccharides is an amino sugar (N-acetylgalactosamine or N-acetylglucosamine) and the other one is a uronic acid. GAGs are present on all animal cell surfaces and in the extracellular matrix where they exist as proteoglycans by covalently linking to proteins (such as growth factors, enzymes, and cytokines). Based on the disaccharide composition, linkage type, and presence of sulfate groups, GAGs may be chondroitin sulfate (CS), hyaluronic acid (HA), dermatan sulfate (DS), heparin, or keratan sulfate (KS). GAGs are significantly present in the cartilage, which serves as a cushion between bones and joints. The cushion structures are formed by a matrix of collagen and elastin associated with proteoglycans consisting of CS, KS, DS, and heparan sulfate. Hyaluronic acid (HA) is the only non-sulfated GAGs, which is not covalently bound to the protein in any tissue, although specific HA-protein interaction is shown. CS and HA are the most commercially valued GAGs because of their physiological functions and high activity. CS plays an important role in the elasticity and function of the articular cartilage and is mainly attached covalently to core proteins in the form of proteoglycans. CS is composed of an alternating sequence of sulfated and/or unsulfated D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine (GalNAc) residues linked through alternating β (1–3) and β (1–4) glycosidic bonds and sulfated in different carbon positions, as shown in Fig. 3. Some GlcA residues are epimerized into iduronic acid, the resulting disaccharide being referred as dermatan sulfate. The classification and type of CS are dependent on sulfate group placing, namely, sulfate at carbon 4 (CS-A), at carbon 6 (CS-C, more common), both at carbon 4 and 6 (CS-E), at carbon positions 6 of GalNAc and 2 of GlcA (CS-D), and at carbon 4 of GalNAc/carbon 2 of GlcA (CS-B). Sulfation in different positions confers specific biological activities to chondroitin [157, 158].

CS from terrestrial and marine sources contains diverse chain lengths and sulfation. Examples are shark, CS-D; dogfish, CS-A and CS-D; squid and salmon, CS-E and CS-E; and ray, CS-A and CS-C [157]. The endoskeletons of shark are composed of cartilage, which contains up to 29% CS. Processes for isolation of CS from shark cartilage and also from the cartilages of ray, swordfish, dogfish, fins of shark and skate, and salmon nasal cartilage have been reported [158–160]. CS was extracted from fish head cartilage employing hydrolysis by alcalase (55.7 °C, pH 8.2) and chemical treatment of the hydrolyzates by alkaline-alcoholic saline solutions (0. 54 M NaOH, 1.17 V EtOH, and 2.5% NaCl) for selective dissolution followed by ultrafiltration [158, 159]. Optimization of skate (*Raja avirostris*) cartilage hydrolysis for the preparation of chondroitin sulfate has been reported [159]. A low-cost two-step process recovery of CS in non-denaturing conditions was reported. It consists of an enzymatic extraction followed by tangential membrane filtration [160]. Figure 4 provides an overview of chondroitin sulfate recovery and

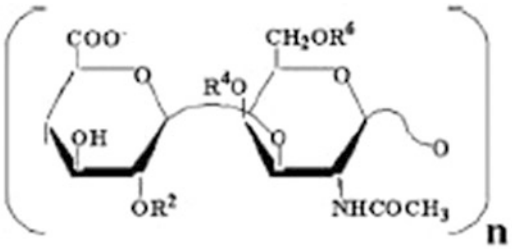


Fig. 3 Chondroitin sulfate derivatives

	R^2	R^4	R^6
i-0S	H	H	H
i-6S	H	H	SO_3^-
i-4S	H	SO_3^-	H
i-2,6 di S	SO_3^-	H	SO_3^-
i-4,6 di S	H	SO_3^-	SO_3^-
i-2,4 di S	SO_3^-	SO_3^-	H
i-2,4,6 tri S	SO_3^-	SO_3^-	SO_3^-

purification processes from marine cartilage by-products. CS is quite stable to heating at temperatures as high as 121 °C for several min. Shark cartilage powder is traditionally used for wound healing and as anti-angiogenic, antiarthritic, and antitumor agent. CS is considered a nutritional supplement that provides support for strong, healthy cartilage and joints [161].

Hyaluronic acid (HA) is a linear, high molecular weight unbranched and non-sulfated GAG made by alternating disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic linked by β-(1 → 3) and β-(1 → 4) glycosidic bonds. It is used in ophthalmological surgeries and cosmetic regeneration of soft tissues. Marine discards have been also explored for HA [158].

The presence of heparin has been reported from different shrimp species. Sulfated glycosaminoglycans that exhibited electrophoretic migration pattern similar to mammalian heparin were recovered (79 ± 2 μg g^{-1} wet processing waste) from Pacific white shrimp (*L. vannamei*) shell discards. Their degradation products suggested the presence of C6-sulfated heparin sulfate [144].

Sea cucumbers are found in shallow water areas of the sea to deep ocean floors, where they live on decaying organic matter. The body wall of the sea cucumber contains high amounts of sulfated glycans, which differ in structure from glycosaminoglycans of animal tissues. The cucumber cell wall polysaccharide was comparable in backbone structure with the mammalian chondroitin sulfate, but some of the glucuronic acid residues displayed sulfated fucose branches. These sulfated glycans exhibit a wide range of biological activities, which include anticoagulant activity, venous antithrombic activity, and recombinant HIV reverse transcriptase activity [4].

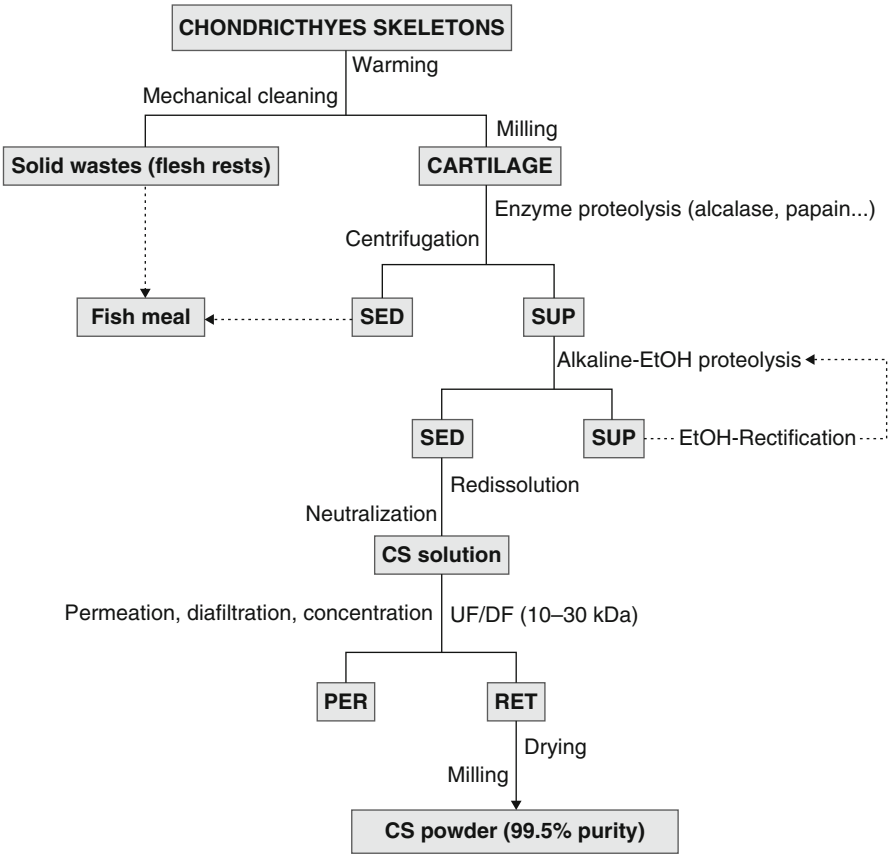


Fig. 4 Overview of chondroitin sulfate recovery and purification processes from marine cartilage by-products. SED, Sediment; SUP, supernatant; PER, permeate; and RET, retentate (Courtesy: Vázquez JA, Ref. 158)

4.3.4 Other Seafood Polysaccharides

Bioactivities and nutraceutical potentials of other polysaccharides from seafood have also been reported in recent times. Mollusks have a wide range of uses in pharmacology; several compounds from mollusks having pharmacological value have been described in the literature [4]. Mytilan, a mussel polysaccharide, possesses antibacterial, antioxidant, and immune-modulating activities. Another mussel polysaccharide, a (1–4)-D-glucan, is known to exhibit antioxidant activity and a protective effect on acute liver injury in mice [137]. The high radical scavenging capacity and total phenolics suggest the nutraceutical potential of the mussel *P. viridis* [83]. Abalone is a source of bioactive compounds having antithrombotic, anticoagulant, anti-inflammatory, antioxidant, and anticancer activities. Polysaccharides and also proteins and fatty acids of abalone provide health benefits beyond basic nutrition [162]. The glucan extract from China white jade snail

(*Achatina fulica*) has significant antioxidant activities, suggesting its potentials as dietary antioxidant [163].

4.4 Mineral-Based Compounds

4.4.1 Bone Powders

Fish bone is a potential source of calcium for use as a fortificant of the mineral. To prepare the product, fish is treated with hot water followed by hot aqueous acetic acid, which softens the bone and converts into an edible form. Superheated steam is preferred to reduce the loss of soluble components from fish tissue, which enables better recovery of the bone within a shorter period. The treated bones are subjected to saponification, degreasing, and degumming. The bone preparation is a good source of bioavailable calcium [164]. Tuna powder is a value-added product that contains a proper balance of calcium and phosphorus; it can be used as a food supplement and for treating osteoporosis [165]. The powder can also combat calcium deficiency in children [166]. The bone preparations of channel catfish have antibacterial activities, with pepsin hydrolyzate of the bone showing the greatest antibacterial activity [167]. A fish bone phosphopeptide (FBP) containing up to 24% of phosphorus has a molecular weight of 3.5 kDa and has high calcium-binding activity, which could be utilized as a nutraceutical [168].

4.5 Marine Biotechnology for the Isolation of Nutraceuticals

Marine biotechnology explores aquatic organisms for the recovery of nutraceuticals, pharmaceuticals, cosmeceuticals, and other products through a multitude of processes. Whereas the conventional processes include liquid–liquid or solid–liquid extraction and enzyme-assisted extraction, advanced biotechnological methods are pressurized liquid extraction, subcritical and supercritical extractions, and microwave- and ultrasound-assisted extractions, among others [93, 169–174]. Membrane separation is beneficial to extract, concentrate, separate, or fractionate bioactive compounds. Membrane bioreactors integrate reaction vessels with membrane separation units for producing materials such as peptides, chito-oligosaccharides, and PUFA from seafood discards [175]. Supercritical carbon dioxide (SC-CO_2) extraction at optimal conditions of 40 MPa and 65 °C was employed to concentrate PUFA from fish oil [176]. Vázquez et al. [158] suggested environment-friendly and sustainable processes combining various microbial, chemical, enzymatic, and also membrane technologies for the recovery of chitin/chitosan, chondroitin sulfate, and hyaluronic acid from marine discards. In this, the conventional process for chitin/chitosan was supplemented with protease treatment and/or lactic acid fermentation for protein removal and also use of fungal deacetylase for conversion of chitin to chitosan. The process appears to have scope for large-scale processing of seafood discards in eco-friendly conditions. The process is depicted in Fig. 5. The authors also reported a pilot plant scale processing of *Penaeus vannamei* by-products using a

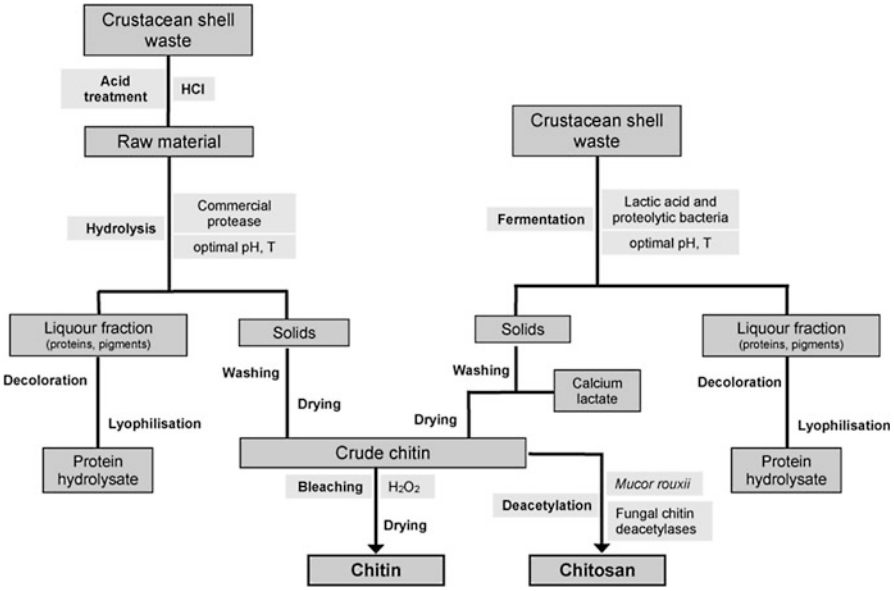


Fig. 5 Eco-friendly process for the preparations of chitin and chitosan from crustacean shell discard (Courtesy: Vázquez JA, Ref. 177)

combination of enzymatic and chemical technologies, which gave chitin with 30% yield. Deacetylation under optimal conditions yielded chitosan having a molecular weight of 82 kDa and 92% deacetylation [177].

4.6 Commercial Status

Interests in marine bioactive products are shown by the fact that in the past 8 years, annually, more than 1000 new compounds from marine organisms have been described [178]. A number of by-products from seafood have already entered commercial markets. Fish trypsin, chymotrypsin, and cold-active chlamysin (lysozyme) are available commercially. Some of the other commercial products include Neptune Aquatein, krill extract (http://ingredientsnetwork.com/neptune-technologies-bioressources-comp249137.html), heat-labile shrimp alkaline phosphatase, uracil-DNA glycosylase from Atlantic cod (http://arcticzymes.com), seafood protein extracts (http://novozymes.com), and protein blends from CIFT, Kochi, India (www.cift.res.in). Animal proteins can be used as a source of bioactive hydrolyzates and peptides with potential for use as functional food ingredients industry [179]. Fish gelatin is available commercially. Collagen or gelatin peptides with a molecular weight around 1–5 kDa containing tripeptides, dipeptides, and also free amino acids are marketed as nutraceuticals for the maintenance of normal bone and tendon integrity, improving joint health. Calcitonin is a hormone (32-amino acid peptide

containing a single disulfide bond), known to participate in calcium and phosphorus metabolism in mammals. The major source of calcitonin is from the parafollicular or C cells in the thyroid gland. Calcitonin produced from salmon is currently commercially available. Ziconotide is a 25-amino acid peptide derived from the ω-conotoxin of cone snail (*Conus magus)* found in tropical waters. Ziconotide has been approved by the US FDA for analgesic use. Other products from fish which are under clinical trials include fish gelatin and Gabolysat and Seacure® (a hydrolyzate of fish proteins) [103, 114].

Annually around 1 million tons of fish oil is produced, with a current global market valued at US$3.9 billion [180]. At present the main use of fish oil is for aquaculture. Current consumption level of fish oil for human nutrition is not adequate [181]. In order to increase the consumption, foods fortified with omega-3 are made available in the markets all over the world. Developments in fortification technology have encouraged more than 200 omega-3 PUFA-fortified foods, such as bread, dairy products, eggs, pasta, biscuits, margarines, and other spreads available commercially in Japan and other countries [182, 183]. Capsules such as Lovaza® which contains ethyl esters of EPA and DHA are available commercially (https://www.drugs.com/pro/lovaza.html). There is, however, concern that because of seafood production reaching a plateau in recent years, the commodity may not be able to fully satisfy future needs. Therefore, other sources such as algae may be required to produce EPA and DHA to meet the increasing demand [181].

Chitosan and its derivatives are finding several end users which, apart from healthcare, medical, food, and beverages, include water treatment, agriculture, biotechnology, and others, encompassing about 30 companies (www.strategyr.com/Chitin_and_Chitosan_Market_Report.asp) with a projection of a market worth of US $4.2 billion by 2020. Anti-inflammatory and antiarthritic dietary supplements such as “Seatone” and “Lyprinol” from mussel are commercially available [137]. Tetrodotoxin (TTX) and saxitoxin are neurotoxins found in many marine organisms including mussels and fish. TTX is currently under clinical trials due to its specific and reversible activity on voltage-dependent sodium channels [181]. CS, hyaluronic acid, and also chitosan have attracted increasing commercial attention in the formulation of cosmeceuticals, nutraceuticals, and food ingredients [103, 158]. Some fish-based cosmeceutical products include Penzim, a natural skin care product from Iceland, derived from natural marine enzymes from the Arctic Ocean from North Atlantic cod (http://www.andra.is/), extracts from male sturgeon gonads, zonase enzyme and gelatin from fish eggs for treatment of psoriasis and eczema, cod sperm as water binder in body lotions, shark cartilage containing chondroitin sulfate used in various creams, squalene from shark liver oil as moisturizer in body lotions, and guanine from fish such as herring and ribbon fish, used in nail polish [103]. A product depicted as “skin food from the sea” contains blend of marine-derived nutrients and is suggested for antiaging (www.nutreeplus.com/antiaging.html). A number of companies like Aquapreneur (www.aquapreneur.com), Sederma (http://www.sederma.fr), NutraIngredients (https://www.nutraingredients.com), SpecialChem (http://www.specialchem4cosmetics.com), Fortitech (fortitech-premixes.com), Copalis (http://www.copalis.fr/), and others have been active in

developing marine ingredients such as collagen, elastin, various peptides, GAGs, alkyl glycerols, fish oil, hydrolyzed proteins, calcium supplements, glucosamine, hyaluronic acid, and chondroitin sulfate for food, pharmaceutical, and other purposes.

5 Conclusions

Seafood items are rich in nutrients that can have positive influence on human health. Nutrient contents depend on the species, and thus consumption of individual species significantly determines the nutritional benefits derived by individual consumers. The health benefits derived from seafood items also depend on the frequencies of their consumption, as well as quantities consumed. Seafood items and also their processing discards are good sources of many nutraceuticals. In recent times, marine biotechnology has made rapid advancements, which could be used to recover nutraceuticals and other bioactive compounds from seafood discards. There is robust evidence that fish oil and its component fatty acids, especially EPA plus DHA, are beneficial in maintaining cardiovascular health in normal adults. Fish protein hydrolyzates contain peptides with significant antihypertensive and other bioactivities. Fish cartilage products such as shark cartilage and chondroitin sulfate, glucosamine, and other glycosaminoglycans are able to alleviate rheumatoid arthritis. Fish skin collagen and gelatin have a potential to replace bovine collagen and gelatin in food, pharmaceuticals, and cosmetics. Chitosan and its derivative offer immense applications in human health and cosmeceutical fields. There is large potential for development of functional foods fortified with nutraceuticals such as fish oils, bioactive peptides, and others. Gelatin, chitosan, and its derivatives can be carriers of nutraceuticals and drugs and food packaging materials. Uses of marine nutraceuticals are increasing in countries such as Japan and others. As Borresen [184] pointed out, there is a need for nutritionists and other scientists to evaluate the nutritional value of fishery items that can lead to total utilization of the commodity for human welfare.

References

1. FAO (2016) The state of world fisheries aquaculture: contributing to food security and nutrition for all. Food Agriculture Organization, Rome, 200 p. Available from: http://www.fao.org/3/a-i5555e.pdf. Accessed 1 Oct 2016
2. Sachindra MM, Mahendrakar MS (2015) Fish processing by-products: quality assessment and applications. Studium Press, Houston, p 413
3. Kelleher K (2005) Discards in the world's marine fisheries: an update. Food and Agriculture Organization, Rome, fisheries technical paper no. 470, p. 131
4. Venugopal V (2009) Marine products for healthcare: functional bioactive nutraceuticals from the ocean. CRC Press, Boca Raton, p 527
5. Kim S-K (ed) (2017) Marine nutraceuticals: prospects and perspectives. CRC Press, Boca Raton, p 464

6. Venugopal VM, Lele S (2015) Nutraceuticals and bioactive compounds from seafood processing discard. In: Kim SK (ed) Springer handbook of marine biotechnology. Springer, Berlin, pp 1405–1425
7. Ohr LM (2007) Nutraceuticals: health foods at forefront. Food Technol 61(6):55–57
8. Olsen RL, Toppe J, Karunasagar I (2014) Challenges and realistic opportunities in the use of by-products from processing of fish and shellfish. Trends Food Sci Technol 36:144–151
9. Gul K, Singh AK, Jabeen R (2014) Nutraceuticals and functional foods: the foods for the future world. Crit Rev Food Sci Nutr 56:2617–2627
10. Eskin NAM, Tamir S (2006) Dictionary of nutraceuticals and functional foods. CRC Press, Boca Raton, p 520
11. Arai S (1996) Studies on functional foods in Japan – state of the art. Biosci Bitech Biochem 60:9–14
12. Hasler CM (1998) Functional foods: their role in disease prevention health promotion. J Food Technol 52:63–68
13. Plaza M, Cifuentes A, Ibáñez E (2008) In the search of new functional food ingredients from algae. Trends Food Sci Technol 19:31–39
14. FAO/INFOODS (2016) Global food composition database for fish and shellfish. Version 10- uFiSh10. Food Agriculture Organization, Rome. Available from: http://www.fao.org/3/a-i6655e.pdf. Accessed 6 Jan 2017
15. USDA (2012) United States Department of Agriculture. 2012. Food composition databases. USDA national nutrient database for stard reference. Available from: https://ndb.nal.usda.gov/ndb/search/list. Accessed 2016 October 4.
16. NMFS (1987) National Marine Fisheries Service. Proximate composition, energy, fatty acids, sodium, cholesterol contents of finfish, shellfish their products. In: Krzynowek J, Murphy J (eds) Technical report no.74. National Oceanic Atmospheric Administration, Washington, DC
17. Department of Health UK (2013) Nutrient analysis of fish and fish products. Available from: www.dh.gov.uk/publications. Accessed 4 Feb 2017
18. FSANZ (2011) Food nutrient database. Available from: http://www.foodstandards.gov.au/science/monitoringnutrients/ausnut/foodnutrient/Pages/default.aspx. Accessed 6 Aug 2017
19. Dong FM (2001) The nutritional value of shellfish. Washington Sea Grant: WSG-MR 09-03. US National Oceanic Atmospheric Administration, Seattle, pp 4–8
20. Nettleton JA, Exler J (1992) Nutrients in wild and farmed fish shellfish. J Food Sci 57:257–260
21. Venugopal V, Shahidi F (1996) Structure and composition of fish muscle. Food Rev Int 12:175–197
22. Venugopal V (2006) Seafood processing: adding value through quick freezing, retortable packaging and cook-chilling. CRC Press, Boca Raton, p 504
23. Venugopal V, Gopakumar K (2017) Shellfish: nutritive value, health benefits and consumer safety. Comp Rev Food Sci Food Safety 16:1219
24. Coppes Petricorena Z (2015) Chemical composition of fish and fishery products. In: Cheung PCK, Mehta BM (eds) Handbook of food chemistry. Springer, Berlin/Heidelberg, pp 403–435
25. SELFNutritionData. Available from: http://nutritiondata.self.com/facts/finfish-and-shellfish-products/4174/2. Accessed 15 Oct 2016
26. Karnjanapratum S, Benjakul S, Kishimura H, Tsar V-H (2013) Chemical composition nutritional value of Asian hard clam *Meretrix lusoria* from the coast of Aman Sea. Food Chem 141:4138–4145
27. Cherif S, Frikha F, Gargouri Y, Miled N (2008) Fatty acid composition of green crab (*Carcinus mediterraneus*) from the Tunisian Mediterranean coasts. Food Chem 111:930–933
28. Turan T, KayaY EME (2011) Proximate composition, cholesterol and fatty acid content of brown shrimp (*Crangon crangon* L.1758) from Sinop Region, Black Sea. J Aquatic Food Prod Technol 20:100–107
29. Sriket P, Benjakul S, Visessanguan W, Kijroongrojan K (2007) Comparative studies on chemical composition: thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) meats. Food Chem 103:1199–1207

30. Zarai Z, Frikha F, Balti R, Miled N, Gargouri N, Mejdoub N (2011) Nutrient composition of the marine snail *Hexaplex trunculus* from the Tunisian Mediterranean coasts. J Sci Food Agri 91:1265–1270
31. Vaz-Pires P, Seixas P, Mota M et al (2004) Sensory, microbiological, physical and nutritional properties of iced whole common octopus, *Octopus vulgaris*. LWT Food Sci Technol 37:105–114
32. Gopakumar K (1997) Biochemical composition of Indian food fishes. Central Institute of Fisheries Technology, Cochin. http://www.cift.res.in. Accessed 3 May 2016
33. Vijaykrishnaraj M, Prabhasankar P (2015) Marine protein hydrolyzates: their present future perspectives in food chemistry – a review. RSC Adv 5:34864–34867
34. Shiau SY (1994) Seafood protein in human and animal nutrition. In: Sikorski ZE, Pan BS, Shahidi F (eds) Seafood proteins. Springer, Boston
35. Weichselbaum E, Coe S, Buttriss J, Stanner S (2013) Fish in the diet: a review. Nutr Bull 38:128–177
36. Hamed I, Ozogul F, Ozogul Y, Regenstein JM (2015) Marine bioactive compounds and their health benefits: a review. Comp Rev Food Sci Food Safety 14:446–465
37. Friedman K (1996) Nutritive value of proteins from different food sources a review. J Agri Food Chem 44:6–29
38. Dayal JS, Ponniah AG, Khan HI, Madhu Babu EP, Ambasankar K, Vasagham KPK (2013) Shrimps – a nutritional perspective. Current Sci (India) 104:1487–1491
39. Dort J, Sirois A, Leblanc N, Coˆte´ CH, Jacques H (2012) Beneficial effects of cod protein on skeletal muscle repair following injury. Appl Physiol Nutr Metab 37:489–498
40. Gigliotti J, Jaczynski J, Tou JC (2008) Determination of the nutritional value, protein quality and safety of krill protein concentrate isolated using an isoelectric solubilization/precipitation technique. Food Chem 111:209–214
41. Venugopal V, Chawla SP, Nair PM (1996) Spray dried protein powder from threadfin bream: preparation, properties and comparison with FPC type-B. J Muscle Foods 7:55–71
42. Kim S-K, Venkatesan J (2015) Introduction to seafood science. In: Kim SK (ed) Seafood science: advances in chemistry, technology and applications. CRC Press, Boca Raton, pp 1–13
43. Elmadfa I, Meyer AL (2017) Animal proteins as important contributors to a healthy human diet. Ann Rev Animal Biosci 5:111–131
44. Phillips SM, Fulgoni III, VL, Heaney RP, Nicklas TA, et al (2015) Commonly consumed protein foods contribute to nutrient intake, diet quality, and nutrient adequacy. Am J Clin Nutr 1S–7S. https://doi.org/10.3945/ajcn.114.084079
45. Konasu S, Yamaguchi K (1982) In: Martin RE, Flick GJ Jr, Hebard CE, Ward DR (eds) Chemistry and biochemistry of marine food products. AVI Publishing, Westport
46. Kim J, Lall S (2000) Amino acid composition of whole body tissue of Atlantic halibut (*Hippoglossus hippoglossus*), yellowtail flounder (*Pleuronectes ferruginea*) Japanese flounder (*Paralichthys olivaceus*). Aquaculture 187:367–373
47. Huss HH, Ababouch L, Gram L (2003) Assessment management of seafood safety and quality. Food and Agriculture Organization, Rome, Technical paper no. 444
48. Rosa R, Nunes ML (2004) Nutritional quality of red shrimp *Aristeus antennatus* Risso, pink shrimp *Parapenaeus longirostris* Lucas, and Norway lobster *Nephrops norvegicus Linnaeus*. J Sci Food Agric 8:89–94
49. Ruiz-Capillas C, Moral A (2004) Free amino acids in muscle of Norway lobster *Nephrops norvegicus* L. in controlled and modified atmospheres during chilled storage. Food Chem 86:85–91
50. Naczk M, Williams J, Brennan K, Chrika L, Shahidi F (2004) Compositional characteristics of green crab *Carcinus maenas*. Food Chem 88:429–434
51. Erkan N, Selcuk A, Ozden O (2010) Amino acid and vitamin composition of raw and cooked horse mackerel. Food Anal Methods 3:269–275
52. Gildberg A (2004) Enzymes and bioactive peptides from fish discard related to fish silage, fish feed and fish sauce production. J Aquatic Food Prod Technol 13:3–11

53. Militante JD, Lombardin JB (2004) Dietary taurine supplementation: hypolipidemic and antiatherogenic effects. Nutr Res 24:787–801
54. Sun Q, Wang B, Li Y, Sun F, Li P et al (2016) Taurine supplementation lowers blood pressure improves vascular function in prehypertension: romized, double-blind, placebo-controlled study. Hypertension 67:541–549
55. Gormley TR, Neumann T, Fagan JD, Brunton NP (2007) Taurine content of raw and processed fish fillets/portions. Eur Food Res Technol 225:837–842
56. Passi S, Cataudella S, Ferrante I, De Simone F, Rastrelli L (2002) Fatty acid composition and antioxidant levels in muscle tissue of different Mediterranean marine species of fish and shellfish. J Agric Food Chem 50:7314–7322
57. Bono G, Gai F, Peiretti PG, Badalucco C, Palmegiano GB (2012) Chemical and nutritional characterization of the Central Mediterranean giant red shrimp *Aristaeomorpha foliacea*: influence of trophic and geographical factors on flesh quality. Food Chem 130:104–110
58. Ozogul O, Duysak O, Ozogul F, Ozkutuk AS, Tureli C (2008) Seasonal effects in the nutritional quality of the body structural tissue of cephalopods. Food Chem 108:847–852
59. Li G, Li J, Li D (2010) Seasonal variation in nutrient composition of *Mytilus coruscus* from China. J Agri Food Chem 58:7831–7837
60. Kris-Etherton PM, Harris WS, Appel LJ (2002) Fish consumption fish oil omega-3 fatty acids and cardiovascular disease. Circulation 106:2747–2757
61. Calder PC (2014) Very long chain omega-3 (n-3) fatty acids and human health. Eur J Lipid Sci Technol 116:1280–1300
62. Wall R, Ross RP, Fitzgerald GF, Stanton C (2010) Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. Nutr Rev 68:280–289
63. de Carvalho CCR, Caramujo MJ (2017) Carotenoids in aquatic ecosystems aquaculture: a colorful business with implications for human health. Front. Mar Sci 4:1–13
64. Soumya R, Sachindra NM (2015) Carotenoids from fishery resources. In: Sachindra NM, Mahendrakar NS (eds) Fish processing byproducts: quality assessment and applications. Studium Press, Houston, pp 273–298
65. Zheng H, Liu H, Zhang T, Wang S, Sun Z, Liu W, Li Y (2010) Total carotenoid differences in scallop tissues of *Chlamys nobilis* (Bivalve: Pectinidae) with regard to gender shell colour. Food Chem 122:1164–1177
66. Choe E, Min DB (2006) Chemistry and reactions of reactive oxygen species in foods. Crit Rev Food Sci Nutr 46:1–35
67. Chuyen VH, Eun J-B (2017) Marine carotenoids: bioactivities and potential benefits to human health. Crit Rev Food Sc Nutr 57:2600–2610
68. Kaulman A, Bohn T (2014) Carotenoids inflammation and oxidative stress: implications of cellular signaling pathways relation to chronic disease prevention. Nutr Res 34:907–929
69. Higuera-Ciapara I, Felix-Valenzuela L, Goycoolea FM (2006) Astaxanthin: a review of its chemistry and applications. Crit Rev Food Sci Nutr 46:185–196
70. Abeynayake R, Mendis E (2014) Anti-aging and immuno-enhancing properties of marine bioactive compounds. In: Kim SK (ed) Seafood science: advances in chemistry technology and applications. CRC Press, Boca Raton, pp 262–275
71. Bogard JR, Thilsted SH, Marks GC, Wahab MA, Hossain MAR, Jakobsen J, Stangoulis J (2015) Nutrient composition of important fish species in Bangladesh potential contribution to recommended nutrient intakes. J Food Comp Anal 42:120–133
72. Sunil Kumar BV, Singh S, Verma B (2017) Anticancer potential of dietary vitamin D ascorbic acid: a review. Crit Rev Food Sci Nutr 57:2623–2635
73. Afonso C, Barra NM, Nunes L, Cardoso C (2014) Tocopherols in seafood and aquaculture products. Crit Rev Food Sci Nutr 56:128–140
74. Gotoh N, Mashimo D, Oka T, Sekiguchi K, Tange M, Watanabe H, Noguchi N, Wada S (2011) Analyses of marine-derived tocopherol in processed foods containing fish. Food Chem 129:279–283

75. Watanabe F, Katsura H, Takenaka S, Enomoto T, Miyamoto E et al (2001) Characterization of vitamin B12 compounds from edible shellfish, clam, oyster, and mussel. Int J Food Sci Nutr 52:263–268
76. Nunes ML, Barra NM, Batista I (2011) Health benefits associated with seafood consumption. In: Alasalvar C, Shahidi F, Miyashita K, Wanasundara U (eds) Handbook of seafood quality, safety and health. Wiley-Blackwell, Ames, Iowa, pp 369–379
77. Küçükgülmez A, Çelik M, Yanar Y, Ersoy B, Cikrikci M (2006) Proximate composition and mineral contents of the blue crab *Callinectes sapidus* breast meat, claw meat and hepatopancreas. Int J Food Sci Technol 41:1023–1026
78. Anonymous (2015) US Department of Health Human Services and the US Department of Agriculture 2015–2020 Dietary guidelines for Americans 8th ed. https://health.gov/dietaryguidelines/2015/guidelines/. Accessed 3 July 2016
79. Wu Y-X, Li W, Jin T (2015) Preparation characterization of protein hydrolyzates from little loligo squid *Uroteuthis chinensis*. J Aquatic Food Prod Technol 24:42–51
80. Barrento S, Marques A, Teixeira B, Anacleto P et al (2009) Effect of season on the chemical composition nutritional quality of the edible crab *Cancer pagurus*. J Agri Food Chem 57:10814–10824
81. Maulvault AJ, Anacleto P, Lourenço HM, Carvallo ML, Nunes ML, Marques A (2012) Nutritional quality and safety of cooked edible crab Cancer Pagurus. Food Chem 133:277–283
82. Barrento S, Marques A, Teixeira B, Vaz-Pirez P, Nunes ML (2009) Nutritional quality of the edible tissues of European lobster *Homarus gammarus* and American lobster *Homarus americanus*. J Agri Food Chem 57:3645–3652
83. Chakraborty K, Chakkalakal SJ, Joseph D, Asokan PK, Vijayan KK (2016) Nutritional and antioxidative attributes of green mussel, *Perna viridis* L from the southwestern coast of India. J Aquatic Food prod Technol 25:968–985
84. Storelli MM, Garofalo R, Giungato D, Giacominelli-Stuffler R (2010) Intake of essential non-essential elements from consumption of octopus cuttlefish squid. Food Addit Cont Part B 3:14–18
85. Chakraborty K, Chakkalakal SJ, Joseph D, Joy M (2016) Nutritional composition of edible oysters (*Crassostrea madrasensis* L.) from the southwest coast of India. J Aquatic Food Prod Technol 25:1172–1189
86. Venugopal V, Shahidi F (1995) Value added products from underutilized fish species. Crit Rev Food Sci Nutr 35:431–453
87. Anal AK (2017) Seafood by-products in applications of biomedicine and cosmeticuals. In: Food processing by-products and their utilization. Wiley Blackwell, Hoboken, p 592
88. Suleria HA, Masci P, Gobe G, Osborne S (2016) Current potential uses of bioactive molecules from marine processing discard. J Sci Food Agric 96:1064–1067
89. Ghaly AE, Ramakrishman VV, Brooks MS, Budge SN, Dave D (2013) Fish processing discards as a potential source of proteins, amino acids and oils: a critical review. J Microb Biochem Technol 5:107–129
90. Gencbay G, Turhan S (2016) Proximate composition and nutritional profile of the black sea anchovy (*Engraulis encrasicholus*) whole fish, fillets, and by-products. Aquatic Food Products Technol 25:864–874
91. Senevirathne M, Kim SK (2012) Utilization of seafood processing by-products: medicinal applications. Adv Food Nutr Res 65:495–512
92. Correia-da-Silva M, Sousa E, Pinto MMM, Kijjoa A (2017) Cancer preventive compounds from edible marine organisms. Semin Cancer Biol. https://doi.org/10.1016/j.semcancer.2017.03.011. pii: S1044-579X(17)30084-6
93. Nguyen TT, Barber AR, Corbin K, Zhang W (2017) Lobster processing by-products as valuable bioresource of marine functional ingredients, nutraceuticals, and pharmaceuticals. Bioresour Bioprocess 4:27. https://doi.org/10.1186/s40643-017-0157-5. Epub 2017 Jun 22
94. Hultin HO, Kristinsson HG, Lanier TC, Park JW (2005) Process for recovery of functional proteins by pH shifts. In: Park JW (ed) Surimi and surimi seafood. Taylor and Francis, Boca Raton, pp 107–139

95. Tahergorabi R, Beamer SK, Matak KE, Jaczynski J (2012) Isoelectric solubilization/precipitation as a means to recover protein isolate from striped bass (*Morone saxatilis*): its physicochemical properties in a nutraceutical seafood product. J Agric Food Chem 60:5979–5987
96. Kristinsson HG, Rasco B (2000) Fish protein hydrolyzates: production, biochemical, and functional properties. Crit Rev Food Sci Nutri 40:43–81
97. Herpandi NH, Rosma A, Wan Nadiah WA (2011) The tuna fishing industry: a new outlook on fish protein hydrolyzates. Comp Rev Food Sci Food Safety 10:195–207
98. Shaviklo AR (2015) Development of fish protein powder as an ingredient for food applications: a review. J Food Sci Technol 52:648–666
99. Sathivel S, Bechtel PJ (2006) Properties of soluble protein powders from Alaska pollock (*Theragra chalcogramma*). Int J Food Sci Technol 41:520–529
100. Rudkowska I, Marcotte B, Pilon G, Lavigne C, Marette A, Vohl MC (2010) Fish nutrients decrease expression levels of tumor necrosis factor-alpha in cultured human macrophages. Physiol Genomics 40:189–194
101. Wergedah H, Liaset B, Gudbrandsen OA et al (2004) Fish protein hydrolysate reduces plasma cholesterol, increases the proportion of HDL-cholesterol and lowers acyl-coA-cholesterol acyltransferase activity in liver of zucker rats. J Nutr 134:1320–1327
102. Mathew S, Ninan G, Hema GS, Shiny K, Lakshmanan PT (2015) Fish collagen and gelatin. In: Sachindra MM, Mahendrakar MS (eds) Fish processing by-products: quality assessment and applications. Studium Press, Houston, pp 173–236
103. Venugopal V (2012) Cosmeceucals from marine fish and shellfish. In: Kim SK (ed) Marine cosmeceuticals trends and prospects. CRC Press, Boca Raton, pp 211–232
104. Bello AE, Oesser S (2006) Collagen hydrolyzate for the treatment of osteo-arthritis and other joint disorders: a review of the literature. Curr Med Res Opin 22:2221–2232
105. Siddiqui KS, Cavicchioli R (2006) Cold-adapted enzymes. Annu Rev Biochem 75:403–412
106. Debashish G, Malay S, Barindra S, Joydeep M (2005) Marine enzymes. Adv Biochem Eng Biotechnol 96:189–218
107. Haard NF, Simpson BK (eds) (2006) Seafood enzymes: Utilization and influence on postharvest seafood quality. Marcel Dekker, New York, p 681
108. Bougatef A (2013) Trypsins from fish processing discard: characteristics and biotechnological applications: comprehensive review. J Clean Prod 57:257–265
109. Venugopal V (2016) Enzymes from seafood processing discard their applications in seafood processing. In: Kim SK, Toldrá F (eds) Advances in food and nutrition research, vol 78. Academic Press, Burlington, pp 47–69
110. Udanigwe CC, Aluko RE (2012) Food proteins-derived bioactive peptides: production, processing and potential health benefits. J Food Sci 87:2353–2357
111. Manikkam V, Vasiljevic T, Donkor ON, Mathai ML (2016) A review of potential marine-derived hypotensive and anti-obesity peptides. Crit Rev Food Sci Nutr 56:92–112
112. Ngo DH, Vo TS, Ngo DN, Wijesekara I, Kim SK (2012) Biological activities and potential health benefits of bioactive peptides derived from marine organisms. Int J Biol Macromol 51:378–383
113. Cheung RC, Ng TB, Wong JH (2015) Marine peptides: bioactivities and applications. Mar Drugs 13:4006–4043
114. Cheung S-H, Kim E-K, Hwang J-W, Kim Y-S, Lee JS et al (2013) Purification of a novel peptide derived from a shellfish *Crassostrea gigas* and evaluation of its anticancer property. J Agric Food Chem 61:11442–11446
115. Mathew M (2015) Fish oils: production and quality aspects. In: Sachindra MM, Mahendrakar MS (eds) Fish processing by-products: quality assessment applications. Studium Press, Houston, pp 77–106
116. Nichols PD, Bakes MJ, Elliott NJ (1998) Oils rich in docosahexaenoic acid in livers of sharks from temperate Australian waters. Mar Freshw Res 49:763–766
117. Bimbo AP (2007) Current and future sources of raw materials for the long-chain omega-3 fatty acid market. Lipid Technol 19:176–181

118. Elavarasan K, Shamsundar BA (2015) Utilization of surimi processing discards for value added products. In: Sachindra MM, Mahendrakar MS (eds) Fish processing by-products: quality assessment and applications. Studium Press, Houston, pp 237–272
119. Okada T, Morrissey MT (2007) Recovery and characterization of sardine oil extracted by pH adjustment. J Agri Food Chem 55:1808–1813
120. Liaset B, Julshamn K, Eape M (2003) Chemical composition and theoretical nutrition of the processed fractions from enzyme hydrolysis of salmon with Protamex™. Process Biochem 38:1747–1759
121. Rodriguez N, Diego SD, Beltran S, Jaime I, Sanz MT, Rovira J (2012) Supercritical fluid extraction of fish oil from fish by-products. A comparison with other extraction methods. J Food Eng 100:238–248
122. Pike IH, Jackson A (2010) Fish oil: production and use now and in the future. Lipid Technol 22:59–56
123. Venugopal V, Kumaran AK, Sekhar Chatterjee N, Kumar S, Kavilakath S, Nair JR, Mathew S (2016) Biochemical characterization of liver oil of *Echinorhinus brucus* (bramble shark) and its cytotoxic evaluation on neuroblastoma cell lines (SHSY-5Y). Scientifica (Cairo). https://doi.org/10.1155/2016/6294030
124. Bunea R, El Farrah K, Deutsch L (2004) Evaluation of the effects of Neptune krill oil on the clinical course of hyperlipidemia. Altern Med Rev 9:420
125. Apostolidis E, Karayannakidis PD, Lee CM (2016) Recovery of bioactive peptides and omega-3 fatty acids-containing phospholipids from squid processing by-product hydrolyzate. J Aquat Food Product Technol 25:496–506
126. Bowen KJ, Harris WS, Kris-Etherton PM (2016) Omega-3 fatty acids and cardiovascular disease: are there benefits? Curr Treat Options Cardi Med 18:69
127. Gobbo LC, Imamura F, Aslibekyan S, Marklund M, Virtanen JK, Wennberg M et al (2016) ω-3 polyunsaturated fatty acid biomarkers coronary heart disease: pooling project of 19 cohort studies. JAMA 176(8):1155–1166
128. Lavie CL, Milani RV, Mehra MR, Ventura HO (2009) Omega-3 polyunsaturated fatty acids and cardiovascular diseases. J Am Coll Cardiol 54:585–594. https://doi.org/10.1016/j.jacc.2009.02.084
129. Mozaffarrian D, Rimm EB (2006) Fish intake, contaminants and human health: evaluating the risks and benefits. JAMA 296:1885–1899
130. Scorletti E, Byrne CD (2013) Omega-3 fatty acids, hepatic lipid metabolism and nonalcoholic fatty liver disease. Ann Rev Nutr 33:231–248
131. Romeo J, Warnberg J, Garcia-Marmol E et al (2011) Daily consumption of milk enriched with fish oil, oleic acid, minerals and vitamins reduces cell adhesion molecules in healthy children. Nutr Metab Cardiovasc Dis 21:113–120
132. Gogus U, Smith C (2010) n-3 omega fatty acids: a review of current knowledge. Int J Food Sci Technol 45:417–436
133. Bao B, Prasad AS, Beck FW et al (2010) Zinc decreases C-reactive protein, lipid peroxidation, inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. The Am J Clin Nutr 91:1634–1641
134. Kim SK, Karadeniz F (2012) Biological importance and applications of squalene and squalane. Adv Food Nutr Res 65:223–233
135. Fu Y, Li G, Zhang X, Xing G, Hu X, Yang L, Li D (2015) Lipid extract from hard-shelled mussel (*Mytilus coruscus)* improves clinical conditions of patients with rheumatoid arthritis: a randomized controlled trial. Forum Nutr 7:625–645
136. Emelyanov A, Fedoseev G, Krasnoschekova O, Abulimity A, Trendeleva T, Barnes PJ (2002) Treatment of asthma with lipid extract of new zeal green-lipped mussel: a romized clinical trial. The Eur Resp J 20:596–600
137. Grienke U, Silke J, Tasdemir D (2014) Bioactive compounds from marine mussels and their effects on human health. Food Chem 142:48–60
138. Sachindra NM, Bhaskar N, Mahendrakar NS (2005) Carotenoids in different body components of Indian shrimps. J Sci Food Agric 85:167–172

139. Li N, Hu J, Wang S, Cheng J, Hu X et al (2010) Isolation identification of the main carotenoid pigment from the rare orange muscle of the Yesso scallop. Food Chem 118:616–619
140. Venugopal V (2011) Chapter 11, Biomedical applications of marine polysaccharides: an overview. In: Marine polysaccharides: Food applications. CRC Press, Boca Raton
141. d'Ayala GG, Malinconico M, Laurienzo P (2008) Marine derived polysaccharides for biomedical applications: chemical modification approaches. Molecules 13:2069–2106
142. Tharanathan RN, Kittur FS (2003) Chitin – the undisputed biomolecule of great potential. Crit Rev Food Sci Nutr 43:61–87
143. Hayes M, Carney B, Slater J, Brück W (2008) Mining marine shellfish discards for bioactive molecules: chitin chitosan – Part A: extraction methods. Biotechnol J 3:871–877
144. Cahu TB, Santos SD, Mendes A, Cordula CR et al (2012) Recovery of protein, chitin, carotenoids glycosaminoglycans from Pacific white shrimp (*Litopenaeus vannamei*) processing discard. Process Biochem 47:570–577
145. No HK, Meyers SP, Prinyawiwatkul W, Xu Z (2007) Applications of chitosan for improvement of quality and shelf life of foods. J. Food Sci 72:R87–R100
146. Mourya VK, Inamdar NN (2008) Chitosan-modifications and applications: opportunities galore. React Funct Polym 68:1013–1051
147. Muxika A, Etxabide A, Uranga J, Guerrero P, de la Caba K (2017) Chitosan as a bioactive polymer: Processing, properties and applications. Int J Biol Macromol 105:1358–1368. pii: S0141-8130(17)3175
148. Prashanth KVH, Tharanathan RN (2007) Chitin/chitosan: modifications and their unlimited application potential – an overview. Trends Food Sci Technol 18:117–131
149. Shahidi F, Abuzaytoon R (2005) Chitin, chitosan and co-products: chemistry, production, applications and health effects. Adv Food Nutr Res 49:93–145
150. Kurita K (2006) Chitin and chitosan: functional biopolymers from marine crustaceans. Mar Biotechnol 8:203–226
151. Muzzarelli RAA (2009) Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. Carbohydr Polym 76:167–182
152. Morganti P, Palombo M, Palombo P, Dziergowski S (2010) Cosmetic science in skin aging: achieving the efficacy by the chitin nano-structured crystallites. SOFW-J 136:14–24
153. Ahsan SM, Thomas M, Reddy KK, Sooraparaju SG, Asthana A, Bhatnagar I (2017) Chitosan as biomaterial in drug delivery tissue engineering. Int J Biol Macromol. https://doi.org/10.1016/j.ijbiomac.2017.08.140. pii: S0141-8130(17)31884-6
154. Lordan S, Ross PR, Stanton C (2011) Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Mar Drugs 9:1056–1100
155. Venkatesan J, Kim SK (2014) Chitosan for bone repair and regeneration. In: Mallick K (ed) Bone substitute materials. Woodhead Publishing, London, pp 244–260
156. Belorkar SA, Gupta AK (2016) Oligosaccharides: a boon from nature's desk. AMB Expr 6(82):1–11
157. Garnjanagoonchorn W, Wongekalak L, Engkagul A (2007) Determination of chondroitin sulfate from different sources of cartilage. Chem Eng Process 46:465–471
158. Vázquez JA, Rodríguez-Amado I, Montemayor MI et al (2013) Chondroitin sulfate, hyaluronic acid chitin/chitosan production using marine discard sources: characteristics, applications and eco-friendly processes: a review. Mar Drugs 11:747–777
159. Jo JH, Park DC, Do J-R, Kim Y-M, Kim D-S, Park Y-K, Lee T-K, Lee CS-M (2004) Optimization of skate (*Raja avirostris*) cartilage hydrolysis for the preparation of chondroitin sulfate. Food Sci Biotechnol 13:622–626
160. Lignot B, Lahogue V, Bourseau P (2003) Enzymatic extraction of chondroitin sulfate from skate cartilage and concentration-desalting by ultrafiltration. J Biotechnol 103:281–284
161. Sim J-S, Im A-R, Cho SM, Jang HJ, Jo HJ, Kim YS (2007) Evaluation of chondroitin sulfate in shark cartilage powder as a dietary supplement: raw materials finished products. Food Chem 101:532–539
162. Suleria HAR, Masci PP, Gobe GC, Osborne SA (2017) Therapeutic potential of abalone and status of bioactive molecules: a comprehensive review. Crit Rev Food Sci Nutri 57:1742–1748

163. Liao N, Chen S, Ye X, Zhong J, Ye X, Yin X, Tian J, Liu D (2014) Structural characterization of a novel glucan from *Achatina fulica* its antioxidant activity. J Agri Food Chem 62:2344–2352
164. Shun-gan X (1996) Calcium powder of freshwater fish bone. J Shanghai Fish Univ 5:246–249
165. Sultanbawa Y, Aksnes A (2006) Tuna process discard – an unexploited resource. Infofish International, www.infofish.org. March issue, pp 37–40
166. Chrasekharan M (2015) Biotechnology for utilization of marine byproducts. In: Sachindra MM, Mahendrakar MS (eds) Fish processing by-products: quality assessment and applications. Studium Press, Houston, pp 43–76
167. Ren X, Ma L, Wang Y-H, Zhuang YP et al (2012) Optimization of enzymatic hydrolysis of channel catfish bones for preparing antimicrobial agents. J Aquatic Food Prod Technol 21:99–110
168. Jung WK, Park P, ByunH MS-H, Kim S-K (2005) Preparation of hoki (*Johnius belengerii*) bone oligophosphopeptide with a high affinity to calcium by carnivorous intestine crude proteinase. Food Chem 91:333–340
169. Balano A (2014) Recovery of biomolecules from food discards – a review. Molecules 17:14821–14842
170. Kiuru P, D'Auria MV, Christian D, Muller CD et al (2014) Exploring marine resources for bioactive compounds. Planta Med 80:1234–1246
171. Freitas AC, Rodrigues D, Rocha-Santos TAP, Gomes AMP, Duarte AC (2012) Marine biotechnology advances towards applications in new functional foods. Biotechnol Adv 30:1562–1574
172. Rasmussen RS, Morrissey MT (2007) Marine biotechnology for production of food ingredients. Adv Food Nutr Res 52:237–292
173. Muffler K, Ulber R (2005) Down-stream processing in marine biotechnology. Adv Biochem Eng Biotechnol 97:63–103
174. Gil-Chavez GJ, Villa JA, Ayala-Zavala FJ, Heredia JB et al (2013) Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. Comp Rev Food Sci Food Safety 12:5–23
175. Kim SK, Senevirathne M (2011) Membrane bioreactor technology for the development of functional materials from sea-food processing discards and their potential health benefits. Membranes 1:327–344
176. Ferdosh S, Sarker Md ZI, Ab Rahman NIM, Akanda Md JH et al (2016) Simultaneous extraction and fractionation of fish oil from tuna by-product using supercritical carbon dioxide ($SC\text{-}CO_2$). J Aquatic Food Prod Technol 25:230–239
177. Vázquez J, Patrícia Ramos R, Mirón J et al (2017) Production of chitin from *Penaeus vannamei* by-products to pilot plant scale using a combination of enzymatic chemical processes and subsequent optimization of the chemical production of chitosan by response surface methodology. Mar Drugs 15(6):180. https://doi.org/10.3390/md15060180
178. Rangel M, Falkenberg M (2015) An overview of the marine natural products in clinical trials on the market. J Coastal Life Med 3(6):421–428
179. Lafarga T, Hayes M (2017) Bioactive protein hydrolyzates in the functional food ingredient industry: overcoming current challenges. Food Rev Int 33:217–246
180. GOED (2017) Organization of EPA and DHA Omega-3s. Available from: http://www.goedomega3.com. Accessed 12 Jan 2017
181. Salem N Jr, Eggersdorfer M (2015) Is the world supply of omega-3 fatty acids adequate for optimal human nutrition? Curr Opin Clin Nutr Metab Care 18:147–154
182. Garg ML, Wood LG, Singh H, Moughan PJ (2006) Means of delivery recommended levels of long chain omega-3 polyunsaturated fatty acids in human diets. J Food Sci 71:R66–R71
183. Ohshima T (2002) Marine nutraceuticals and functional foods in Japan. In: Alasalvar C, Taylor T (eds) Seafoods: quality, technology and nutraceutical applications. Springer, Berlin/Heidelberg, pp 205–220
184. Borresen T (2016) Fish lipids and peptides in nutrition. J Aquatic Food Prod Technol 25:1171

Roots and Tubers as Functional Foods

48

Anoma Chandrasekara

Contents

A. Chandrasekara (✉)
Department of Applied Nutrition, Faculty of Livestock Fisheries and Nutrition, Wayamba University of Sri Lanka, Gonawila, Sri Lanka
e-mail: anomapriyan@yahoo.ca

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_37

Abstract

Starchy roots and tuber crops are important components in the human diet. There are number of roots and tubers belonging to several species and make an extensive biodiversity even within the same geographical location. From the ancient time of human evolution starchy roots and tubers have been a part of food choices and add variety to the modern diet in addition to offering numerous desirable nutritional and health benefits such as anti-obesity, antioxidative, hypoglycemic, hypocholesterolemic, antimicrobial, and immunomodulatory activities, among others. There are a number of bioactive constituents, namely, phenolic compounds, saponins, bioactive proteins, glycoalkaloids, phytic acids, and hydroxycoumarins, reported in tuber crops. Except the common potatoes, sweet potatoes, and cassava, other starchy tuber crops are yet to be explored for their nutritional and health benefits to use as functional foods. Some edible tubers are served for traditional and alternative medicinal sources. Tubers and roots are potential functional foods and nutraceutical ingredients to manage a number of ailments and to ensure general wellness.

Keywords

Antioxidative · Hypoglycemic · Hypocholesterolemic · Phenolic compounds · Saponins

List of Abbreviations

AMPK	Adenosine monophosphate-activated protein kinase
ACC	Acetyl coenzyme A carboxylase
DPPH	2,2, diphenyl-1-picrylhydrazyl
ERK	Extracellular signal-regulated protein kinase
FAO	Food and Agriculture Organization
GAE	Gallic acid equivalents
GGT	Glutamyltransferase
HCC	Hepatocellular carcinoma
LPS	Lipopolysaccharide
MTT	Microculture tetrazolium treatment assay
NCDs	Noncommunicable diseases
NASA	National Aeronautics and Space Administration
NO	Nitric oxide
IFN-γ	Interferon- γ
ORAC	Oxygen radical absorbance capacity
OGTT	Oral glucose tolerance test
SHBG	Sex hormone binding globulin
SOD	Superoxide dismutase
TPC	Total phenolic content
t-BHP	Tert-butylhydroperoxide
WSSP	White skinned sweet potatoes

1 Introduction

Roots and tuber crops are important cultivated staple carbohydrate sources, second to cereals, generally in tropical regions in the world. During fast few decades the roles of roots and tubers in the economy and food systems of developing countries have been changed. They are subsistence crops of pivotal importance in consumers and producers in low-income countries. In addition, they provide a substantial input for animal feeds and industrial products such as distilled spirits, starches, fermented foods, and other minor products. Economically important roots and tubers include potatoes, cassava, sweet potatoes, yams, and aroids.

An important agronomic advantage of root and tuber crops as staple foods is their favorable adaptation to diverse soil and environmental conditions and a variety of farming systems with minimum agricultural inputs. Further, the variation in the growth pattern and production requirements of root and tubers make their way into distinct production systems and varied consumption uses. However, roots and tuber crops are bulky in nature with high moisture content of 60–90% leading them to be associated with high transportation cost, short shelf life, and limited market margin in developing countries where they are produced.

The contribution of starchy roots and tubers to the energy supply in different populations varies with the region and the country. Their annual global production is approximately 845 million tons [1]. Asia is the main producer of starchy roots and tubers followed by Africa, Europe, and America. Asian and African regions produced 43% and 33%, respectively, in the global production of roots and tubers [1]. Cassava, potatoes, and sweet potatoes consist of 90% global production of root and tuber crops though a number of species and varieties are consumed [1]. Roots and tubers provide dietary energy in the form of carbohydrates, dietary fiber, vitamins, and minerals (Table 1; [2]). It is interesting to note that high yields of roots and tubers give more energy per land unit per day than those of cereal grains [3]. In general, roots and tubers provide low protein contents ranging from 1% to 2% on a dry weight basis [3]. However, potatoes and yams contain high amounts of proteins among other tubers. Sulfur-containing amino acids, namely, methionine and cystine, are the limiting ones in root crop proteins. Cassava, sweet potatoes, potatoes, and yam contain some vitamin C, and yellow varieties of sweet potatoes, yam, and cassava contain β-carotene. Roots and tubers are deficient in most other vitamins and minerals but contain significant amounts of dietary fiber [3]. Nutritional value of roots and tubers vary with variety, location, soil type, and agricultural practices, among others.

Noncommunicable diseases (NCDs) are in rise in developed as well as developing countries on concurrence with increasing aging population worldwide. Dietary and lifestyle factors play a prominent role in oxidative stress which contributes immensely to the etiology of NCDs as well as the aging process. Several epidemiological studies showed the association between plant food intake and reduced NCDs [4–7]. Furthermore, identification of specific plant

Table 1 Nutrient composition of major starchy roots

	Cassava	Potato	Sweet potato	Yams
Crude analysis				
Dry matter (DM) (g/100 g fresh weight (FW)	31.3	22.2	30.8	31.1
Protein (g/100 of DM)	2.7	9.2	5.3	6.4
Protein (g/100 of FW)	0.8	2.0	1.6	2.0
Fat (g/100 of DM)	0.62	0.5	1.95	0.42
Available carbohydrates (g/100 of DM)	86.9	66.7	78.2	72.8
Fiber (g/100 of DM)	7.9	9.3	10.2	17.9
Energy (kcal/100 g of DM)	364	316	351	318
***Amino acid composition (mg/g crude protein)**				
Ile	28	39	37	37
Leucine	40	59	54	65
Lysine	41	60	34	41
Methionine + Cystine	27	30	28	28
Phenyl alanine + tyrosine	41	78	62	80
Threonine	26	39	38	36
Tryptophan	12	14	14	13
Valine	33	45	45	47
Minerals (mg/100 kcal)				
Calcium	23.8	8.8	32.3	25.3
Phosphorus	28	71	42	44
Magnesium	48	28	23	–
Iron	0.88	0.57	0.78	0.91
Zinc	0.41	0.49	0.83	0.11
Vitamins				
Vitamin A (μg eq ret./100 kcal)	3.7	1.2	1321.1	1.7
Vitamin B1(μg /100 kcal)	45	157	59	91
Vitamin B2 (μg /100 kcal)	22	67	46	30
Nicotinamide (μg /100 kcal)	446	1737	554	607
Vitamin C (μg /100 kcal)	22.3	24.2	27.7	10.1

Sources: Souci et al. [118]; * FAO [119]

constituents which convey health benefits is of much interest. Foods of plant origin consist of a wide range of nonnutrient phytochemicals which are synthesized as secondary metabolites and serve a wide range of ecological roles in plants [8].

Tubers and root crops contain several bioactive compounds such as saponins, phenolic compounds, glycoalkaloids, phytic acids, carotenoids, and ascorbic acid among others. A number of bioactivities, namely antioxidant, immunomodulatory, antimicrobial, antidiabetic, antiobesity, and hypocholesterolemic activities are reported for tubers and root crops. This chapter discusses the distribution and bioactivities of several selected nutrient and nonnutrient phytochemicals in starchy roots and tuber crops.

Table 2 Common tuber crops worldwide

	Botanical name	Family	Common name
Potatoes	*Solanum tuberosum*	*Solanaceae*	
Country potato Hausa potato	*Solenostemon rotundifolius*	*Lamiaceae* (mint family)	Innala, ratala (Sri Lanka)
Cannas	Canna edulis	Cannaceae	Buthsarana (Sri Lanka)
	Maranta arundinacea L	Marantaceae	Arrow root Hulankeeriya (Sri Lanka) Aru aru,Arawak (India)
Taro	*Xanthosoma sagittifolium*	Araceae	Kiriala (Sri Lanka) Keladi (Malasia) Phueak (Thailand) Khoai mon (Vietnam) Sato-imo (Japan)
Yam	*Dioscorea alata*	*Dioscoreaceae*	Purple yam; Greater yam Guyana;Water yam Winged yam; Raja ala (Sri Lanka); Ube (Philippines)
Sweet potatoes	*Ipomoea batatas*	*Convolvulaceae*	Camote; Batata Shakarkand
Cassava	*Manihot esculenta*	*Euphorbiaceae*	Yuxco; Mogo; Manioc Mandioca; Kamoteng kahoy
Elephant foot yam	*Amorphophallus paeoniifolius*	*Araceae*	White pot giant arum; Stink lily

2 Starchy Roots and Tuber Crops

Roots and tubers are belonging to different botanical families but are grouped together as all types produce and store edible food in underground parts. Starchy roots and tubers store starch in subterranean stems, roots, rhizomes, corms, and tubers. Potatoes and yams are tubers whereas taro and cocoyams are derived from corms, underground stems, and swollen hypocotyls. Cassava and sweet potatoes are storage roots whereas canna and arrowroots are edible rhizomes. Vegetative propagation is a common characteristic of roots and tubers. These plant parts include tubers (potatoes and yams), stem cuttings (cassava), vine cuttings (sweet potatoes), and side shoots, stolons, or corm heads (taro and cocoyam). Table 2 presents commonly consumed starchy tuber and root crops worldwide.

2.1 Potato (*Solanum tuberosum*)

Potato is ranked currently at the fourth place of the highly consumed crops through the world followed by maize, wheat, and rice. The average annual potato production is about

381 million tones [1]. Potato has a highland origin and has been domesticated in the high Andes of South America. It is a popular food crop in the cool highland areas of South America, Asia, and Central and Eastern Africa [9]. China is the biggest potato producer followed by Russia and India. The average energy contribution from potato in the world ranged from 41 to 130 Kcal per day for developing and developed countries, respectively [10]. Potato is a high yielding cash crop with a short cropping cycle of 3–4 months. Further, potato is a significant source of carbohydrates and they can be processed into a variety of foods such as mashed potato, chips and fries, deep frozen and dehydrated products. Starch derived from potato has shown a great economic importance. The productivity of potato in terms of nutrients is higher compared to maize, wheat, and rice.

Potato produces high amount of calories, protein, and calcium from the same land than those of other major crops leading its significance as a food security crop. Potatoes have several secondary metabolites which demonstrated antioxidant as well as other bioactivities [11].

2.2 Sweet Potato (*Ipomea batatas* L.)

Sweet potato originated from the Central America and the cultivation has been spread through the tropical, subtropical, and warm temperate countries. In Asia it is predominate in lowland conditions. Sweet potato is known as the seventh largest food crop with an annual production of 106 million tones [1]. They are known as an insurance crop as the cultivation can be extended throughout the year under suitable climatic conditions and its greater capacity to withstand the adverse climatic changes. In addition, the crop can be harvested for an extended period of time giving advantages in the poor communities. Further, the short growing cycle of sweet potato is an advantage for the flexible planting and harvesting conditions in spite of the climatic changes. Rice and sweet potato can be grown with crop rotation. China is the largest producer of sweet potato [12]. National Aeronautics and Space Administration (NASA) has selected sweet potatoes as a potential crop to be grown and incorporated into the menus for astronauts on space missions due to its unique features and nutritional value [13]. It can be consumed in many ways such as boiled, baked, fried, boiled canned or frozen [14]. The biofortified sweet potatoes provide considerable amounts of beta-carotene, and it has been proved as a cost-effective strategy to provide vitamin A for the vulnerable communities including young children, pregnant and lactating mothers. Sweet potato is an important source of many vitamins, minerals, and dietary fiber. Further it is a good source of nonnutritive bioactive compounds such as phenolic acids and anthocyanins [15–19]. The different flesh colors such as cream, deep yellow, orange, and purple are attributed to the degree of anthocyanin and carotenoids content of sweet potatoes [15–18, 20].

2.3 Cassava (*Manihot esculenta*)

Cassava is a perennial shrub belonging to the family of *Euphorbiaceae*. Nigeria, Thailand, Indonesia, and Brazil are the leading cassava producers in the world. The

*Manihot g*enus comprises of 98 species and the most prominent species is *M. esculenta* [21]. The crop is originated in South America and has speared through tropical and subtropical regions of Africa and Asia [22]. Cassava is the sixth most important food crop in the world with about 268 million tones annual production [1]. It is the most widely cultivated root crop and contains nearly the maximum theoretical concentration of starch on a dry weight basis among food crops. The edible roots can store the foods underground for up to 3 years contributing for the food security consistently.

Different range of food products are derived from cassava such as traditional and novel food products, fermented products, ethanol, and livestock feeds. The starch extracted from cassava is used as the raw material for the production of bread, crackers, pasta, and tapioca. Cassava leaves are also a nutritious food addition. Through the west, steamed and boiled cassava roots are pounded in to sticky dough and consumed as *fufu*. The Philippines consume cassava pulp made into pellet as *landing* or cassava rice [23]. Cassava starches are popular in the industry due to its high availability (75–80% of the dry matter content of roots) and the easily extractable nature due to the lower content of protein and fat. Further, cassava is used for the manufacturing of paper, textiles, adhesives, and high fructose corn syrup [14].

Cassava is well known for the production of hydrogen cyanide (HCN) in toxic quantities. The presence of two types of cyanogenic glycosides such as linamarin (93% of the cyanide content) and lotaustralin has been reported [24]. High doses of HCN about 0.5–3.5 mg/Kg of body weight can exert lethal effects. Further, non-cyanogenic glycosides, hydroxycoumarins such as scopoletin, terpenoids, and flavonoids, have been investigated in cassava roots. Both roots and leaves contain the cyanogenic glycosides and the content is not significantly different from the roots. Despite that cassava leaves are a good source of calcium and protein [23].

2.4 Yams (*Dioscorea* sp.)

Yams are a group of many species of plants bearing rhizomes or carbohydrate rich underground tubers belonging to the genus *Dioscorea.* They are mainly distributed through the tropics and only a few numbers can be found in temperate regions of the world [25]. Yams are staple foods in West Africa, Southeast Asia, and the Caribbean regions in the world and help to ensure the food security and livelihood of at least 60 million people in West Africa [26]. They rank as the third important source of calorie and protein contributor for the livelihood of Benin and Ghana populations. The contribution for the protein intake is even higher than the widely grown and available cassava. They are classified into the genus *Dioscorea* of the family of *Dioscoreaceae*, which is one of the oldest members of the monocot group [27, 28]. Even though there are many varieties present, only few numbers of species are popular throughout regions in the world. About 603 species have been identified and from them 50 species are edible. *Dioscorea alata*, *Dioscorea esculenta Dioscorea rotundata*, *Dioscorea hispida*, *Dioscorea bulbifera*, *Dioscorea trifida*, *Dioscorea nummularia*, and *Dioscorea pentaphylla* are prominent yam species, among others.

A wide variation in morphological characteristics presents within the same species of yams and these include size of tuber, color of flesh, presence of roots, and thickness of peel (Fig. 1).

The yams are usually grown in intercropping systems with coconut palms. Further, yams can be easily grown due to their specific ability to thrive under low agricultural inputs, having tolerance to stress environmental conditions, ability to resist pest attacks, and high adaptability to mixed cropping systems. They are considered as seasonal crops which are generally planted in the end of March to April [29]. The harvesting season begins usually during the period of December to February. After harvesting, these yams would be on a dormancy period until the favorable conditions are met for the growth of new plant.

Dioscorea alata is called as winged yam, greater yam, or water yam and includes a wide array of tubers referred in several local names in different countries. It has been reported that *D. alata* are originated in Asia [30]. They are widely cultivated in India, Sri Lanka, South East Asia, and Pacific Islands and they prefer the low lands up to 800 m. The tubers are famous for the sweetness and the delicacy of the tubers. *Dioscorea alata* contains only a single large tuber per vine [30]. The shapes of the tubers have significant differences. They may be globular, long, and flattened or palmate. Sometimes they may be branched or lobed but usually large in size.

Yam tubers have various bioactive components, namely, mucin, dioscin, dioscorin, allantoin, choline, polyphenols, diosgenin, and vitamins such as carotenoids and tocopherols [31, 32]. Mucilage of yam tuber contains soluble glycoprotein and dietary fiber. There are evidences to show hypoglycemic, antimicrobial, and antioxidant activities of yam extracts [33, 34]. Yams may stimulate the proliferation of gastric epithelial cells and enhance digestive enzyme activities in the small intestine [35]. *Dioscorea esculenta* (Chinese yam, lesser yam, sweet yam) is one of the main yam species found in Sri Lanka [29]. They are native to Indo-China and at present they have widely been distributed through Madagascar and New Guinea. Usually the yams are cultivated in the lowland up to 700 m. Their stems contain a large number of tubers and they are generally ovoid and cylindrical in shape [29]. In addition, the tubers may be intermingled and covered with numerous spiny roots. The color of the tuber flesh varies from white to yellowish white. Leaves are broadly ovate with a chordate base. The raw tubers are known for a number of medicinal properties [30].

*Dioscorea pentaphyll*a is known as five-fingered yam and buck yam. These tubers are variable in shape such as finger shaped, cylindrical, or shapeless. The skin of the tuber is brown in color and the flesh ranges from white to yellow. Sometimes there may be violet spots in the flesh. The species is native to South East Asia and is grown in teak forests or forest borders with altitudes of 500–1000 m. Tubers are consumed as staple food especially as an alternative to maize in the dry areas. It is used as a raw material for the production of starch and alcohol. The origin of *Dioscorea bulbifera L*. is evidenced from Asia, Africa, and Oceania and are known as air potato, aerial yam, or potato yam. The characteristics of the vine are documented as stem round, twining to the left, without spines subterranean tuber small or absent, and spongy.

Fig. 1 Different accessions of *Dioscorea alata*

Leaves are alternate, broad, round, heart-shaped, and leaf blade embossed with well-marked veins. In addition, less known *Dioscorea* species have been reported and they include *Dioscorea rotundata Poir.; D. cayenensis* (African yam, white yam); *Dioscorea trifida L.* (*Cushcush*, Indian yam); and *Dioscorea nummularia Lam.* (Pacific yam, hard yam).

2.5 Aroids

Aroids are tuber or underground stem-bearing plants belonging to the family Araceae. Aroids can be grown in both rain-fed and irrigated climates. Taro is used as the generic name for the four related species belonging to *Araceae* including edible tubers such as taro (*Colocasia*), giant taro (*Alocasia*), tannia or yautia (*Xanthosoma*), elephant foot yam (*Amorphophallus*), and swamp taro (*Cyrtosperma*) [36]. They serve as a staple food for many islands of the South Pacific, such as Tonga and Western Samoa, and in Papua New Guinea. The cultivation has been extended to Asia, Africa, Pacific and Caribbean islands. Aroids originated from the Indo–Malayan region between Myanmar and Bangladesh. They are known to have thousands of different cultivars and most of them prefer the humid environments. The young leaves are eaten as vegetables. They are well cultivated in the low lands and highlands up to 2.7 m above the sea level. Aroids are considered as the staple in Irian Jaya, the Moluccas, and Mentawai Islands in West Sumatra. They can be consumed as chips as well as steamed forms among others.

3 Nutrients in Roots and Tubers

Starchy roots are major contributors to the supply of carbohydrates in the diet. The protein content of the tubers varies from 1% to 2% on fresh weight basis. The limiting amino acids of the tubers are identified as sulfur-containing amino acids, namely, methionine and cystine, whereas sweet potato contains lysine [3]. Vitamin C are available in cassava, sweet potato, and potato. Further, potato is a dietary source of micronutrients such as zinc and iron. Sweet potato cultivars are known for the dietary fiber, mineral, and vitamins including β-carotene and tocopherols. Especially the yellow-fleshed sweet potato varieties are known for their high β-carotene content. Taro and potato have appreciable level of potassium. All most all the tuber species are rich with dietary fiber (Table 2).

3.1 Proteins

Roots and tubers are not considered as appraisable sources of proteins and the contents are variable. In populations worldwide, global contribution of proteins from roots and tubers in the diet is less than 3%. However, this contribution varies from 5 to 15% depending on the quantity consumed as a staple in African

countries [9]. Cassava contains only 1–2% of protein on dry weight basis with low content of sulfur-containing amino acids [37].

Dioscorin is the main storage protein known to present in *Dioscorea* yams and is about 90% of water extractable soluble proteins in a majority of *Dioscorea* species. Among many activities reported dioscorin has shown carbonic anhydrase and trypsin inhibitor activities [38]. Further, dehydroascorbate reductase (DHA) and monodehydroascorbate reductase (MDA) and immunomodulatory activities of dioscorin in the presence of glutathione have been reported [39]. Dioscorin from fresh yam (*Dioscorea batatas*) showed 2,2, diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [40]. Further, dioscorin demonstrated angiotensin converting enzyme (ACE) inhibitory and antihypertensive activities on spontaneously hypertensive rats [31, 41, 42].

Sporamin is a soluble protein and serves as the main storage protein of sweet potato tubers. It accounts for 60–80% of tubers total proteins [43]. The sporamin of sweet potatoes was initially known as ipomoein and is a nonglycoprotein without glycan. The sporamin is generally stored in vacuoles in the monomeric form. This protein is initially produced as preprosporamin, which is synthesized by the membrane-bound polysomes in the endoplasmic reticulum (ER) [44]. Sporamin is a trypsin inhibitor with a kunitz-type trypsin inhibitory activity which has potential application in the transgenic insect-resistant plants [45]. Furthermore, sporamin showed various antioxidant activities related to stress tolerance, such as DHA and MDA reductase activities [46].

Patatin is a storage protein in potato tubers and contributes to about 40% of soluble proteins [47]. It is a glycoprotein in the storage of parenchyma cells and exhibited a number of bioactivities [48].

3.2 Carotenoids

Carotenoids serve as the most extensively available natural pigments with yellow, orange, and red colors in plants. Carotenoids, either isolated from natural sources or chemically synthesized, have been widely utilized as natural nontoxic colorants in manufactured foods, drinks, and cosmetics due to the distinctive coloring properties. The majority of carotenoids are unsaturated tetraterpenes with the same basic C 40 isoprenoid skeleton resulting from the joining of eight isoprene units in a head-to-tail manner with the exception of the tail-to-tail connection at the center. They are hydrocarbons and are soluble in nonpolar solvents such as hexane and petroleum ether. However, the oxygenated derivatives of carotenes, such as xanthophyll, dissolve better in polar solvents such as alcohols. Carotenoids are important molecules in living organisms. They participate in a variety of photochemical reactions in photosynthetic systems of higher plants, algae, and phototrophic bacteria [49].

Carotenoids possess numerous bioactivities and are well known for provitamin A activity. In addition, they play important roles in human health and nutrition, namely antioxidant activity, regulation of gene expression, and induction of cell-to-cell communication [50]. It has been demonstrated that zeaxanthin and lutein are stable

throughout artificial digestion, whereas β-carotene and all-trans lycopene are degraded in the jejunal and ileal compartments. Among the isomers, the stability of 5-cis lycopene is superior to that of all-trans, and 9-cis lycopene [51]. Yellow to orange varieties of sweet potatoes and yams are good sources of carotenoids and lutein, zeaxanthin, violaxanthin and neoxanthin are major carotenoids in potatoes [52]. Further, digestive stability of lutein and zeaxanthin of yellow-fleshed potatoes were reported to be high ranging from 70% to 95% [53].

3.3 Ascorbic Acid

Ascorbic acid, also known as vitamin C, is a water-soluble vitamin. Ascorbic acid naturally occurs in plant tissues, commonly in fruits and vegetables but considerable quantities also present in several root crops. However, the level could be reduced during cooking of roots unless skins and cooking water are utilized [54]. Root crops, if carefully prepared, can make a significant contribution to the vitamin C content of the diet. Potatoes serve as a principal source of vitamin C in British diets, providing 19.4% of the total requirement [3]. In general, yams contain 6–10 mg of vitamin C/100 g and may vary up to 21 mg/100 g. Further, the vitamin C content of potatoes is very similar to those of sweet potatoes, and cassava. The concentration of ascorbic acid varies with the species, location, crop year, maturity at harvest, soil, nitrogen and phosphate fertilizers [3].

4 Nonnutrient Compounds in Roots and Tubers

Bioactive compounds in plants are secondary metabolites having pharmacological or toxicological effects in humans and animals. Secondary metabolites are produced within the plants besides the primary biosynthesis associated with growth and development. These compounds perform several essential functions in plants, including protection from undesirable effects, attraction of pollinators, or signaling of essential functions, among others. Roots and tubers are rich with nonnutritive phytochemicals. Yam tubers contain mucin, dioscin, dioscorin, allantoin, choline, polyphenols, diosgenin, and saponins [32]. Potatoes are rich with phenolic acids including chlorogenic acids and flavonoids and some varieties are rich sources of anthocyanins [55]. Cultivation location, climatic factors, soil type, agricultural practices, and environmental stress are the key important determinants of the nutritional and phytochemical profile of tubers.

4.1 Phenolic Compounds

Phenolic compounds are important molecules with an aromatic ring attached with one or more hydroxyl groups. They are derived from biosynthetic precursors such as pyruvate and acetate, amino acids such as phenyl alanine and tyrosine, and acetyl

CoA and malonyl CoA by following the pentose phosphate, shikimate, and phenylpropanoid metabolism pathways [8, 56]. Among groups of phenolic compounds abundantly found in plants simple phenolics, phenolic acids, flavonoids, coumarins, stilbenes, tannins, lignans, and lignins are reported to possess myriad of health benefits [8]. Cultivar, environmental conditions, cultural practices, post-harvest practices, processing and storage conditions affect the quantity of phenolic compounds present in a given species of plant material, among others [57]. Phenolic acids found in plants are categorized as hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids include gallic, *p*-hydroxybenzoic, vanillic, syringic, and protocatechuic acids, among others. The hydroxycinnamic acids include *p*-coumaric, caffeic, ferulic, and sinapic acids. These latter compounds with a phenyl ring (C_6) and a C_3 side chain are known as phenylpropanoids and serve as precursors for the synthesis of other phenolic compounds. Flavonoids are common and known constituents synthesized by condensation of a phenylpropanoid compound with three molecules of malonyl coenzyme A. The subclasses such as flavones, flavonols, flavonones, flavononols, isoflavones, flavans (catechins and anthocyanidins), and flavanols are common molecules of flavonoids, among others. Flavones and flavonols are present as aglycones in foods [57]. The phenolics present in tubers render several health benefits, namely, antibacterial, anti-inflammatory, and antimutagenic activities.

4.2 Saponins and Sapogenins

Saponins are high molecular weight secondary metabolites and are glycosides consisting of a sugar moiety linked to a triterpene or steroid aglycone. The common biological feature of saponins include ability to lyse erythrocytes and foam [58]. The aglycone portion of the saponin molecule is called sapogenin which is hydrophobic. The hydrophilic sugar moiety present in saponins include one to three straight or branched sugar chains and they are composed of D-glucose, L-rhamnose, D-galactose, D-glucuronic acid, L-arabinose, D-xylose, and D-fucose [59]. Depending on the type of genin present, saponins are divided into three groups, namely, triterpenoid saponins, steroidal saponins, and steroidal alkaloid saponins. Steroidal saponins are precursors for the chemical synthesis of birth control pills (with progesterone and estrogen), similar hormones, and corticosteroids [60]. According to recent findings steroidal saponins could be a novel class of prebiotics to lactic acid bacteria and are effective candidates for treating fungal and yeast infections in humans and animals [61].

4.3 Steroidal Glycoalkaloids

Glycoalkaloids are belonging to phytochemicals found in species of the genus *Solanum* and *Veratrum* [62, 63]. Alkalioids are nitrogen-containing secondary metabolites and are found in several higher plants as well as microorganisms and

animals [64]. The skeleton of alkaloids is derived from amino acids and moieties from other pathways, such as those originating from terpenoids. The alkaloids in plants act as phytotoxins, antibactricides, insecticides, fungicides, and as feeding deterrents to insects, herbivorous mammals, and mollusks [65]. The two main glycoalkaloids present in commercially cultivated potatoes include α-chaconine and α-solanine which are glycosylated derivatives of the aglycone solonidine. Wild potatoes (*Solanum chacoense*) contain leptinine 1, leptinine 11, leptine 1, leptine 11, and dehydrocommersonine [66]. The major glycoalkaloid reported in tomatoes is α-tomatine which is a glycosylated derivative of aglycone tomatidine. Steroidal alkaloids and their glycosides present in several species of Solanum are known to possess a variety of biological activities such as antitumor, antifungal, teratogenic, antiviral, and antiestrogenic activities. Certain glycoalkaloids are used as anticancer agents [67, 68]. The steroidal alkaloid glycosides showed cytotoxic activity against various tumor cell lines [69].

5 Bioactivities of Phytochemicals in Roots and Tubers

5.1 Hormonal Activities

Some roots and tubers especially yams belonging to *Dioscorea* have demonstrated health effects due to the bioactivities affected on hormones. According to Wu et al. [70] yam diet has ability to reduce the risk of cancer and cardiovascular diseases in postmenopausal women. They showed that the levels of serum estrogen and sex hormone binding globulin (SHBG) increased significantly after subjects had been on a yam diet for 30 days. Further, serum hormone parameters such as estrogen, estradiol, and SHBG did not change in controls who had sweet potatoes compared to those who had yam diet. The risk of breast cancer increased by estrogens might be balanced by the elevated SHB and the ratio of estrogen plus estradiol to SHBG. The study further showed that increased SHBG levels had a protective effect against the occurrence of type 2 diabetes mellitus and coronary heart diseases in postmenopausal women [70].

Chen et al. [71] showed that chronic administration of *Dioscorea* may enhance the bone strength. Further it shed light on the role of *Dioscorea* in bone remodeling and osteoporosis during the menopause [71]. Administration of *Dioscorea* to ovariectomized rats decreased the porosity effect on bones and increased the ultimate force of bones. The changes in biochemical and physiological functions seen in these animals were similar to those in menopausal women [71].

5.2 Antioxidant Activity

Antioxidants play a pivotal role in attenuating several chronic diseases such as different types of cancer, cardiovascular diseases, arthritis, diabetes, autoimmune and neurodegenerative disorders, and aging in which oxidative stress plays a major

role in the development of the diseases. Several studies provide evidences for the antioxidant activities of roots and tuber crops.

Potato is a rich source of a number of phytochemicals and exhibit antioxidant activities to varying degrees. Methanolic extract of potatoes demonstrated high antioxidant activities as determined by DPPH radical scavenging activity [72]. Further, the total phenolic content (TPC) ranged from 16.6 to 32 mg gallic acid equivalents (GAE)/100 g dry matter, and EC_{50} of DPPH radical scavenging activity of potatoes extract was 94 mg/mL [72]. The major phenolic compound reported in potato was chlorogenic acid which constituted more than 90% of its phenolics [73]. Extracts of flavonoids and flavones of potatoes showed high scavenging activities of reactive oxygen species. Potatoes scavenged 94% of hydroxyl radicals [74]. In addition, a range of hydrophilic oxygen radical absorbance capacity (ORAC) of 200–1400 μmol trolox equivalents/100 g fresh weight (FW) and the lipophilic ORAC range of 5–30 nmol alpha-tocopherol equivalents/100 g FW were reported for potatoes [75]. Further the TPC of water soluble and insoluble extracts of 15 Andean potato cultivars ranged from 2.4 to 28 μmol GAE/g (dm) and TFC varied between 0.9 and 4.8 μmol CE/g (dm) [76]. Antioxidant activities varied between cultivars. In addition, five phenolic compounds, namely, gallic acid, protocatechuic acid, cholorogenic acid, syringaldehyde, and epicatechin, were identified as major compounds.

Han et al. [77] showed that ethanolic extracts of purple-fleshed potato flakes had effective free radical scavenging activity and inhibition of linoleic acid oxidation in a rat model. Further, potato extracts enhanced hepatic manganese superoxide dismutase (SOD), Cu/Zn-SOD, and glutathione peroxidase (GSH-Px) activities as well as mRNA expression, suggesting a reduced hepatic lipid peroxidation and an improved antioxidant potential [77]. Potatoes contain water-soluble antioxidant compounds such as glutathione and ascorbic acids [52].

Ji et al. [78] reported the contents of phenolic compounds and glycoalkaloids of 20 potato clones and their antioxidant, cholesterol uptake, and neuroprotective activities in vitro. Peels of purple and red pigmented potato clones showed higher phenolic content than those of yellow and un-pigmented clones [78]. Chlorogenic acid (50–70%) and anthocyanins, namely, pelargonidin and petunidin, were identified as major phenolic compounds present in potatoes. Major glycoalkaloids reported to present in potatoes included α-chacoine and α-solanin and their contents were reduced by granulation process. Peels of potato clones showed the highest DPPH radical scavenging activity followed by flesh and granules [78]. Thus, potato peels can be utilized as a potential source of nutraceuticals. In addition, carotenoids such as lutein, zeaxanthine, and violoxanthine present in potatoes contribute to the antioxidant activity. The contents of carotenoids range from 50 to 100 μg in white-fleshed varieties to 2000 μg per 100 g FW in orange-fleshed cultivars [79].

The peels of sweet potato possessed a potent wound-healing effect which appears to be related to the free radical scavenging activity of the bioactive constituents and their ability in lipid oxidation inhibition [80–82]. According to Suzuki et al. [80] sweet potato fiber was effective in healing of burns or decubital wounds in a rat model. They further observed the reduction of wound area and changes of the quality

of the wounds after treating with sweet potato fiber compared to those of the control. Later, Chimkode et al. [81] also showed that petroleum ether extract of sweet potato had a significant effect on the closure of scar area for the complete epithelialization compared to the control.

The methanolic extracts of the peels and peel bandage of sweet potato tubers were screened for wound-healing effect by excision and incision wound models on Wistar rats [82]. It was found that hydroxyproline content was significantly increased in the test group than that of wounded control. This may improve collagen synthesis which affects the increased wound healing. Further, the content of malondialdehyde decreased in the test groups compared to that of wounded control, indicating lipid oxidation inhibitory effect of sweet potato peels [82].

The flavonoid contents of different selected tuber crops such as sweet potato, potato, coco yam, dasheen (*Colocasia esculenta*), St Vincent yam, yellow yam (*Dioscorea cayenensis*), and water yam (*Dioscorea alata*) varied from 71.5 to 390.7 CE/100 mg. Water yam (*Dioscorea alata*) reported the highest DPPH radical scavenging activity of 96% among others [83].

Phenolic content and antioxidant activities of yam vary with the cultivar. Cornago et al. [84] showed that purple yam (*Dioscorea alata)* and lesser yam (*Dioscorea esculenta)* had a TPC ranging from 69.9 to 421.8 mg GAE/100 g dry weight. The purple yam variety *Daking* showed the highest TPC and antioxidant activities as measured by DPPH radical scavenging activity, reducing power and ferrous ion chelating capacity whereas varieties *Sampero* and *Kimabajo* showed the least.

Water and ethanolic extracts of yam peel showed the antioxidant activity on tert-butylhydroperoxide (t-BHP)-induced oxidative stress in mouse liver cells, namely Hepa 1–6 and FL83B [85]. Ethanolic extracts (EE) of yam peel exhibited a better protective effect on t-BHP-treated cells than that of water extracts (WE). Further, EE had increased catalase activity whereas WE decreased it.

Chen and Lin [86] showed that heating affected the TPC, antioxidant capacity, and the stability of dioscorin of various yam tubers. Cooked yams showed lower TPC than their raw counterparts. Further, the DPPH radical scavenging activities declined with increasing temperature. TPC and dioscorin content of yam cultivars (*Dioscorea alata* L. var. Tainung No. 2) and Keelung yam (*D. japonica* Thunb. var. pseudojaponica (Hay.) Yamam) correlated with DPPH radical scavenging activity and ferrous ion chelating activity [86]. Phytochemicals of yams seem to enhance the activities of endogenous antioxidant enzymes. The administration of yams decreased the levels of γ-glutamino transpeptidase (GGT), low-density lipoprotein, and triacylglycerol in serum of rats in which hepatic fibrosis was induced by carbon tetrachloride [87]. Treatment of rats with yams increased the antioxidant activities of hepatic enzymes, namely, glutathione peroxidase and superoxide dismutase [87].

Limited studies have reported the antioxidant activities of cassava roots. The antioxidant activities of organically grown cassava tubers were higher than those of mineral-base fertilized roots. They further found that TPC and flavonoid content (FC) were significantly higher for organic cassava compared to that of cassava grown with inorganic fertilizers [88].

5.3 Antiulcerative Activities

The antiulcerative activity of sweet potato tubers was studied in a rat model [89]. The extract of sweet potatoes did not show any toxic or deleterious effects by oral route up to 2000 mg/kg. Further, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) activities were significantly elevated by administration of tuber extracts in treated rats, indicating the ability of restoring enzyme activities compared to the control. Kim et al. [16] showed that butanol fraction of sweet potato could be a better source for treating gastric ulcers induced by excessive alcohol intake.

5.4 Anticancer Activities

In the contemporary society cancer has been a leading cause of death mainly due to unhealthy food habits and lifestyle. Cancer is a multistage disease and tapping at any initial juncture could help to attenuate the disease condition. There are evidences to show that dietary components, specifically present in plant foods, are important to reduce and prevent the risk of cancers. A number of phytochemicals found in root and tubers have demonstrated anticancer effects in several types of carcinoma cell lines and animal models.

Extracts of sweet potatoes demonstrated antiproliferative activity in human lymphoma NB4 cells [90]. Further it has been shown that aqueous extract of sweet potatoes had higher antiproliferative activity than that of ethanol extracts. Cell proliferation was analyzed at 48 h after in human lymphoma NB4 cells which had been cultured with several concentrations of extracts 0, 25, 50, 100, 200, 400, 800, or 1000 μg dm/mL in the media using the microculture tetrazolium treatment assay (MTT).

Further it has been shown that the phytochemicals present in sweet potato roots may exert a significant effect on antioxidant as well as anticancer activities [90]. Antioxidant activity is directly related to the phenolics and flavonoid contents of the sweet potato extracts. There was an additive role of phytochemicals contributing significantly to the potent antioxidant activity and antiproliferative activity in vitro [90]. Two anthocyanin pigments, namely, 3-(6,6′-caffeylferulylsophoroside)-5-glucoside of cyanidin (YGM-3) and peonidin (YGM-6), purified from purple sweet potato inhibited the reverse mutation induced by mutagenic pyrolysates of tryptophan (Trp-P-1, Trp-P-2) and imidazoquinoline (IQ) in the presence of rat liver microsomal activation systems [91]. Red colored potatoes demonstrated higher inhibition of carcinogenesis compared to counterparts of white in breast cancer-induced rats [92]. In addition, the red cultivar of potatoes reported high levels of anthocyanins and chlorogenic acid derivatives which could be contributors for observed effects.

Further Madiwale et al. [93] demonstrated that purple-fleshed potato had higher potential in suppressing proliferation and elevated apoptosis of HT-29 human colon cancer cell lines compared to those of white-fleshed potato.

Storage of potatoes affected their antioxidant and anticancer activities and TPC. The extracts of fresh and stored potatoes inhibited cancer cell proliferation and elevated apoptosis. However, anticancer effects were higher in fresh potatoes than those of stored tubers. The investigators further demonstrated that storage duration of potatoes had a strong positive correlation with antioxidant activity and percentage of viable cancer cells but a negative correlation with apoptosis induction. These findings further elaborated that antioxidant activity and phenolic content of potatoes increased with storage, but antiproliferative and proapoptotic activities were decreased [93].

Apart from phenolics in roots and tubers, saponins play a pivotal role as anticancer/antitumor agents. A number of groups of saponins, namely, cycloartanes, ammaranes, oleananes, lupanes, and steroids, demonstrated antitumor effects on different types of cancers. Auyeung et al. [94] showed that cycloartanes possess antitumor properties in human colon cancer cells and tumor xenografts. They downregulated expression of the hepatocellular carcinoma (HCC) tumor marker α-fetoprotein and suppressed HepG2 cell growth by inducing apoptosis and modulating an extracellular signal-regulated protein kinase (ERK)-independent NF-κB signaling pathway [94]. Furthermore, oleananes exerted their antitumor effect through various pathways, such as anticancer, anti-metastasis, immunostimulation, and chemoprevention [94].

A number of studies reported that glycoalkaloids, namely, α-chaconine and α-solanine, present in some tubers are potential anticarcinogenic agents [95]. Glycoalkaloids showed antiproliferative activities against human colon (HT29) and liver (HepG2) cancer cells as assessed by the MTT assay [95].

The aqueous extract of *Dioscorea alata* inhibited the H_2O_2-$CuSO_4$-induced damage of calf thymus DNA, and protected human lymphoblastoid cells from $CuSO_4$-induced DNA damage [96]. Phenolic compounds saponins and polysaccharide mucilage present in yams are responsible for the observed bioactivities of yams extract. Water-soluble mucilage polysaccharides are the most important copper chelators in the extract of water yam. Thus *Dioscorea alata* aqueous extracts could serve as potential agents in the management of copper-mediated oxidative disorders [96].

5.5 Hypoglycemic Activities

Diabetes mellitus is among the NCDs marked by elevated levels of glucose in the blood leading to complications that can untimely cause death. Evidences are emerging that a number of phytochemicals in foods are effective agents in the prevention as well as management of type 2 diabetes. The extract of white-skinned sweet potatoes (WSSP) reduced hyperinsulinemia in Zucker fatty rats by 23, 26, 60, and 50%, after 3, 4, 6, and 8 weeks of oral administration, respectively, of WSSP similar to troglitazone (insulin sensitizer) [97]. Further lipid parameters such as blood triacylglycerol (TG) and free fatty acid (FFA) lactate levels were also lowered by the oral administration of WSSP. Further histological examinations of the pancreas

of Zucker fatty rats showed a remarkable regranulation of pancreatic islet of B-cells in the WSSP and troglitazone treated groups after 8 weeks. These evidences further reinforced that WSSP was likely to improve the abnormal glucose and lipid metabolism in insulin-resistant diabetes mellitus. In addition, supporting these observations extracts of sweet potato peels have shown reduced plasma glucose levels of diabetic patients [98]. Ingestion of 4 g of Caiapo, the extract of WSSP, per day for 6 weeks reduced fasting blood glucose and total as well as low density lipoprotein (LDL) cholesterol in male Caucasian type 2 diabetic patients who were previously treated by dietary management alone [98]. The improvement of insulin sensitivity, as determined by the frequently sampled intravenous glucose tolerance test (FSIGT), indicated that Caiapo extracts showed beneficial effects via reducing insulin resistance. Further Ludvik et al. [98] confirmed the beneficial effects of Caiapo on glucose and serum cholesterol levels in type 2 diabetic patients treated by diet alone for 3 months after recruitment for the study. Improved fasting blood glucose levels and glucose levels during an OGTT and in the postprandial state as well as improvement in long-term glucose control was also observed as expressed by the significant decrease in HbA1c [98].

The ethanolic extract of *Dioscorea alata* showed antidiabetic effects on alloxan-induced diabetic rats [99]. It was observed that diabetic rats who administered *yam* extract exhibited significantly lower creatinine levels which could be a result of improved renal function by reduced plasma glucose level and subsequent glycosylation of renal basement membranes. A number of bioactives, including phenolics, was identified in the ethanolic extract of *D. alata*. These include hydro-Q9 chromene, γ-tocopherol, α-tocopherol, -feruloyl glycerol, dioscorin, cyanidin-3-glucoside, catechin, procyanidin, cyanidin, peonidin 3-gentiobioside, and alatanins A, B, and C [99].

5.6 Hypocholesterolemic Activity

Diet plays an important role in the regulation of cholesterol homeostasis and CVDs are among the leading causes of death worldwide. External agents possessing anticholesteromic activities continuously show beneficial effects on risk reduction and management of CVDs.

Diosgenin, a steroidal saponin of yam (*Dioscorea*), demonstrated antioxidative and hypolipidemic effects in a rat model [100]. Rats fed with a high-cholesterol diet were supplemented with either 0.1 or 0.5% diosgenin for 6 weeks. The lipid profile of the plasma and liver, lipid peroxidation and antioxidant enzyme activities in the plasma, erythrocyte, and gene expression of antioxidative enzymes in the liver and the oxidative DNA damage in lymphocytes were measured. Diosgenin showed pancreatic lipase inhibitory activity, protective effect of liver under high cholesterol diet, reduced total cholesterol level, and protection against the oxidative damaging effects of polyunsaturated fatty acids [100].

Steroidal saponins of yams are used for industrial drug processing. Saponins, such as dioscin and gracilin, and prosapogenins of dioscin have long been identified

from yam. The content of the dioscin was about 2.7% (w/w). Diosgenin content was about 0.004 and 0.12–0.48% in cultivated yams and wild yams, respectively. The anti-hypercholesterolemic effect of yam saponin is related to its inhibitory activity against cholesterol absorption [101].

The hypocholesterolemic effect of yam diosgenin had been reported by an in vivo study. Rats fed with yam (*Dioscorea*) showed a decreased cholesterol absorption, increased hepatic cholesterol synthesis, and increased bilary cholesterol secretion without affecting serum cholesterol level [102]. In agreement with this finding, several studies showed that diosgenin, present in some *Dioscorea,* could enhance fecal bile acid secretion and decrease intestinal cholesterol absorption [103, 104]. The relative contribution of bilary secretion and intestinal absorption of cholesterol in diosgenin-stimulated fecal cholesterol excretion were studied in wild-type (WT) and Niemann-Pick C1 Like 1 (NPC1L1) knockout (LIKO) mice. NPCiL1 was recently identified as an essential protein for intestinal cholesterol absorption [105]. Diosgenin significantly increased bilary cholesterol and hepatic expression of cholesterol synthetic genes in both WT and LIKO mice. In addition, diosgenin stimulation of fecal cholesterol excretion was primarily attributable to its impact on hepatic cholesterol metabolism rather than NPC1L1-dependent intestinal cholesterol absorption [105].

Native protein of dioscorin purified from *D. alata* (cv. Tainung No. 1) (TN1-dioscorin) and its peptic hydrolysates showed ACE inhibitory activities in a dose-dependent manner [41]. The kinetic analysis of dioscorin showed a mixed noncompetitive inhibition against ACE. Their results suggested that dioscorin isolated from *Dioscorea* might be beneficial in controlling high blood pressure [105, 106].

Chen et al. [35] reported the effects of Taiwanese yam (*Dioscorea alata* Cv Tainung No.2) on mucosal hydrolase activities and lipid metabolism in male Balb/c mice. High level of Tainung No.2 yam in the diet (50% w/w) reduced plasma and hepatic cholesterol levels and increased fecal steroid excretions in the mice model. This could be due to the loss of bile acid in the enterohepatic cycle to fecal excretion [35]. They further suggested that the increased viscosity of the digest and the thickness of the unstirred layer in the small intestine caused by Tainung No.2 yam fiber (and/or mucilage) decreased the absorption of fat, cholesterol, and bile acid. Short-term (3 week) consumption of 25% Tainung No.2 yam in the diet could reduce the anthrogenic index, but not total cholesterol level in nonhypercholesteromic mice. However, additional dietary yam (50% yam diet) could consistently exert hypocholesterolemic effects in these mice [35]. However, diosgenin was not elucidated in Tainung No.2 used in this study [35]. Thus, authors suggested that diosgenin might not be involved in the cholesterol lowering effect of Tainung No.2 yam. Dietary fiber and viscous mucilage could be active components for the beneficial cholesterol lowering effects of yam. Further, short-term consumption (3 week) of 25% uncooked keelung yam effectively reduced total blood cholesterol levels and the atherogenic index in mice. Authors indicated that the active components for the lipid lowering effects may be attributed to dietary fiber, mucilage, plant sterols, or synergism of these active components [35].

5.7 Antiobesity

Obesity resulting from the chronic disruption of energy balance in the body has become an epidemic globally. Hypertrophy and hyperplasia of fat cells is clearly explained for their unique contribution in pathology of obesity. There are a number of behavioral, metabolic, and biological influences leading to obesity [107]. In addressing the management of obesity phytochemicals may be potential agents targeting increasing energy expenditure, fat oxidation or inhibiting adipose tissue proliferation.

Differentiation and proliferation inhibitory activities of sporamin of sweet potato tubers were reported in 3 T3-L1 preadipocytes [41]. It should be noteworthy that sporamin did not exhibit any cytotoxic activity toward the model cell line 3 T3-L1 preadipocytes which have frequently been used to study differentiation of adipocytes in vitro. Concentration range of 0.025–1 mg/ml sporamin showed antidifferentiation and antiproliferation effects on 3 T3-L1 cells similarly to 0.02 mg/ml berberine. Berberine is a traditional Chinese medicine used as an antimicrobial and antitumor agent [41]. Hwang et al. [108] demonstrated that purple sweet potato is a potential food, which can be used for the prevention of obesity. Anthocyanin fractions of purple sweet potato inhibited hepatic lipid accumulation through the induction of adenosine monophosphate activated protein kinase (AMPK) signaling pathways. AMPK plays an important role in the regulation of lipid synthesis in metabolic tissues [108]. Anthocyanin dose of 200 mg/kg of body weight per day reduced weight gain, hepatic triacylglycerol accumulation and improved serum lipid parameters in mice fed for 4 weeks with purple sweet potatoes. Furthermore, anthocyanin administration increased the phosphorylation of AMPK and acetyl coenzyme A carboxylase (ACC) in the liver and HepG2 hepatocytes. These authors further suggested that anthocyanin found in purple sweet potatoes may improve high fat diet induced fatty liver disease and regulate hepatic lipid metabolism [108].

5.8 Immunomodulatory Activities

Phytochemicals are reported to possess a number of bioactivities in management and risk reduction of NCDs and immunomodulatory and anti-inflammatory properties are reported among others. The mechanisms of action include effects on enzyme function involved in the generation of inflammatory mediators such as nitric oxide (NO), prostanoids and leukotrienes, regulation of gene, and protein expression [109].

The effects of purified dioscorin from yam tubers on native BALB/c mice spleen cell proliferation were assayed by MTT assay [26]. Dioscorin stimulated mouse macrophage-like cells RAW264.7 to produce nitric oxide (NO), in the absence of lipopolysaccharide (LPS) contaminations. Yam dioscorin exhibited immunomodulatory activities by the innate immunity which is a nonspecific immune system which comprises the cells and mechanisms that defend the host from infection by other

organisms in a nonspecific manner. Dioscorin was reported to stimulate cytokine production and to enhance phagocytosis. Furthermore, the released cytokines may act synergistically with phytohemagglutinin (PHA) which is a lectin found in plants that stimulate the proliferation of splenocytes [26].

Yam mucopolysaccharides (YMP) demonstrated the immune activity in several cell lines. In vitro cytotoxic activity of murine splenocyte against leukemia cells was increased in the presence of YMP of *Dioscorea batatas* at 10 μg/ml [110]. Furthermore, the production of interferon-γ (IFN-γ) was significantly increased in the YMP-treated splenocytes, suggesting their capability of inducing cell-mediated immune responses. In addition, YMP at a concentration of 50 μg/ml increased uptake capacity and lysosomal phosphatase activity of peritoneal macrophages [110].

Bioactive compounds from *Dioscorea* enhanced murine splenocyte proliferation ex vivo and improved regeneration of bone marrow cells in vivo [111]. Mice which were fed with a *Dioscorea* extract recovered damaged bone marrow progenitor cells that had been depleted by large doses of 5-flourouracil (5-FU). In addition, they found that the compound(s) responsible for these bioactivities had a high molecular weight ($\geq$100 kDa) and were most likely polysaccharides. The high molecular weight polysaccharides in *Dioscorea* crude extract–II (DsCE-II) may act on specific target cell types such as dendritic cells, intestinal epithelial cells, and T-cells in the GI tract to mediate a cascade of immune regulatory activities. This may lead to the recovery of damaged cell populations exposed to 5-FU or other chemical insults in the bone marrow, spleen, or other immune cell systems [111].

Oral administration of *Dioscorea* tuber mucilage from Taiwanese yams (*Dioscorea Japonica* Thunb var) showed significant effects on the innate immunity and adaptive immunity on BALB/c mice [112]. It was interesting to note that the specific antibodies rapidly responded against foreign proteins (or antigens) in the presence of yam mucilage. Mucilage from these yam varieties exhibited a stimulatory effect on phagocytic activity by granulocyte and monocyte (ex vivo), on peritoneal macrophages, and on the RAW 264.7 cells (in vivo) of mice [112].

Yams (*Dioscorea esculenta*) demonstrated anti-inflammatory activities on carrageenan-induced edema in the right hind paw of Wistar rats [113]. However, it was noted that the activity was short lived as it was quickly removed from the system after reaching the peak within 2 hours. Phytochemical screening of *D. esculenta* confirmed the presence of saponins, β-sitosterol, stigmasterol, cardiac glycosides, fats, starch, and diosgenin, which could be responsible for the observed activity [113]. Diosgenin contained in Chinese yam was an immunoactive steroidal saponin which also showed prebiotic effects. Diosgenin had also beneficial effects on the growth of enteric lactic acid bacteria [61].

In a human trial the impact of the consumption of pigmented potatoes on oxidative stress and inflammatory damages has been demonstrated [114]. In this study participants were administrated white-, yellow- (high concentrations in phenolic acids and carotenoids), or purple-fleshed (high content of anthocyanin and phenolic acids) potato once per day in a randomized 6-week trial which reported good compliance. The results showed that the consumption of pigmented potatoes

was responsible for elevated antioxidant status and reduced inflammation and DNA damage, which was observed through the reduction of inflammatory cytokines and C-reactive protein concentrations [114].

Methanolic extracts of *Amorphophallus campanulatus* (Suran) tuber also showed immunomodulatory activities in Wistar albino mouse model [115]. The mice were immunized with sheep red blood cells to assess delayed type hypersensitivity and charcoal clearance test. Active phagocytosis is the major defense mechanism against infection. They indicated that the presence of steroids and flavonoids in *Amorphophallus campanulatus* (Suran) tuber may be responsible for the observed immunomodulatory activity [115].

5.9 Antimicrobial Activity

Yam varieties with their phenolic compounds are potential agents with antimicrobial efficacy. Sonibare and Abegunde [116] reported that the methanolic extracts of *Dioscorea* yams (*Dioscorea dumetorum* and *Dioscorea hirtiflora*) showed antioxidant and antimicrobial activities. Antimicrobial activity was determined by the agar diffusion method (for bacteria) and pour plate method (for fungi). Nonedible *D. dumetorum* showed the highest in vitro antibacterial activity against *Proteus mirabilis*. The methanolic extracts from *D. hirtiflora* demonstrated antimicrobial activity against all tested organisms, namely *Staphylococcus aureus*, *E-Coli*, *Bacillus subtilis, Proteus mirabilis, Salmonella typhi, Candida albicans, Aspergillus niger,* and *Penicillium chrysogenum*. Saponin present in yams (*Dioscorea)* also has tendency to ward off microbes [117]. These compounds serve as natural antibiotics, helping the body to fight against infections and microbial invasion [117].

6 Conclusions

Foregoing evidences support the fact that roots and tubers are important components in the everyday diet for humans adding variety in the plate and a number of health implications to attenuate NCDs. They perform a pivotal role as an energy contributor while providing many desirable nutritional and health benefits, namely, antioxidative, hypoglycemic, hypocholesterolemic, antimicrobial, and immunomodulatory activities among others. There are a wide array of roots and tubers which can be utilized in preparation of numerous commercial foods though usage may vary with the country and region. Thus, tubers may serve as functional foods and nutraceutical ingredients to attenuate NCDs and to maintain wellness.

Acknowledgment This research was supported by the Research Grant Scheme of Wayamba University of Sri Lanka through a grant (SRHDC/RP/04/13-09) to AC. The author wishes to thank members of the research team Apeksha Herath, Jayani Wijerathne, Upuli Dahanayake, Thamilini Joshepkumar, and Saman Ranasinghe at Wayamba University of Sri Lanka.

References

1. FAOSTAT (2014) Agricultural data. Rome, Italy: Food and Agriculture Organization of the United Nations. Available from http://www.faostat.fao.org. Accessed on 12 Feb 2017
2. USDA NAL (2015) https://fnic.nal.usda.gov/food-composition. Accessed on 12 Feb 2016
3. FAO (1990) Roots, tubers, plantains and bananas in human nutrition. Food and nutrition series, no. 24. Food and Agriculture Organization, Rome
4. Jacobs DR, Meyer KA, Kushi LH, Folsom AR (1998) Whole grain intake may reduce risk of coronary heart disease death in postmenopausal women: The Iowa Women's Health Study. Am J Clin Nutr 68:248–257
5. Liu S, Stampfer MJ, Hu FB, Giovanucci E, Rimm E, Manson JE, Hennekens CH, Willett WC (1999) Whole grain consumption and risk of coronary heart disease: results from the Nurses' Health study. Am J Clin Nutr 70:412–419
6. Liu S, Manson JE, Stampfer MJ, Hu FB, Giovanucci E, Colditz GA, Hennekens CH, Willett WC (2000) A prospective study of whole grain intake and risk of type 2 diabetes mellitus in US women. Am J Public Health 90:1409–1415
7. Meyer KA, Kushi LH, Jacobs DR, Slavin J, Sellers TA, Folsom AR (2000) Carbohydrates, dietary fiber, incident type 2 diabetes mellitus in older women. Am J Clin Nutr 71:921–930
8. Naczk M, Shahidi F (2006) Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. J Pharm Biomed Anal 41:1523–1542
9. FAO (1999) Production year book, vol 53. Food and Agriculture Organization, Rome
10. Burlingame B, Mouille B, Charrondiere R (2009) Nutrients, bioactive nonnutrients and anti-nutrients in potatoes. J Food Compos Anal 22:494–502
11. Ezekiel R, Singh B (2007) Changes in contents of sugars, free amino acids and phenols in four varieties of potato tubers stored at five temperatures for 180 days. J Food Sci Technol 44:471–477
12. Scott GJ (1992) Transforming traditional food crops: product development for roots and tubers. Asia Int Potato Center 1:3–20
13. Benjamin A (2007) Sweet potato: a review of its past, present and future role in human nutrition. Adv Food Nutr Res 52:1–59
14. Odebunmi EO, Oluwaniyi OO, Sanda AM, Kolade BO (2007) Nutritional compositions of selected tubers and root crops used in Nigerian food preparations. Int J Chem 17:37–43
15. Bengtsson A, Namutebib A, Almingera ML, Svanberga U (2008) Effects of various traditional processing methods on the all-trans-b-carotene content of orange-fleshed sweet potato. J Food Compos Anal 21:134–143
16. Kim JJ, Kim CW, Park DS, Shih SH, Jeon JH, Jang MJ, Ji HJ, Song JG, Lee JS, Kim BY, Choi EK, Joo SS, Hwang SY, Kim YB (2008) Effects of sweet potato fractions on alcoholic hangover and gastric ulcer. Lab Anim Res 24:209–216
17. van Jaarsveld P, Marais D, Harmse E, Nestel P, Amaya D (2006) Retention of beta carotene in boiled, mashed orange fleshed sweet potato. J Food Compos Anal 19:321–329
18. Tokusoglu O, Yildirim Z, Durucasu I (2005) Nutraceutical phenolics (total polyphenols, chlorogenic [5-*O*-Caffeoylquinic] acid) in tubers, leaves, stalks and stems of new developed sweetpotato (Ipomea Batatas L.): alterations in tubers during short-term storage. J Food Technol 3:444–448
19. Yildirim Z, Tokusoglu O, Ozturk G (2011) Determination of sweet potato [Ipomoea batatas (L.) Lam] genotypes suitable to the Aegean region of Turkey. Turk J Field Crops 16:48–52
20. Kosambo LM, Carey EE, Misra AK, Wilkes J, Hagenimana V (1998) Influence of age, farming site, and boiling on pro-vitamin A content in sweet potato (Ipomoea batatas (L.) Lam.) storage roots. J Food Compos Anal 11:305–321
21. Nassar NMA, Hashimoto DYC, Fernandes SDC (2008) Wild Manihot species: botanical aspects, geographic distribution and economic value. Genet Mol Res 7:16–28
22. Blagbrough IS, Bayoumi SAL, Rowan MG, Beeching JR (2010) Cassava: an appraisal of its phytochemistry and its biotechnological prospects- review. Phytochemistry 71:1940–1951

23. Lebot V, Champagne A, Malapa R, Shiley D (2009) NIR determination of major constituents in tropical root and tuber crop flours. J Agric Food Chem 57:10539–10547
24. Prawat H, Mahidol C, Ruchirawat S, Prawat U, Tuntiwachwuttikul P, Tooptakong U, Taylor WT, Pakwatchal C, Skeleton BW, White AH (1995) Cyanogenic and non cyanogenic glycosides from *Manihot esculenta*. Phytochemistry 40:1167–1173
25. Okwu DE, Ndu CU (2006) Evaluation of the phytonutrients, mineral and vitamin contents of some varieties of yam (*Dioscorea* sp). Int J Mol Med Adv Sci 2:199–203
26. Liu YW, Shang HF, Wang CK, Hsu FL, Hou WC (2007) Immunomodulatory activity of dioscorin, the storage protein of yam (*Dioscorea alata* cv. Tainung No 1) tuber. Food Chem Toxicol 45:2312–2318
27. Behera KK, Sahoo S, Prusti A (2010) Biochemical quantification of diosgenin and ascorbic acid from the tubers of different *Dioscorea* species found in Odisha. Libyan Agric Res Cent J Int 1:123–127
28. Chen YT, Kao WT, Lin KW (2008) Effects of pH on the total phenolic compound, anti-oxidative ability and the stability of dioscorin of various yam cultivars. Food Chem 107:250–257
29. Senanayake SA, Ranaweera KKDS, Gunaratne A, Bamunuarachchi A (2013) Comparative analysis of nutritional quality of five different cultivars of sweet potatoes (*Ipomea batatas* (L) Lam) in Sri Lanka. Food Sci Nutr 1(4):284–291
30. Hermann M, Heller J (1997) Andean roots and tubers: ahipa, arracacha, maca and yacon. Promoting the conservation and use of underutilized and neglected crops, vol 21. Co published by the Institute of Plant Genetics and Crop Plant Research, Gatersleben, and the International Plant Genetic Resources Institute (IPGRI), Rome
31. Iwu MM, Okunji CO, Ohiaeri GO, Akah P, Corley D, Tempesta MS (1999) Hypoglycemic activity of dioscoretine from tubers of *Dioscorea dumetorum* in normal and alloxan diabetic rabbits. Planta Med 56:264–267
32. Bhandari MR, Kasai T, Kawabata J (2003) Nutritional evaluation of wild edible yam (*Dioscorea* spp) tubers of Nepal. Food Chem 82:619–623
33. Kelmanson JE, Jager AK, van Staden J (2000) Zulu medicinal plants with antibacterial activity. J Ethnopharmacol 69:241–246
34. Chan YC, Hsu CK, Wang MF, Su TY (2004) A diet containing yam reduces the cognitive deterioration and brain lipid peroxidation in mice with senescence accelerated. Int J Food Sci Technol 39:99–107
35. Chen HL, Wang CH, Chang CT, Wang TC (2003) Effects of Taiwanese Yam (*Dioscorea japonica* Thunb var. pseudo japonica Yamamoto) on upper gut function and lipid metabolism in Balb/c mice. Nutrition 19:646–651
36. Shewry PR (2003) Tuber storage proteins. Ann Bot 91:755–769
37. Yeoh HH, Chew MY (1977) Protein content and acid composition of cassava seed and tuber. Malays Agric J 51:1–6
38. Hou WC, Chen HJ, Lin YH (1999) Dioscorins, the major tuber storage proteins of yam (*Dioscorea batatas* Decne), with dehydroascorbatereductase and mono-dehydroascorbatereductase activities. Plant Sci 149:151–156
39. Hou WC, Chen HJ, Lin YH (2000) Dioscorins from different *Dioscorea* species all exhibit both carbonic anhydrase and trypsin inhibitor activities. Bot Bull Acad Sin 41:191–196
40. Hou WC, Lee MH, Chen HJ, Liang WL, Han CH, Liu YW, Lin YH (2001) Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas* Decne) tuber. J Agric Food Chem 49:4956–4960
41. Hsu FL, Lin YH, Lee MH, Lin CL, Hou WC (2002) Both dioscorin, the tuber storage protein of yam (*Dioscorea alata* cv. Tainong No 1) and its peptic hydrosylates exhibited angiotensin converting enzyme inhibitory activities. J Agric Food Chem 50:6109–6113
42. Lin JY, Lu S, Liou YL, Liou HL (2006) Antioxidant and hypolipidaemic effects of a novel yam-boxthorn noodle in an in vivo murine model. Food Chem 94:377–384

43. Zhi-Dong X, Peng-Gao L, Tai-Hua M (2009) The differentiation- and proliferation-inhibitory effects of sporamin from sweet potato in 3T3-L1 preadipocytes. Agric Sci China 8:671–677
44. Senthilkumar R, Yeh KW (2012) Multiple biological functions of sporamin relate to stress tolerance in sweet potato (Ipomoea batatas Lam). Biotechnol Adv 30:1309–1317
45. Yeh K, Chen J, Lin M, Chen Y, Lin C (1997) Functional activity of sporamin from sweet potato (*Ipomoea batatas* Lam): a tuber storage protein with trypsin inhibitory activity. Plant Mol Biol 33:565–570
46. Hou WC, Lin YH (1997) Dehydroascorbate reductase and monodehydroascorbate reductase activities of trypsin inhibitors, the major sweet potato (*Ipomoea batatas* [L] Lam) root storage protein. Plant Sci 128:151–158
47. Paiva E, Lister RM, Park WD (1983) Induction and accumulation of major tuber proteins of potato stems and petioles. Plant Physiol 71:161–168
48. Pots AM, Gruppen H, Hessing M, van Boekel MA, Voragen AG (1999) Isolation and characterization of patatin isoforms. J Agric Food Chem 47:4587–4592
49. Cogdell RJ, Frank HA (1987) The function of carotenoids in photosynthesis. Biochim Biophys Acta 815:63–79
50. Paiva SA, Russell RM (1999) Beta-carotene content and other carotenoids as antioxidants. J Am Coll Nutr 18:426–433
51. Blanquet-Diot S, Soufi M, Rambeau M, Rock E, Alric M (2009) Digestive stability of xanthophylls exceeds that of carotenes as studied in a dynamic in vitro gastrointestinal system. J Nutr 139:876–883
52. Ezekiel R, Singh N, Sharma S, Kaur A (2013) Beneficial phytochemicals in potato- a review. Food Res Int 50:487–496
53. Burgos G, Muñoa L, Sosa P, Bonierbale M, Felde TZ, Díaz C (2013) In vitro bioaccessibility of lutein and zeaxanthin of yellow fleshed boiled potatoes. Plant Foods Hum Nutr 68:385–390
54. Eka OU (1998) Root and tubers. In: Osagie AU, Eka OU (eds) Nutritional quality of plant foods. Post Harvest Research Unit University of Benin Nigeria, Benin, pp 1–31
55. Schieber A, Saldaña MDA (2009) Potato peels: a source of nutritionally and pharmacologically interesting compounds – a review. FoodReview 3:23–29
56. Shahidi F (2002) Phytochemicals in oilseeds. In: Phytochemicals in nutrition and health. CRC Press, Boca Raton, pp 139–156
57. Shahidi F, Naczk M (2004) Phenolics in food and nutraceuticals. CRC press, Boca Raton, pp 1–82
58. Francis G, Kerem Z, Makkar HPS et al (2002) The biological action of saponins in animal systems: a review. Br J Nutr 88:587–605
59. Vincken JP, Heng L, de Groot A et al (2007) Saponins, classification and occurrence in the plant kingdom. Phytochemistry 68:275–297
60. Sparg SG, Light ME, van Staden J (2004) Biological activities and distribution of plant saponins. J Ethnopharmacol 94:219–243
61. Huang CH, Cheng JY, Deng MC, Chou CH, Jan TR (2012) Prebiotic effect of diosgenin, an immunoactive steroidal sapogenin of the Chinese yam. Food Chem 132:428–432
62. Kaneko K, Tanaka MW, Mitsuhashi H (1977) Dormantinol, a possible precursor in solanidine biosynthesis, from budding *Veratrum grandiflorum*. Phytochemistry 16:1247–1251
63. Milner SE, Brunton NP, Jones PW, O'Brien NM, Collins SG, Maguire AR (2011) Bioactivities of glycoalkaloids and their aglycones from *Solanum* species. J Agric Food Chem 59:3454–3484
64. Peksa A, Golubowska G, Rytel E, Lisinska G, Aniolowski K (2002) Influence of harvest date on glycoalkaloid contents of three potato varieties. Food Chem 78:313–317
65. Craik DJ, Daly LN, Plan RM, Salim AA, Sando L (2002) Structure and function of plant toxins (with emphasis on cystine knot toxins). J Toxicol Toxin Rev 21:229–271
66. Mweetwa AM, Hunter D, Poe R, Harich KC, Ginzberg I, Veilleux RE, Tokuhisa JG (2012) Steroidal glycoalkaloids in *Solanum chacoense*. Phytochemistry 75:32–40
67. Kuo KW, Hsu SH, Li YP, Lin WL, Liu LF, Chang LC, Lin CC, Lin CN, Sheu HM (2000) Anticancer activity evaluation of the solanum glycoalkaloid solamargine. Triggering apoptosis in human hepatoma cells. Biochem Pharmacol 60:1865–1873

68. Liu LF, Liang CH, Shiu LY, Lin WL, Lin CC, Kuo KW (2004) Action of solamargine on human lung cancer cells-enhancement of the susceptibility of cancer cells to TNFs. FEBS Lett 577:67–74
69. Ikeda T, Tsumagari H, Honbu T, Nohara T (2003) Cytotoxic activity of steroidal glycosides from Solanum plants. Biol Pharm Bull 26:1198–1201
70. Wu WH, Liu LY, Chung CJ, Joe HJ, Wang TA (2005) Estrogenic effect of yam ingestion in healthy postmenopausal women. J Am Coll Nutr 24:235–243
71. Chen JH, JSS W, Lin HC, Wu SL, Wang WF, Huang SK, Ho YJ (2008) *Dioscorea* improves the morphometric and mechanical properties of bone in ovariectomised rats. J Sci Food Agric 88:2700–2706
72. Hesam F, Balali GR, Tehrani RT (2012) Evaluation of antioxidant activity of three common potato (*Solanum tuberosum)* cultivars in Iran Avicenna. J Phytomed 2:79–85
73. Malmberg AG, Theander O (1985) Determination of chlorogenic acid in potato tubers. J Agric Food Chem 33:549–551
74. Chu YH, Chang CL (2000) Flavonoid content of several vegetables and their antioxidant activity. J Sci Food Agric 80:561–556
75. Brown CR (2008) Breeding for phytonutrient enhancement of potato. Am J Pot Res 85: 298–307
76. Penarrieta JM, Salluca T, Tejeda L, Alvarado JA, Bergensta B (2011) Changes in phenolic antioxidants during chuno production (traditional Andean freeze and sun-dried potato). J Food Compos Anal 24:580–587
77. Han K, Shimado K, Sekikawa M, Fukushima M (2007) Anthocyanin rich potato flakes affect serum lipid peroxidation and hepatic SOD mRNA level in rats. Biosci Biotechnol Biochem 71:1356–1359
78. Ji X, Rivers L, Zielinski Z, Xu M, Macdougall E, Stephen J, Zhang S, Wang Y, Chapman R, Keddy P, Robertson G, Kirby C, Embleton J, Worall K, Murphy A, Koeyer D, Tai H, Yu L, Charter E, Zhang J (2012) Quantitative analysis of phenolic components and glycoalkaloids from 20 potato clones and in vitro evaluation of antioxidant, cholesterol uptake and neuroprotectiveactivities. Food Chem 133:1177–1187
79. Brown CR (2005) Antioxidants in potato. Am J Potato Res 82:163–172
80. Suzuki T, Tada H, Sato E, Sagae Y (1996) Application of sweet potato fibre to skin wound in rat. Biol Pharmacol Bull 19:977–983
81. Chimkode R, Patil MB, Jalalpure SS (2009) Wound healing activity of tuberous root extracts of Ipomoea batatas. Adv Pharmacol Toxicol 10:69–72
82. Panda V, Sonkamble M (2011) Anti-ulcer activity of *Ipomoea batatas* tubers (sweet potato). Funct Foods Health Dis 2:48–61
83. Dilworth L, Brown K, Wright R, Oliver M, Hall S, Asemota H (2012) Antioxidants, minerals and bioactive compounds in tropical staples. Afr J Food Sci Technol 3:90–98
84. Cornago DF, Rumbaoa RCO, Geronimo IM (2011) Philippine yam (*Dioscorea* spp) tubers phenolic content and antioxidant capacity. Philipp J Sci 140:145–152
85. Hsu CK, Yeh JY, Wei JH (2011) Protective effects of the ceude extracts from (*Dioscorea alata*) peel on tert-butylhydroperoxide-induced oxidative stress in mouse liver cells. Food Chem 126:429–434
86. Chen YT, Lin KW (2007) Effects of heating temperature on the total phenolic compound, antioxidative ability and the stability of dioscorin of various yam cultivars. Food Chem 101:955–963
87. Chan YC, Chang SC, Liu SY, Yang HL, Hseu YC, Liao JW (2010) Beneficial effects of yam on carbon-tetrachloride- induced hepatic fibrosis in rats. J Sci Food Agric 90:161–167
88. Omar NF, Hassan SA, Yusoff UK, Abdullah NAP, Wahab PEM, Sinnaiah UR (2012) Phenolics, flavonoids, antioxidant activity and cyanogenic glycosides of organic and mineral-base fertilized cassava tubers. Molecules 17:2378–2387
89. Panda V, Sonkamble M (2012) Phytochemical constituents and pharmacological activities of Ipomoea batatas L.(lam)- A review. Int J Res Phytochem Pharmacol 2:25–34

90. Huang D, Lin C, Chen H, Lin Y (2004) Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas* (L.) Lam 'Tainong 57') constituents. Bot Bull Acad Sin 45:179–186
91. Yoshimoto M, Okuno S, Yoshinaga M, Yamakawa O, Yamaguchi M, Yamada J (1999) Antimutagenicity of sweet potato (*Ipomoea batatas*) roots. Biosci Biotechnol Biochem 63:537–541
92. Thompson MD, Thompson HJ, McGinley JN, Neil ES, Rush DK, Holm DG (2009) Functional food characteristics of potato cultivars (*Solanum tuberosum* L.): photochemical composition and inhibition of 1-methyl-1-nitrosourea induced breast cancer in rats. J Food Compos Anal 22:571–576
93. Madiwale PG, Reddivari L, Holm GD, Vanamala J (2011) Storage elevates phenolic content and antioxidant activity but suppresses antiproliferative and pro-apoptopic properties of colored flesh potatoes against human colon cancer cell lines. J Agric Food Chem 59:8155–8166
94. Auyeung KK, Law P, Ko JK (2009) *Astragalus* saponins induce apoptosis via an ERK-independent NF-κB signaling pathway in the human hepatocellular HepG2 cell line. Int J Mol Med 23:189–196
95. Lee K-R, Kozukue N, Han J-S, Park J-H, Chang E-Y, Baek E-J, Chang J-S, Friedman M (2004) Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. J Agric Food Chem 52:2832–2839
96. Wang TS, Lii CK, Huang YC, Chang JY, Yang FY (2011) Anticlastogenic effect of aquaeous extract from water yam (*Dioscorea alata* L.) J Med Plants Res 5:6192–6202
97. Kusano S, Abe H (2000) Antidiabetic activity of white skinned sweet potato (*Ipomoea batatas* L.) in obese Zucker fatty rats. Biol Pharm Bull 23:23–26
98. Ludvik B, Mahdjoobian K, Waldhaeusl W, Hofer A, Parger R, Willer A, Pacini G (2002) The effect of *Ipomoea batatas* (Caiapo) on glucose metabolism and serum cholesterol in patients with type 2 diabetes: observations. Diabetes Care 25:239–240
99. Maithili V, Dhanabal SP, Mahendran S, Vadivelan R (2011) Antidiabetic activity of ethanolic extract of tubers of *Dioscorea alata* in alloxan induced diabetic rats. Indian J Pharmacol 43:455–459
100. Son IS, Kim JH, Sohn HY, Son KH, Kim JS, Kwon CS (2007) Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (*Dioscorea* spp), on high-cholesterol fed rats. Biosci Biotechnol Biochem 71:306–3071
101. Ma HY, Zhao ZT, Wang LJ, Wang Y, Zhou QL, Wang BX (2002) Comparative study on anti-hypercholesterolemia activity of diosgenin and total saponin of *Dioscorea panthaica*. China J Chin Mater Med 27:528–531
102. Cayen MN, Dvornik D (1979) Effects of diosgenin on lipid metabolism in rats. J Lipid Res 20:162–174
103. Thewles A, Parslow RA, Coleman R (1993) Effect of diosgenin on bilary cholesterol transport in the rat. Biochem J 291:793–798
104. Uchida K, Takse H, Nomura Y, Takeda K, Takeuchi N, Ishikawa Y (1984) Effects of diosgenin and B-sisterol on bile acids. J Lipid Res 25:236–245
105. Temel RE, Brown JM, Ma Y, Tang W, Rudel LL, Ioannou YA, Davies JP, Yu L (2009) Diosgenin stimulation of fecal cholesterol excretion in mice is not NPC1L1 dependant. J Lipid Res 50:915–923
106. Lu YL, Chia CY, Liu YW, Hou W (2012) Biological activities and applications of dioscorins, the major tuber storage proteins of yam. J Tradit Complement Med 2:41–46
107. Salbe AD, Ravussin E (2000) The determinants of obesity. In: Bouchard C (ed) Physical activity and obesity. Human Kinetics Publishers, Champaign, pp 67–102
108. Hwang Y, Choi J, Han E, Kim H, Wee J, Jung K, Jung K, Kwon K, Jeong T, Chung Y, Jeong H (2011) Purple sweet potato anthocyanins attenuate hepatic lipid accumulation through activating adenosine monophosphate- activated protein kinase in human Hep G2 cells and obese mice. Nutr Res 31:896–906

109. Gonzalez-Gallego J, Garcıa-Mediavilla MV, Sanche S, Tunon MJ (2010) Fruit polyphenols, immunity and inflammation. Br J Nutr 104:S15–S27
110. Choi EM, Koo SJ, Hwang J-K (2004) Immune cell stimulating activity of mucopolysaccharide isolated from yam (*Dioscorea batatas*). J Ethnopharmacol 91:1–6
111. Su PF, Li CJ, Hsu CC, Benson S, Wang SY, Aravindaram K, Chan SI, Wu SH, Yang FL, Huang WC, Shyur LF, Yang NS (2011) *Dioscorea* phytocompounds enhance murine splenocyte proliferation ex vivo and improve regeneration of bone marrow cells in vivo. Evid Based Complement Alternat Med 2011:731308. PMCID:PMC3137395
112. Shang H-F, Cheng H-C, Liang H-J, Liu H-Y, Liu S-Y, Hou W-C (2007) Immunostimulatory activities of yam tuber mucilages. Bot Stud 48:63–70
113. Olayemi JO, Ajaiyeoba EO (2007) Anti-inflammatory studies of yam (*Dioscorea esculenta*) extract on Wistar rats. Afr J Biotechnol 6:1913–1915
114. Kaspar KL, Park JS, Brown CR, Mathison B, Naverre DA, Chew BP (2011) Pigmented potato consumption alters oxidative stress and inflammatory damage in men. J Nutr 141:108–111
115. Tripathi AS, Chitra V, Sheikh NW, Mohale DS, Dewani AP (2010) Immunomodulatory activity of the methanol extract of *Amorphophallus campanulatus* (Araceae) tuber. Trop J Pharm Res 9:451–454
116. Sonibare MA, Abegunde RB (2012) *In vitro* antimicrobial and antioxidant analysis of *Dioscorea dumetorum* (Kunth) Pax and *Dioscorea hirtiflora* (Linn.) and their bioactive metabolites from Nigeria. J Appl Biosci 51:3583–3590
117. Sodipo OA, Akiniyi JA, Ogunbamosu JU (2000) Studies on certain characteristics of extracts of bark of pansinystalia macruceras (K schemp) pierre Exbeille. Global J Pure Appl Sci 6:83–87
118. Souci SW, Fachmann W, Krut H (1994) Food composition and nutrition tables, 5th edn. Medpharm Stuttgart Medpharm GmbH, Scientific Publishers/CRC Press, Stuttgart/Boca Raton
119. FAO (1972) Food composition tables for use in East Asia. FAO, Rome

Betalains: Application in Functional Foods 49

Wee Sim Choo

Contents

Abstract

Betalains are plant derived natural pigments that are presently gaining popularity for use as natural colorants in the food industry. Although betalains from red beet are one of the most widely used food colorant, betalains are not as well studied as compared to other natural pigments such as anthocyanins, carotenoids, and chlorophylls. This chapter describes the chemical properties of betalains including their biosynthesis. Two main classes of betalains, betacyanins and betaxanthins, are described. Current reports on the biological and pharmacological properties of betalains from four main sources such as red beet, amaranth, prickly pear, and red pitahaya are also described. The biological and pharmacological properties of betalains that are covered in this chapter include antioxidant,

W. S. Choo (✉)
School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Selangor, Malaysia
e-mail: choo.wee.sim@monash.edu

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_38

anticancer, anti-lipidemic, and antimicrobial activities. Lastly, this chapter describes the application of betalains as natural colorants and other potential application in functional foods.

Keywords
Amaranth · Beetroot · Betacyanin · Betaxanthin · Cactus pear · Colorant · Prickly pear · Red dragon fruit · Red pitahaya

1 Introduction

Betalains are one of the most common plant pigments found in nature besides carotenoids, chlorophylls, and anthocyanins. Unlike anthocyanins or chlorophylls which are ubiquitous in the plant kingdom, betalains are found in a much smaller group of plants. It has been established that in the plant kingdom there is mutual exclusivity with respect to betalains and anthocyanins whereby both pigments have never been reported in the same plant [1]. The occurrence of betalains are restricted to the order Caryophyllales and some higher fungi such as *Amanita muscaria* (fly agaric) [2]. Betalains can be found in the families of the Caryophyllales (Achatocarpaceae, Aizoaceae, Amaranthaceae, Basellaceae, Cactaceae, Chenopodiaceae, Didiereaceae, Halophytaceae, Hectorellaceae, Nyctaginaceae, Phytolaccaceae, Portulacaceae, and Stegnospermataceae). Only two families of the Caryophyllales, the Caryophyllaceae and Molluginaceae produce anthocyanins [3].

The most common and widely known source of betalains are those belonging to the Amaranthaceae (namely, *Beta vulgaris* L. and *Amaranthus* sp.) and Cactaceae families (namely, *Opuntia* sp. and *Hylocereus* sp.) [4, 5]. *Beta vulgaris* subsp. *vulgaris* or commonly known as red beet or beet root (Fig. 1a) is mostly cultivated in North America, Central America, and Europe. Red beet is the major commercially exploited betalain crop and a vegetable characteristic of the Eastern and Central European diet. Red beets are not only consumed as fresh vegetables but are also processed to obtain desiccated or frozen products, juices, and their concentrates as well as natural pigments used as food additives [6]. Amaranthus or collectively known as amaranth (Fig. 1b) is a cosmopolitan genus of annual or short-lived perennial plants made up of about 60 species. Some amaranthus are cultivated as leafy vegetables, grains, or ornamental plants, while others are weeds. Amaranthus are cultivated and consumed as a leafy vegetable in many parts of the world. Certain Amaranthus species are consumed as cooked or fresh [7, 8]. Several Amaranth species are raised as grains in Asia and America. *Opuntia* sp. fruits or commonly known as prickly pears or cactus pears (Fig. 1c) are widely distributed in Mexico, Central and Southern America, Australia, South Africa, and the Mediterranean. Prickly pears have edible cladodes, but its sweet and colored fruits are the most desirable and commercially valuable parts of this plant and present economic relevance throughout arid and semiarid areas of the world [9, 10]. *Hylocereus polyrhizus* or commonly known as red pitahaya or red dragon fruit (Fig. 1d) is well known for its aesthetically pleasing deep purple color pulp with numerous small

Fig. 1 Common sources of betalains (**a**) Red beet, (**b**) Amaranth (Source: iStock photos), (**c**) Prickly pear (Source: Peterson [11]), (**d**) Red pitahaya (Source: iStock photos)

soft seeds. This fruit has high appeal in the European and United States market and is cultivated in Asia and Australia.

Stenocereus sp. is an emerging common source of betalains belonging to the Cactaceae family. *S. pruinosus, S. stellatus,* and *S. queretaroensis* are endemic of Mexico. These species produce fruits named pitayas [12]. Fruits are spherical or ellipsoid, spiny, green or red peeled berries [13] with juicy flesh of different colors such as white, yellow, pink, orange, red, or purple [12]. In several references [14–17], the name pitaya has also been given to fruit of the species *Hylocereus* sp., but there are notable differences between the fruits from these two genera. The fruits of *Hylocereus* sp., named pitahayas, do not have spines, possess certain type of bracts, and their size is higher than that of the fruits of *Stenocereus* sp. [12].

Betalains are present in other plant families in interesting amount, such as Baselaceae, Asparagaceae, Nictaginaceae, Pandanaceae, Phytolaccaceae, and Portulacaceae. *Basella rubra* L. and *Ullucus tuberosus* are the most studied sources of betalains belonging to the from the Basellaceae family; *Cordyline fruticosa* belonging to the Asparagaceae family, *Mirabilis jalapa* L. from the Nyctaginaceae family, *Pandannus amaryllifolius* from the Pandanaceae family, *Rivina humilis* L. from the Phytolaccaceae family, and *Talinum triangulare* from the Portulacaceae family. In a plant, betalains can be present in seeds, leaves, flowers, sprouts, aerial parts, roots, and even in fruits [18]. The occurrence of betalains in different parts of plants has been discussed comprehensively in a recent review by Martin et al. [18]. Betalains play important roles in plant physiology and visual attraction for pollinators and seed dispersers [19]. In some plants they also have specific functions. Betalains were discussed to provide protection from the harmful effects of ultraviolet radiation in the ice plant *(Mesembryanthemum crystallinum* L.) [20]. In cactus thorns betalains provide insect-repelling signals whereas for underground plant like red beet, they can improve the resistance to soil pathogens [19]. In red beet, betalains act as reactive oxygen species scavengers, limiting damage caused by wounding and pathogen infiltration in the plant tissues [21]. In food, betalains serve two functions. Betalains improve the aesthetic value of foods and contribute to consumers' health and well-being [19].

In this chapter, the chemical properties of betalains including their biosynthesis are described. The biological and pharmacological properties of betalains from the four main common sources, namely, red beet, amaranthus, prickly pear, and red pitahaya and their application in functional foods or foods that potentially offer health benefits beyond basic nutrition are also covered in this chapter.

2 Chemical Properties of Betalains

Betalains are water-soluble nitrogen-containing pigments which can exist as the red-violet betacyanins or yellow betaxanthins. These two main classes of betalains can occur in the same plant part, despite the difference in coloration. However, betacyanins occur with greater frequency than betaxanthins. In early days, betacyanins were addressed erroneously as nitrogenous anthocyanins whereas betaxanthins were called flavonoids [22]. The term "betalains" was first introduced by Mabry and Dreiding in 1968 [23]. Betalains are immoniun derivatives of betalamic acid (Fig. 2) that is based on the protonated 1,2,4,7,7-pentasubstituted 1,7-diazaheptamethin system with a betalain color being attributable to the resonating double bonds (Fig. 2) [24].

When the basic structure is substituted with an aromatic nucleus, a change in the absorption maximum from 540 nm (red-violet betacyanins; Table 1) to 480 nm (yellow betaxanthins; Table 2) is observed [24]. To date, there are 51 and 23 different betacyanin and betaxanthin structures identified, respectively [18]. Common names of betacyanins or betaxanthins are assigned in relation to the plant from where they were first isolated. Examples are given in Tables 1 and 2 [22, 24, 25]. Betacyanin

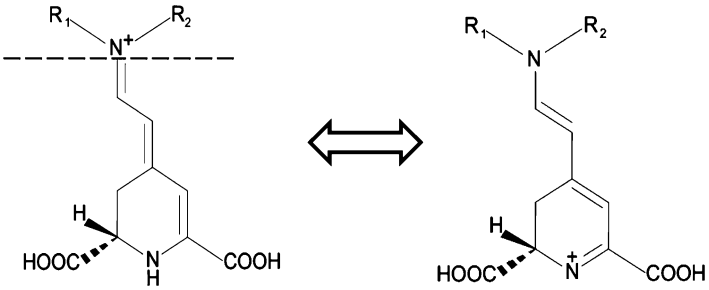
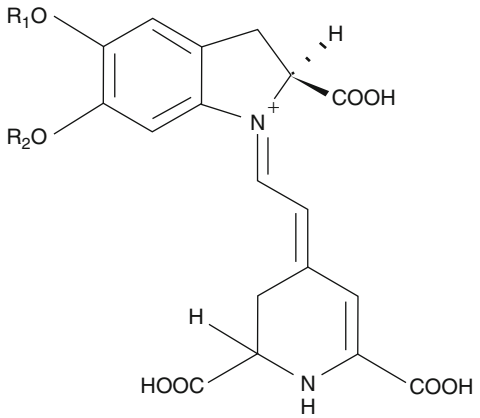


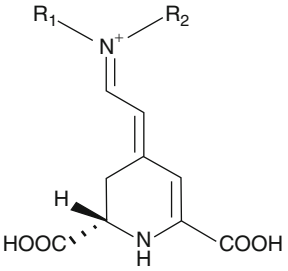
Fig. 2 Resonance structure of betalain

Table 1 Examples of betacyanins



	Substituent group		
Name	R_1	R_2	Plant source
Betanin	β-glucose	H	*Beta vulgaris*
Amaranthin	2′-*O*-(β-glucuronic acid)-β-glucose	H	*Amaranthus tricolor*
Hylocerenin	3-methyl-3-hydroxy methyl glutaryl	H	*Hylocereus polyrhizus*
Phyllocactin	6′-O-(malonyl)-β-glucose	H	*Phyllocactus Hybridus*
Celosianin-I	2′-*O*-[*O*-(p-coumaroyl)-β-glucuronic acid]-β-glucose	H	*Celosia cristata*
Gomphrenin-I	H	β-glucose	*Gomphrena globosa*
Iresinin-I	2′-*O*-(β-glucuronic acid)-6′- *O* –(3-hydroxy-3-methylglutaryl)-β-glucose	H	*Iresine herbstii*
Rivinianin	3′-*O*-(SO_3H)-β-glucose	H	*Rivinia humilis*

Table 2 Examples of betaxanthins



	Substituent group		
Name	R_1	R_2	Plant source
Indicaxanthin	Proline	Proline	*Opuntia ficus-indica*
Portulacaxanthin-I	Hydroxyproline	Hydroxyproline	*Portulaca grandiflora*
Vulgaxanthin-I	Glutamine	H	*Beta vulgaris*
Vulgaxanthin-II	Glutamic acid	H	*Beta vulgaris*
Miraxanthin-II	Aspartic acid	H	*Mirabilis jalapa*
Humilixanthin	Hydroxynorvaline	H	*Rivinia humilis*

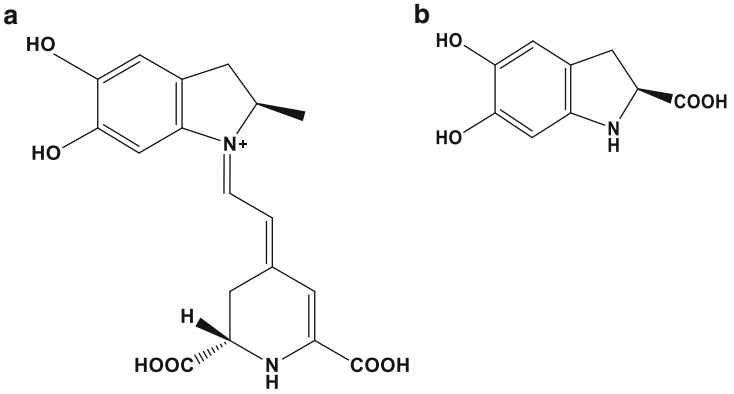


Fig. 3 (**a**) Betanidin (**b**) *cylco*-DOPA

structures show variations in their sugar or acyl groups whereas betaxanthins show conjugation with a wide range of amines or amino acids in their structures [26].

The aglycon betanidin (Fig. 3a) is the backbone of all betacyanins. The occurrence of different betacyanin structures is due to the glycosylation and acylation of the resulting 5-*O*- or 6-*O*-glucosides. The most common glycosyl moiety is glucose, although sophorose and rhamnose could also occur, but less frequently. The most common acyl groups are sulfuric, malonic, 3-hydroxy-3-methylglutaric, citric, p-coumaric, ferulic, caffeic, and sinapic acids [27]. Betanin (betanidin 5-*O*-ß-glucoside) from red beet was the most studied betacyanin pigment [22]. Betacyanins can be further classified into four groups: betanin, gomphrenin, amaranthin, and

Table 3 General classification of betalains

Betacyanin	Betaxanthin
Betanin-type group Betanin Prebetanin Neobetanin Phyllocactin 2′-apiosyl-phyllocactin Hylocerenin Lampranthin I, II Rivinianin	*Amino-acid-derived conjugate group* Portulacaxanthin II Portulacaxanthin III Tryptophan-betaxanthin Tyrosine-betaxanthin
Amaranthin-type group Amaranthin Iresinin Celosianin	*Amine-derived conjugate group* Vulgaxanthin-I 3-Methoxytyroamine-betaxanthin
Gomphrenin-type group Gomphrenin class I, II, III,IV Betaninidin 6-O-sophorosides derives	
Bougainvillein-type group Bougainvillein r-I, r-III Bougainvillein-v Mammillarinin	

Adapted from Slimen et al. [22] and Pavokovic and Krsnik-Rasol [28]

bougainvillein (Table 3). These groups differ by the attachment of glucosyl groups to the oxygen atoms in the ortho position on the *cyclo*-DOPA (Fig. 3b) moiety. The betanin-type group has a hydroxyl group linked to the C6 carbon and a glucosyl group on the C5 one. Gomphrenin- type group is known as structural isomers of betanins with a hydroxyl group attached to the C5 carbon and a glucosyl linked to the C6 carbon [2]. Amaranthin-type group differs from the betanin-type group by having two contiguous glucosyl groups attached to the C5 carbon [28]. Bougainvillein-type group is known as carboxylated or decarboxylated betanins [22]. Betacyanins are known to display two absorption maxima, one in the UV-range between 270 and 280 nm due to the *cyclo*-DOPA structure and a second one in the visible range at about 540 nm [19].

In betaxanthins, the *cyclo*-DOPA unit of betacyanins is replaced by an amino acid or amine. Hence, betaxanthins can be classified into two groups: amino-acid-derived conjugate group and amine-derived conjugate group (Table 3). Indicaxanthin from cactus pear was the first yellow pigment structurally characterized, having proline as substituent. It is also the most commonly studied betaxanthins besides vulgaxanthin I with glutamine as substituent. Vulgaxanthin I is the most abundant betaxantin found in *Beta vulgaris* [22]. Condensation of betalamic acid with various amino acids or amines in betaxanthins produce hypso- and bathochromic shifts [19]. The maximum absorption of betaxanthins varies from 460 to 480 nm and is associated with the structure of the amino or amine conjugates. Amine conjugates display a lower absorption maximum than their respective amino acids [29]. The

highest absorption maximum was displayed by indicaxanthin and portulacaxanthin I with proline and hydroxyproline as a substituent, respectively [29, 30].

The biosynthesis of betalains was studied since the 1950s and their biosynthesis is summarized in a number of reviews [2, 31–36]. Betalains arise from arogenate of the shikimate pathway whereby arogenate is converted to L-tyrosine, an amino acid, via arogenate dehydrogenase [37, 38]. Betalains are therefore secondary metabolites derived from the amino acid L-tyrosine. The pathway involved in the biosynthesis of betalains begins with the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine or better known as L-DOPA [39] through the monophenolase activity of the enzyme tyrosinase (or polyphenoloxidase) [31, 40]. Betalamic acid is derived from L-DOPA by spontaneous intramolecular condensation through the activity of the enzyme 4,5-DOPA-extradiol-dioxygenase [31]. Condensation of betalamic acid with another DOPA derivative such as *cyclo*-DOPA produce red colored betacyanins whereas condensation of betalamic acid with an amino acid or amine produce yellow betaxanthins [1]. Figure 4 shows the biosynthetic pathway of betanidin, a betacyanin and indicaxanthin, a betaxanthin as examples of betalains [1].

3 Biological and Pharmacological Properties of Betalains

Betalains play an important role in human health because of their biological and pharmacological properties/activities such as antioxidant, anticancer, anti-lipidemic, antimicrobial, etc. [5]. Although various pharmacological activities of betalains have been observed in vivo, the use of extracts limits the conclusions drawn, the hypothesis on the mechanisms involved, and the therapeutic potential of the assays. To date, studies with purified pigments are scarce, but they provide exciting conclusions [41]. Also, the likely optimum concentration for daily intake may only be decided after systematically addressing stability and bioavailability issues of betalains [42]. There are lack of stability and bioavailability studies on different groups of betalains. Current knowledge on the stability or bioavailability of betalains has been discussed in a number of reviews [4, 18, 42–46]. Current reports on the antioxidant, anticancer, anti-lipidemic, and antimicrobial activities of betalains from red beet, prickly pear, amaranth, and red pitahaya are covered in this section.

3.1 Antioxidant Activity

A broad definition of antioxidant is “any substrate that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of the substrate” [47]. The term “oxidizable substrate” includes almost everything found in foods and in living tissues including proteins, lipids, carbohydrates, and DNA. The target chosen and the source of oxidative damage therefore play paramount importance in characterizing an antioxidant [47]. The bioavailability of betalains is at least as high as flavonoids, which are well-accepted natural antioxidants. Betalains, as natural antioxidants, may provide protection

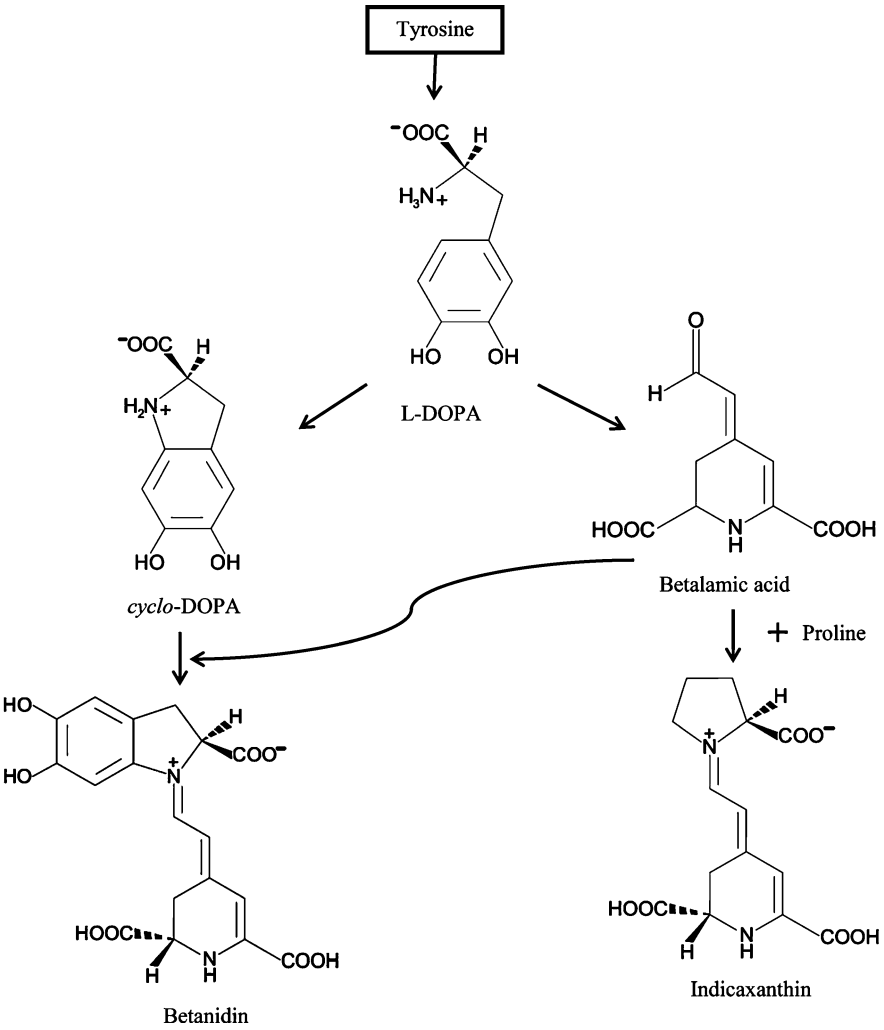


Fig. 4 Biosynthetic pathway of betanidin and indicaxanthin

against oxidative stress-related disorders [6, 48]. The antioxidant activity of betalains has been studied extensively and shown in several chemical and biological models [6, 22, 49–54] including the ex vivo oxidation of human low-density lipoproteins (LDL). However, ex vivo oxidation studies of human LDL or lipid inhibition studies are covered in a separate following section. The antioxidant activity of betalains in vitro was investigated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, ABTS (2,2′-azinobis(3-ethylbenzothiozoline-6- sulfonic acid) diammonium salt) radical scavenging assay, electron spin resonance spectroscopy, etc. Some studies measured the TEAC (trolox equivalent antioxidant capacity) assay that is based on the ability of an antioxidant to

scavenge ABTS radical cation relative to that of the water-soluble vitamin E analogue, Trolox [55].

In vitro studies of betalains from red beets have demonstrated that they possess high antiradical and antioxidant activity. The free radical-scavenging activity of betanin from red beet measured in a TEAC assay at pH 7.4 and in a DPPH assay was about 7.5- and 3.0-fold higher, respectively, than that of vitamin C, which is commonly accepted as an effective natural water soluble antioxidant [50, 56]. Betalains from red beet in a TEAC assay were also found to have 1.5–2.0-fold greater free radical scavenging activity than some anthocyanins [56] such as cyanidin-3-*O*-glucoside and cyanidin, above pH 4 [57]. Purified betanin and indicaxanthin from prickly pear were more effective than Trolox at scavenging ABTS radical [49]. Similarly, EC_{50} values determined using the DPPH radical-scavenging activity method for pulp and peel extracts of red pitahaya containing betalains were 22.4 ± 0.29 and 118 ± 4.12 mol vitamin C equivalents/g, respectively [58]. However, betalain extracts of red pitahaya also have high content of ascorbic acid which acts as potential antioxidant. Isolated betacyanins such as betanin, phyllocactin, hylocerenin, and their isomers are therefore required to assess their antioxidant capacity separately [59]. Betalains from Amaranthaceae determined using DPPH method were found to have EC_{50} values ranging from 3.4 to 8.4 μM. Gomphrenin type betacyanins and betaxanthins assessed using the DPPH method demonstrated the strongest antioxidant activity which was three to four times stronger than ascorbic acid and also stronger than other potent antioxidants such as rutin and catechin [50]. In addition, betalain pigments from various plants of the family Amaranthaceae exhibited strong antioxidant activity presented as mean EC_{50} value (μM): amaranthine or isoamaranthine (8.37 or 8.35), iresinins (8.08), celosianins (7.13), betanin or isobetanin (4.88 or 4.89), 3-methoxy-tyramine-betaxanthin (4.21), (S)-tryptophan-betaxanthin, dopamine-betaxanthin (4.08), acylated gomphrenins (4.11), and simple gomphrenins (3.35) [50]. Although free radical scavenging activity investigated using the DPPH- or TEAC-assay can provide valuable insights on the mechanisms of action, it is important to note that in vitro chemical assays bear no similarity to biological systems including the absorption of antioxidant compounds by the human body [60]. The measurements of the in vitro antioxidant activity of betalains therefore needs to be carefully interpreted [5].

Betanin from red beet was reported to dose-dependently scavenge DPPH-, galvinoxyl-, superoxide-, and hydroxyl-radicals in electron spin resonance spectroscopy and spin trapping studies and prevented hydrogen peroxide induced cellular DNA damage in cultured HT-29 cells. Furthermore, betanin treatment induced the transcription factor Nrf2 and resulted in an increase of heme oxygenase 1 (HO-1) protein levels, PON1-transactivation and cellular glutathione (GSH). Nrf2 is a transcription factor regulating the expression of genes encoding proteins important in antioxidant defense. The antioxidant enzyme HO-1 is a Nrf2 target gene. The hepatic enzyme PON1 also exhibits antioxidant and antiatherogenic activity. GSH is an important cytosolic antioxidant centrally involved in redox signaling and stress response. These studies suggested that betanin is both a free radical scavenger and an inducer of antioxidant defense mechanism in cultured cells [61]. Red beet pomace containing betalains was evaluated on three different free radical species: DPPH·, ·OH and O_2^- using ESR

spectroscopy and was found to show better scavenging activity (EC_{50} OH = 0.0655 mg/mL, EC_{50}DPPH· = 0.0797 mg/mL, and $EC_{50}O_2^-$ = 1.0625 mg/mL) than those of butylated hydroxyanisole (BHA) (EC_{50}DPPH· = 0.180 mg/mL, EC_{50}·OH = 1.505 mg/mL, and $EC_{50}O_2^-$ = 2.680 mg/mL) [62].

The antioxidant capacity of betacyanins from red pitahaya was evaluated using the peroxyl radical generating system in the presence of AAPH and the peroxyl radical scavenging capacity was dose-dependent in the low concentration range (25–100 nM). The mol-Trolox equivalent activity/mol compound (mol-TEA/mol-compound) as an index of the antioxidant capacity indicated 3.31 and 2.83 mol-TEA/mol-compound for betanin and phyllocactin, respectively [54]. Betalains from red beets inhibited peroxynitrate-mediated tyrosin nitration and DNA strand cleavage [63]. It was also found that pre-treatment of HT-29 cells with betalains significantly reduced hydrogen peroxide induced DNA damage [61]. The nitrogen scavenging activity of betacyanins was examined using NOR3 as an NO donor, and it was found that the IC_{50} values of nitrogen radical scavenging activity were 24.48, 17.51, 6.81 for betanin, phyllocactin, and betanidin, respectively [54]. The reduction of the nitrite level when treated with betacyanins was high in comparison to flavonoids such as aromadendrin, quercitrin, and loliolide [64]. These studies demonstrated the potential DNA-protective effect of betalains against reactive oxygen and nitrogen radicals.

Sources of betalains such as cacti fruits and red beet exhibited strong antioxidant activities in biological environment [4] by inhibiting lipid peroxidation and heme decomposition at very low betalain concentrations. Betalains have been shown to protect vascular endothelium cells, which are direct targets of oxidative stress in inflammation from cytokine-induced redox state alteration [65]. Betalains also counteract lipoperoxidases which may damage gastrointestinal cells during food digestion [6, 50]. Human ingestion of a single dose of red beet juice resulted in an increase of antioxidants in the system with only 0.28% of betalains excreted in urine within the 24 h after consumption [66]. The oral administration of betalains from red beets (5, 20 or 80 mg/kg body weight) in mice exposed to gamma irradiation significantly enhanced the activity of antioxidant enzymes such as superoxide dismutase (SOD) and gluthathione peroxidase (GPx) in the liver, spleen, and kidney, in a dose-dependent manner [67]. *Opuntia ficus-indica* extracts containing betalains were also found to counteract the release of acetaldehyde in blood due to chronic alcohol intake by restoring the malondialdehyde (MDA) and GSH level in the erythrocytes [68]. The use of in vivo assays using mice models and human trials permit the detection of gross effects resulting from multiple mechanisms but they reveal little about the individual mechanisms of activity [69]. A combination of in vitro and in vivo studies may be able to provide an efficient and effective approach to screen the potential antioxidant activity of betalains [5].

3.2 Anticancer Properties

Cancer is a growing problem worldwide that had surpassed common infectious diseases as the second leading cause of death globally [70]. Different approaches

are being explored for the prevention and treatment of cancer [71]. Exploring natural compounds for their anticarcinogenic properties is one of the major approaches to prevent or treat cancer. In this context, the establishment of health benefits of edible fruits through in vitro anticancer assays would substantiate their potential as functional foods [72].

Betanin from red beet showed excellent growth inhibition of MCF-7 (breast), HCT-116 (colon), AGS (stomach), SF-268 (CNS), and NCI-H460 (lung) cancer cell lines with IC_{50} values of 162, 142, 158, 164, and 147 μg/mL, respectively [73]. Betanin/isobetanin concentrate from red beet was reported to significantly decrease cancer cell proliferation and viability of MCF-7-treated cells. The expressions of apoptosis-related proteins (Bad, TRAILR4, FAS, p53) were strongly increased and the mitochondrial membrane potential was altered, demonstrating the involvement of both intrinsic and extrinsic apoptotic pathways [74]. Betanin from red beet resulted in a 49% inhibition of HepG2 cell proliferation at 200 μg/mL, and betaine yielded a 25% inhibition at 800 μg/mL [75]. The in vitro inhibitory effect of red beet extract (betalains) on Epstein-Barr virus early antigen (EBV-EA) induced by the tumor promoter 12-*O*-tetradecanoylphorbol-I3-acetate revealed a high order of activity compared to paprika (capsanthin), cranberry (anthocyanins), red onion skin (anthocyanins), and short and long red bell peppers (carotenoids). An in vivo anti-tumor promoting activity evaluation against the mice skin and lung bioassays also revealed a significant tumor inhibitory effect [76]. Red beet extract containing betalains exhibited a lower cytotoxic profile compared to doxorubicin (a red colored anticancer antibiotic drug) in in vitro studies utilizing two cancer cell lines of human origin (prostate PC-3 and breast MCF-7). However, it had minimal effect on the growth of normal human skin (FC) and liver (HC) cells in contrast to doxorubicin. It was suggested that as a dietary supplement, the red beet extract may have the potential to lower the serious adverse effects associated with doxorubicin (adriamycin) therapy [77]. Red beet extracts containing betalains were also found to counteract the lethal effects of the chemical carcinogen 7,12-dimethylbenz[a] anthracene (DMBA) in the livers of Sprague- Dawley rats. This was achieved by a reduction in activity of EROD (CYP1A1 marker) and MROD (marker of CYP1A2) and an increase in the activities of hepatic phase II enzymes, gluthathione S- transferase (GST) and NAD(P)H:quinone oxidoreductase-1 (NQO1) [78]. Similarly, the effect of red beet extracts containing betalains on MCF-7 and MRC-5 (fetal lungs) cell lines determined by using the sulforhodamine B (SRB) assay resulted in inhibiting concentration (IC_{50}) values in the range of 362–504 and 383–588 μg/mL for MRC-5 and MCF-7, respectively [79]. Red beet pomace extract containing betalains exhibited cytotoxic properties against Ehrlich carcinoma (EAC) cells in vivo. The EAC cell numbers were decreased in all extract-treated groups compared with the untreated EAC control group. The largest decreases in EAC cell numbers were observed in the pretreated male (approximately 53%) and female (approximately 47%) mice, and also the EAC cell viability was decreased after administration of red beet pomace extract containing betalains [62]. In three different experimental tumor models in mice, a very low dose of betanin (0.0025%) from red beet acted as a potent chemopreventive agent [80]. Red beet extract containing

betalains consistently reduced multiorgan tumor formations in various animal models when administered in drinking water (25–78 μg/mL, equivalent to a daily intake of about 6–20 μg of beetroot betacyanins/kg body weight) [76, 81]. A long-term daily exposure to low doses of red beet extract through diet was postulated to be safe and sufficient to produce cancer chemopreventive effect in humans [82].

Immortalized ovarian and cervical epithelial cells as well as ovarian, cervical, and bladder cancer cells that were exposed to prickly pear (*Opuntia ficus-indica*) extracts containing betalains displayed a significant increase in apoptosis and growth inhibition in a dose- and time-dependent manner. The extracts containing betalains affected cell cycle of cancer cells by increasing G1 and decreasing G2 and S phases, significantly suppressed tumor growth in nude mice, increased annexin IV expression, and decreased VEGF by modulating the expression of tumor-related genes [83]. Betanin from *Opuntia ficus-indica* fruits were found to possess an antiproliferative activity in K562 cells with an IC_{50} of 40 μM. Betanin induced apoptosis in K562 cells through the intrinsic pathway and is mediated by the release of cytochrome c from mitochondria into the cytosol and poly (ADP) ribose polymerase (PARP) cleavage [84]. Another study showed that prickly pear extracts containing betalains exhibited a significant growth inhibitory effect on human ovarian cancer cells similar to a synthetic chemopreventive agent, 4- HPR, in nude mice [83]. Extracts containing betalains from prickly pears was found to be less effective in inhibiting colon cancer cell (HT29) growth compared to doxorubicin, an anticancer drug. Doxorubicin induced HT29 cycle arrest in G2/M phase [85] and thus combining the drug treatment with extracts from prickly pears that arrested cells on different cell cycle checkpoints (G1 and/or S phases) could be a promising strategy to improve the anticancer effect and diminished chemoresistance as an adjuvant of chemotherapy [86]. Screening of antiproliferative activity of extract containing betalains from the fruit of red pitahaya on AGS (a human gastric adenocarcinoma cell line), HeLa (a human cervical adenocarcinoma cell line), MCF-7 and B16F10 melanoma cell [87] was carried out but the results were not significant. To date, there are no reported studies on the anticancer or antiproliferative activity of extract containing betalains from Amaranthus. The use of cancer cell lines allowed the investigation of potential anticancer compounds in a simplified, controlled, and reproducible environment [88, 89]. However, the inability of cell cultures to behave like tumors and their interactions with the host [89] means that the efficacy of betalains as an anticancer agent in human clinical trials may not be the same. Further in vivo studies in a model that closely approximates the targeted human cancer are therefore needed [5].

3.3 Anti-Lipidemic and Lipid Oxidation Inhibition Effect

Betalains exhibited antioxidant effect to inhibit lipid oxidation. In a linoleate peroxidation assay by cytochrome c, linoleate peroxidation was inhibited by betanin, betanidin, catechin, and α-tocopherol with IC_{50} values of 0.4, 0.8, 1.2, and 5.0 mM, respectively. Betacyanins from red beet were therefore more potent

antioxidants than catechin or tocopherol in inhibiting linoleate peroxidation [6]. Betaxanthins from red beet were found to inhibit lipid peroxidation by cytochrome c at an IC_{50} of ~1.0 μM. In addition, betanin was almost twice as effective as catechin in preventing LDL oxidation by H_2O_2-activated metmyoglobin [6]. Betanin from red beet was found to inhibit lipid peroxidation in a model liposome oxidation assay using fluorescence spectroscopy [73]. Betalains from prickly pears inhibited the myeloperoxidase/nitrate-induced oxidation of human LDL by scavenging the initiator radical nitrogen dioxide and lipoperoxyl radical [90]. A short-term supplementation study on healthy volunteers with prickly pear fruit showed a significant decrease in 8-epiprostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) and malondialdehyde in plasma, the ratio of reduced to oxidized glutathione (GSH:GSSG) in erythrocytes, and lipid hydroperoxides in LDL (these are biomarkers of oxidative stress) whereas supplementation with 75 mg vitamin C did not significantly affect any marker of oxidative stress [91]. Betalains in prickly pear were suggested to be responsible in the protection of oxidative damage to lipid [6]. The lipid peroxidation inhibitory effects of prickly pear fruit extracts containing betalains on fish oil, fish oil-in-water emulsion, and linoleic acid were investigated using conjugated diene hydroperoxides, weight gaining, peroxide value, and thiobabituric acid reactive substances assays. Prickly pear extracts containing betalains were able to show multiple lipid peroxidation inhibitory effect on different stages of lipid oxidation products generation in oils and emulsion model systems [92]. In an ex vivo study in which pooled human plasma from 10 healthy volunteers was incubated with either 25–100 μM betanin or indicaxanthin isolated from prickly pear, incorporation of both compounds in LDL was observed, with a maximum binding of 0:52 and 0:51 nmoles of indicaxanthin and betanin, respectively, per mg LDL protein. Indicaxanthin-enriched and betanin-enriched LDL were also more resistant than homologous native LDL to copper-induced oxidation [93].

Lipid-lowering drugs are used for primary and secondary prevention of cardiovascular diseases. The use of plant extracts to treat or regulate the conditions of lipidemia are gaining interest as they are known to have no side effects or toxicity compared to common synthetic lipid lowering drugs [5]. Administration of an aqueous extract of *Amaranthus tricolor* containing betalains at 200 mg/kg and 400 mg/kg body weight to diabetic rats significantly reduced blood cholesterol, triglyceride, and LDL levels, and increased HDL level [94].

A study on hypercholesterolemic rats found that force feeding of 300 mg/kg (body weight) of betalain containing extracts from pulps of *Hylocereus polyrhizus* reduced their total cholesterol level by 43.5% [95], probably by increasing excretion of bile acids. Feeding of betalains enriched beet crisps in mice resulted in a significant decrease in serum glucose level, atherogenic index, isovaleric acid concentration in the caecal, and body weight in rats. Furthermore, oral administration of betalains suppressed production of short-chain fatty acids, prevented the rise in serum total cholesterol and triacylglycerol levels in dyslipidemic rat models [96]. Rats fed with a control diet containing mixture of powders containing red beet as a source of betalains showed a significant reduction in their plasma total lipids, total cholesterol, LDL, triglycerides, and the ratio of total cholesterol/HDL in different

degrees, while HDL increased significantly [97]. Betalains may therefore have potential application in the management of hyperglycemia and associated lipidemia [5].

3.4 Antimicrobial Activity

Betalains have been reported to exhibit antimalarial and antimicrobial effects, but their effects are dependent on the dose and type of betalains present. Betalain rich *Amaranthus spinosus* showed significant antimalarial activity in mice due to high levels of betanin and amaranthin. These compounds were able to chelate the indispensable inner cations (Ca^{2+}, Fe^{2+} and Mg^{2+}) in the parasite and block the parasites choline intracellular transport which is crucial for malarial parasite growth [98]. Beetroot pomace containing betalains induced zones of reduced growth in *Salmonella* Typhymurium, *Staphylococcus aureus,* and *Bacillus cereus* [99]. Beet root pomace containing betalains was also found to inhibit Gram-negative bacteria *(Escherichia coli, Pseudomonas aeruginosa, Citrobacter freundii, Citrobacter youngae, Enterobacter cloacae,* S. Typhimurium) with S. Typhimurium and *C. freundii* being most susceptible [100]. Red beet pomace extract containing betalains showed inhibitory activity against Gram-negative bacteria in which the most susceptible strains are S. Typhimurium and *C. freundii.* Among the Gram-positive bacteria, *S. aureus, Staphylococcus sciuri,* and *B. cereus* showed high susceptibility. However, the extract did not exert any activities towards wild strains Bacillus sp., *Enterococcus faecalis* and *Listeria monocytogenes* and did not inhibit the growth of microorganisms with eukaryotic type of cells such as *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus niger,* and *Penicillium aurantiogriseum* [62]. Slight antibacterial activity of methanolic extracts of red beet was also obtained against *E. coli* and *S. aureus* and absence of antifungal activity against *A. niger* and *C. albicans* were also reported by Rauha et al. [101].

Extracts of xoconostle pear (*Opuntia matudae*) containing betalains had a significant inhibitory effect on the growth of *E. coli* O157:H7 with an increase in xoconostle pear extract concentration associated with a slower growth rate and reduction in bacterial populations [102]. Betalains-rich fraction extracted from the pulp of red pitahaya exerted a broad antimicrobial spectrum by inhibiting Gram-positive bacteria (*B. cereus, S. aureus, Escherichia faecalis, L. monocytogenes*) at 7.8 μg/mL, Gram-negative bacteria (E. *coli, Proteus mirabilis, Proteus vulgaris, P. aeruginosa, Salmonella typhi Ty2, Yersinia enterocolitica, Klebsiella pneumonia, Enterobacter cloacae, Enterobacter aerogenes)* at 15.6–62.5 μg/mL, yeasts (C. *albicans, Rhizoctonia solani)* at 125–250 μg/mL and molds *(Fusarium oxysporum, Cladosporium herbarum, Botrytis cinerea, Aspergillus flavus)* at 500 μg/mL [103]. Betacyanin rich extract from red spinach *(Amaranthus dubius)* (minimum inhibitory concentration [MIC] values: 0.78–3.13 mg/mL) demonstrated a better antimicrobial activity profile than that of red pitahaya (MIC values: 3.13–6.25 mg/mL) against nine Gram-positive bacterial strains (*S. aureus,* Enterococcus sp., Bacillus sp.). Similarly, betacyanin rich extract from red spinach (MIC values: 1.56–3.13 mg/mL) was more active than that of red pitahaya (MIC

values: 3.13–6.25 mg/mL) against five Gram-negative bacterial strains *(Shigella flexneri, S.* Typhimurium, *K. pneumoniae, E. coli, P. aeruginosa)* [104]. The antimicrobial activity of betalains may be due to their adverse effects on the structure, function, and permeability of the cellular membranes of the microbes which eventually leads to cell death [100]. Although betalains were found to inhibit a wide antimicrobial spectrum, very little is reported in the literature on the mechanism of microbial inhibition by betalains. The specific underlying cellular and molecular mechanism of antimicrobial activity of betalains should be investigated in future studies [5].

4 Application of Betalains

The main application of betalains to date is as natural colorants. Other potential application of betalains in functional foods is also covered in this section.

4.1 Natural Colorants

Colorants or color additives can be used to serve several purposes in food processing, including standardizing raw ingredient colors, providing color identities to otherwise colorless foods, and accounting for loss during processing or storage [105]. There is growing interest in the use of natural pigments for food coloring which are considered to be harmless or even healthy. Synthetic colorants are becoming more and more critically assessed by the consumer. There were numerous regulation changes worldwide in terms of permitted colorings throughout the years. Many advanced countries have their own regulations and a list of approved food colors that can be used, including maximum daily intake. For instance, the USA permitted list of synthetic colorants was reduced from 700 to only seven until the beginning of the twenty-first century [106]. The last synthetic food colorant to be approved by the Food and Drug Administration (FDA) was FD&C Red No. 40 (Allura Red) in 1971 and there are not likely to be any more in the near future [107]. Colored natural extracts are preferred over purified colors because declaration of the former allows clean labeling [108]. Betalains are less commonly used than anthocyanins in food processing despite being more pH stable than the anthocyanins [39]. Betalains are water-soluble pigments that are stable between pH 3 and 7 and well suited for coloring from sour (low acid) to neutral foods [4, 19] whereas the use of anthocyanins is not possible due to the instability at pH values over 3 [28]. Betalains can be effectively stabilized by ascorbic acid, which on the other hand is known to facilitate anthocyanin degradation [109]. Application of betalains instead of anthocyanins for coloring food with high vitamin C content or vitamin C-supplemented products may be of particular interest [44].

Betalains have been used as food colorants at least since the turn of the twentieth century [24]. The early application involved the use of pokeberry (*Phytolacca Americana*) juice to improve the color of red wine [24, 110]. The addition of pokeberry juice to wine was forbidden in France in 1892 because the juice also

contains a purgatory and emetic saponin called phytolaccatoxin [110, 111]. The red pigment from pokeberry was called phytolaccanin until research established that it was identical to betanin in red beets [111, 112]. Today, the food colorant known as "beetroot red," extracted from red beet is commercialized in European Union and USA as food colorant. This food colorant is also called betanin or known as E-162 in the European Union and as 73.40 in the 21 CFR section of the FDA in the USA [113]. Betanin from red beet is one of the successful natural colorants used in the food industry [114] and approved for use as colorants in dairy products, sauces, soups, cosmetics, and pharmaceuticals by the US FDA and European Union [115]. Red beet colorants can be effectively used to color dairy products such as yoghurt and ice cream, salad dressings, hard candies and fruit chews, frostings, cake mixes, gelatin desserts, starch-based puddings, meat substitutes, poultry meat sausages, gravy mixes, soft drink and powdered drink mixes [116, 117]. Commercial red beet colorants are available as either juice concentrates (produced by vacuum-concentration of red beet juice to 60–65% total solids) or powders (produced by freeze or spray drying), containing from 0.3% to 1% of pigment [24, 117, 118]. The concentration of pure pigment required to obtain the desired hue is relatively small, rarely exceeding 50 mg/kg, calculated as betanin [26] as betalains have good tinctorial strength [108]. Betanin from red beets are, however, afflicted with a narrow color spectrum [108]. The typical earthy flavor caused by geosmin [119] may also deter the commercial use of E-162 in food application. Furthermore, the risk of carry-over earth bound microorganism from the raw material red beetroot is an important consideration [120]. Red beets are notorious accumulators of nitrates and nitrites during growing, and there is a necessity to reduce these levels in red beets [110].

The use of *Opuntia* sp. as colorants has several advantages such as neutral smell and/or taste, low nitrate content [115, 121], a broad color range [19, 121], and represents a lower risk for microbiological carry-over [9]. In particular, the yellow orange prickly pear fruits are of promising potential because of the scarceness of yellow water-soluble pigments [121]. Moreover, the low levels of colorless phenolic compounds in prickly pear fruits make them very promising since potential interactions of betalains with these phenolics are avoided [9]. However, prickly pears contain high amounts of reducing sugars and amino acids and thereby may enhance Maillard product formation [9]. *Opuntia ficus-indica* is the most extensively studied Opuntia species. Recent research carried out with various Opuntia species has revealed *Opuntia stricta* to be a promising source of betacyanin pigments with high betanin levels (800 mg/kg) which are five times higher than those found in red fruits of *O. ficus-indica* and even higher than those shown by commercial red beets used for their purple color [115]. *Opuntia stricta* extracts lack the yellow betaxanthins, and betanin and isobetanin are the betacyanins present [122]. Concentrated betacyanin extracts from the fruits of *O. stricta* with its pleasant taste and flavor showed high potential to be used as a natural colorant [113, 122]. Red- purple powder from *O. stricta* fruit juice was successfully applied as a colorant in two food model systems: a yogurt and a soft-drink [123]. On the other hand, Amaranthus plants, which can grow in a wider range of environments are a potential source of

red-violet betacyanin pigments and an alternative to the use of red beet [124]. Some Amaranthus genotypes produce particularly high biomass and contain high levels of pigment [125]. Betacyanins from Amaranthus showed high potential to be used as natural colorants in jelly, ice cream, and beverage [126, 127]. Red pitahaya is high in betacyanins and devoid of betaxanthins (which are present in E-162) providing colorants with a better tinctorial strength. Therefore, the red pitahaya can be used as an alternative source of betacyanins in regions where it is grown [128]. Betacyanin from red pitahaya showed promising application as a natural colorant in dairy products such as milk [128] and yogurt [129] whereby sensory acceptability of panelists of using colorant preparations from red pitahaya were similar or higher than that of E-162 [128, 129].

4.2 Other Applications of Betalains

Commercially, fruits and cladodes of Opuntia species are made into products such as juices, jams, and dehydrated fruits [130]. Many research have been carried out to expand the use of betalains, particularly betalains from the four main sources, namely, red beet, prickly pear, amaranthus, and red pitahaya. Effort has been made to produce various types of red beet juice [131–138], prickly pear beverages or juices [121, 139–144], and red pitahaya juices [16, 145, 146]. In addition, spray drying or other drying and encapsulation techniques have been investigated to produce dried form (powder) of betalains or to further stabilize betalains [126, 138, 147–156]. Powdered or dried red beet containing betalains has been incorporated into edible or intelligent films [157, 158], emulsified pork sausage [159], and cheese [160]. Red beet juice containing betalains has been investigated to produce nutritious pasta [161] and added as part of a curing system to produce chicken frankfurters [162]. Red beet chips as healthy fried snack foods were also investigated [163]. Prickly pear containing betalains has been investigated to produce enriched cereal-based extrudates [164] whereas *Opuntia ficus-indica* cladodes as a functional ingredient was incorporated into bread [165]. The effect of betacyanin pigments from *Amaranthus tricolor* and *Amaranthus cruentus* on commercial wheat flours was investigated as a basis to expand the applications of betalains as natural quality enhancers of wheat-based foods [166].

5 Conclusions

The biological and pharmacological properties of betalains-rich foods such as red beetroot, amaranth, prickly pear, and red pitahaya show their great potential as functional foods. Betalains should be used regularly in the diet. Consumers are most likely to benefit from the regular consumption of products rich in betalains such as juice and other products made of foods colored with betalains. The lack of commercially available standards has limited betalain analysis. There is a need for more highly purified betalains and increasing of studies using purified compound

instead of betalain-rich extracts would help to establish the actual role played by betalains alone or in cooperation with other compounds. The underlying cellular and molecular mechanisms of betalains need to be addressed in more detail in future studies. Further research is also needed to determine the concentration, extractability, and stability of other source of betalains in order to expand the use of betalains. Plant sources of betalains other than red beet should be grown in sufficient quantities to ensure that betalains could be produced at a large scale for food industry use.

References

1. Stafford HA (1994) Anthocyanins and betalains: evolution of the mutually exclusive pathways. Plant Sci 101:91–98. https://doi.org/10.1016/0168-9452(94)90244-5
2. Strack D, Vogt T, Schliemann W (2003) Recent advances in betalain research. Phytochemistry 62:247–269. https://doi.org/10.1016/s0031-9422(02)00564-2
3. Mabry TJ (2001) Selected topics from forty years of natural products research: Betalains to flavonoids, antiviral proteins, and neurotoxic nonprotein amino acids. J Nat Prod 64: 1596–1604. https://doi.org/10.1021/np010524s
4. Azeredo HMC (2009) Betalains: properties, sources, applications, and stability – a review. Int J Food Sci Technol 44:2365–2376. https://doi.org/10.1111/j.1365-2621.2007.01668.x
5. Gengatharan A, Dykes GA, Choo WS (2015) Betalains: natural plant pigments with potential application in functional foods. LWT Food Sci Technol 64:645–649. https://doi.org/10.1016/j.lwt.2015.06.052
6. Kanner J, Harel S, Granit R (2001) Betalains – a new class of dietary cationized antioxidants. J Agric Food Chem 49:5178–5185. https://doi.org/10.1021/jf010456f
7. Amin I, Norazaidah Y, Hainida KIE (2006) Antioxidant activity and phenolic content of raw and blanched Amaranthus species. Food Chem 94:47–52. https://doi.org/10.1016/j.foodchem.2004.10.048
8. Chon S-U, Heo B-G, Park Y-S, Kim D-K, Gorinstein S (2009) Total phenolics level, antioxidant activities and cytotoxicity of young sprouts of some traditional Korean salad plants. Plant Foods Hum Nutr 64:25–31. https://doi.org/10.1007/s11130-008-0092-x
9. Stintzing FC, Schieber A, Carle R (2001) Phytochemical and nutritional significance of cactus pear. Eur Food Res Technol 212:396–407. https://doi.org/10.1007/s002170000219
10. Yahia EM, Mondragon-Jacobo C (2011) Nutritional components and anti-oxidant capacity of ten cultivars and lines of cactus pear fruit (*Opuntia* spp.) Food Res Int 44:2311–2318. https://doi.org/10.1016/j.foodres.2011.02.042
11. Peterson JS (2004) Opuntia ficus-indica (L.) Mill. – Barbary fig. http://plants.usda.gov/core/profile?symbol=OPFI&photoID=opfi_006_ahp.tif#. Accessed 30 Aug 2017
12. Garcia-Cruz L, Duenas M, Santos-Buelgas C, Valle-Guadarrama S, Salinas-Moreno Y (2017) Betalains and phenolic compounds profiling and antioxidant capacity of pitaya (*Stenocereus* spp.) fruit from two species (*S. Pruinosus and S. stellatus*). Food Chem 234:111–118. https://doi.org/10.1016/j.foodchem.2017.04.174
13. Casas A, Cruse-Sanders J, Morales E, Otero-Arnaiz A, Valiente-Banuet A (2006) Maintenance of phenotypic and genotypic diversity in managed populations of *Stenocereus stellatus* (Cactaceae) by indigenous peoples in Central Mexico. Biodivers Conserv 15:879–898. https://doi.org/10.1007/s10531-004-2934-7
14. Stintzing FC, Schieber A, Carle R (2002) Betacyanins in fruits from red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose. Food Chem 77:101–106. https://doi.org/10.1016/s0308-8146(01)00374-0
15. Adnan L, Osman A, Hamid AA (2011) Antioxidant activity of different extracts of red pitaya (*Hylocereus polyrhizus*) seed. Int J Food Prop 14:1171–1181. https://doi.org/10.1080/10942911003592787

16. Herbach KM, Maier C, Stintzing FC (2007) Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (*Hylocereus polyrhizus*) juice. Eur Food Res Technol 224:649–658. https://doi.org/10.1007/s00217-006-0354-5
17. Schweiggert RM, Villalobos-Gutierrez MG, Esquivel P, Carle R (2009) Development and optimization of low temperature enzyme-assisted liquefaction for the production of colouring foodstuff from purple pitaya (*Hylocereus* sp Weber Britton & Rose). Eur Food Res Technol 230:269–280. https://doi.org/10.1007/s00217-009-1167-0
18. Martins N, Roriz CL, Morales P, Barros L, Ferreira ICFR (2017) Coloring attributes of betalains: a key emphasis on stability and future applications. Food Funct 8:1357–1372. https://doi.org/10.1039/c7fo00144d
19. Stintzing FC, Carle R (2004) Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends Food Sci Technol 15:19–38. https://doi.org/10.1016/j.tifs.2003.07.004
20. Ibdah M, Krins A, Seidlitz HK, Heller W, Strack D, Vogt T (2002) Spectral dependence of flavonol and betacyanin accumulation in *Mesembryanthemum crystallinum* under enhanced ultraviolet radiation. Plant Cell Environ 25:1145–1154. https://doi.org/10.1046/j.1365-3040.2002.00895.x
21. Sepúlveda-Jiménez G, Rueda-Benítez P, Porta H, Rocha-Sosa M (2005) A red beet (*Beta vulgaris*) UDP-glucosyltransferase gene induced by wounding, bacterial infiltration and oxidative stress. J Exp Bot 56:605–611. https://doi.org/10.1093/jxb/eri036
22. Slimen IB, Najar T, Abderrabba M (2017) Chemical and antioxidant properties of betalains. J Agric Food Chem 65:675–689. https://doi.org/10.1021/acs.jafc.6b04208
23. Mabry TJ, Dreiding AS (1968) The betalains. In: Mabry TJ, Alston RE, Runeckles VC (eds) Recent advances in phytochemistry. Appleton, New York
24. Delgado-Vargas F, Paredes-Lopez O (2002) Anthocyanins and betalains. In: Natural colorants for food and nutraceutical uses. CRC Press, Boca Raton
25. Jackman RL, Smith JL (1996) In: Hendry GAF, Houghton JD (eds) Natural food colorants. Blackie Academic and Professional, London
26. Delgado-Vargas F, Jimenez AR, Paredes-Lopez O (2000) Natural pigments: carotenoids, anthocyanins, and betalains – characteristics, biosynthesis, processing, and stability. Crit Rev Food Sci Nutr 40:173–289. https://doi.org/10.1080/10408690091189257
27. Piattelli M, Minale L (1964) Pigments of centrospermae – II. Phytochemistry 3:547–557. https://doi.org/10.1016/S0031-9422(00)82927-1
28. Pavokovic D, Krsnik-Rasol M (2011) Complex biochemistry and biotechnological production of betalains. Food Technol Biotechnol 49:145–155
29. Stintzing FC, Schieber A, Carle R (2002) Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear *Opuntia ficus-indica* (L.) Mill. by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Agric Food Chem 50:2302–2307. https://doi.org/10.1021/jf011305f
30. Schliemann W, Kobayashi N, Strack D (1999) The decisive step in betaxanthin biosynthesis is a spontaneous reaction. Plant Physiol 119:1217–1232. https://doi.org/10.1104/pp.119.4.1217
31. Gandía-Herrero F, García-Carmona F (2013) Biosynthesis of betalains: yellow and violet plant pigments. Trends Plant Sci 18:334–343. https://doi.org/10.1016/j.tplants.2013.01.003
32. Davies KM (2004) Plant pigments and their manipulation. Blackwell, Oxford
33. Stintzing FC, Carle R (2008) N-heterocyclic pigments: betalains. In: Socaciu C (ed) Food colorants: chemical and functional properties. CRC Press, Boca Raton
34. Grotewold E (2006) The genetics and biochemistry of floral pigments. Annu Rev Plant Biol 57:761–780. https://doi.org/10.1146/annurev.arplant.57.032905.105248
35. Khan MI, Giridhar P (2015) Plant betalains: chemistry and biochemistry. Phytochemistry 117:267–295. https://doi.org/10.1016/j.phytochem.2015.06.008
36. Strack D, Steglich W, Wray V (1993) Betalains. In: Dey PM, Harbone JB (eds) Methods in plant biochemistry. Academic Press, London
37. Piatelli M (1976) Betalains. In: Goodwin TW (ed) Chemistry and biochemistry of plant pigments. Academic Press, New York

38. Steglich W, Strack D (1990) Betalains. In: Arnold B (ed) The alkaloids: chemistry and pharmacology. Academic Press, San Diego
39. Dawson TL (2009) Biosynthesis and synthesis of natural colours. Color Technol 125:61–73. https://doi.org/10.1111/j.1478-4408.2009.00177.x
40. Steiner U (1999) Tyrosinase involved in betalain biosynthesis of higher plants. Planta 208:114–124. https://doi.org/10.1007/s004250050541
41. Gandía-Herrero F, Escribano J, García-Carmona F (2016) Biological activities of plant pigments betalains. Crit Rev Food Sci Nutr 56:937–945. https://doi.org/10.1080/10408398.2012.740103
42. Khan MI (2016) Plant betalains: safety, antioxidant activity, clinical efficacy, and bioavailability. Compr Rev Food Sci Food Saf 15:316–330. https://doi.org/10.1111/1541-4337.12185
43. During A (2009) Bioavailability of natural (plant) food colorants. Agro Food Ind Hi Tech 20:38–41
44. Herbach KM, Stintzing FC, Carle R (2006) Betalain stability and degradation – structural and chromatic aspects. J Food Sci 71:R41–R50. https://doi.org/10.1111/j.1750-3841.2006.00022.x
45. Khan MI (2016) Stabilization of betalains: a review. Food Chem 197:1280–1285. https://doi.org/10.1016/j.foodchem.2015.11.043
46. Ngamwonglumlert L, Devahastin S, Chiewchan N (2017) Natural colorants: pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods. Crit Rev Food Sci Nutr 57:3243–3259. https://doi.org/10.1080/10408398.2015.1109498
47. Halliwell B (1990) How to characterize a biological antioxidant. Free Radic Res Commun 9:1–32. https://doi.org/10.3109/10715769009148569
48. Tesoriere L, Butera D, Allegra M, Fazzari M, Livrea MA (2005) Distribution of betalain pigments in red blood cells after consumption of cactus pear fruits and increased resistance of the cells to ex vivo induced oxidative hemolysis in humans. J Agric Food Chem 53:1266–1270. https://doi.org/10.1021/jf048134+
49. Butera D, Tesoriere L, Di Gaudio F, Bongiorno A, Allegra M, Pintaudi AM, Kohen R, Livrea MA (2002) Antioxidant activities of Sicilian prickly pear (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: Betanin and indicaxanthin. J Agric Food Chem 50:6895–6901. https://doi.org/10.1021/jf025696p
50. Cai YZ, Sun M, Corke H (2003) Antioxidant activity of betalains from plants of the Amaranthaceae. J Agric Food Chem 51:2288–2294. https://doi.org/10.1021/jf030045u
51. Li HY, Deng ZY, Liu RH, Zhu HH, Draves J, Marcone M, Sun Y, Tsao R (2015) Characterization of phenolics, betacyanins and antioxidant activities of the seed, leaf, sprout, flower and stalk extracts of three Amaranthus species. J Food Compos Anal 37:75–81. https://doi.org/10.1016/j.jfca.2014.09.003
52. Pinedo-Espinoza JM, Aguirre-Mancilla CL, Jimenez-Alvarado R, Raya-Perez JC, Iturriaga G, Ramirez-Pimentel JG, Hernandez-Fuentes AD (2017) Bioactive compounds and antioxidant activity evolution during the ripening process of 12 *Opuntia spp*. fruit accessions. Emir J Food Agric 29:138–148. https://doi.org/10.9755/ejfa.2016-09-1324
53. Sawicki T, Baczek N, Wiczkowski W (2016) Betalain profile, content and antioxidant capacity of red beetroot dependent on the genotype and root part. J Funct Foods 27:249–261. https://doi.org/10.1016/j.jff.2016.09.004
54. Taira J, Tsuchida E, Katon MC, Uehara M, Ogi T (2015) Antioxidant capacity of betacyanins as radical scavengers for peroxyl radical and nitric oxide. Food Chem 166:531–536. https://doi.org/10.1016/j.foodchem.2014.05.102
55. Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20:933–956. https://doi.org/10.1016/0891-5849(95)02227-9
56. Gliszczynska-Swiglo A, Szymusiak H, Malinowska P (2006) Betanin, the main pigment of red beet: molecular origin of its exceptionally high free radical-scavenging activity. Food Addit Contam 23:1079–1087. https://doi.org/10.1080/02652030600986032

57. Borkowski T, Szymusiak H, Gliszczynska-Swiglo A, Rietjens IMCM, Tyrakowska B (2005) Radical scavenging capacity of wine anthocyanins is strongly pH-dependent. J Agric Food Chem 53:5526–5534. https://doi.org/10.1021/jf0478556
58. Wu L-c, Hsu H-W, Chen Y-C, Chiu C-C, Lin Y-I, Ho J-a A (2006) Antioxidant and antiproliferative activities of red pitaya. Food Chem 95:319–327. https://doi.org/10.1016/j.foodchem.2005.01.002
59. Mahattanatawee K, Manthey JA, Luzio G, Talcott ST, Goodner K, Baldwin EA (2006) Total antioxidant activity and fiber content of select Florida-grown tropical fruits. J Agric Food Chem 54:7355–7363. https://doi.org/10.1021/jf060566s
60. Bjelakovic G, Nikolova D, Gluud L, Simonetti RG, Gluud C (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 297:842–857. https://doi.org/10.1001/jama.297.8.842
61. Esatbeyoglu T, Wagner AE, Motafakkerazad R, Nakajima Y, Matsugo S, Rimbach G (2014) Free radical scavenging and antioxidant activity of betanin: electron spin resonance spectroscopy studies and studies in cultured cells. Food Chem Toxicol 73:119–126. https://doi.org/10.1016/j.fct.2014.08.007
62. Vulic JJ, Cebovic TN, Canadanovic VM, Cetkovic GS, Djilas SM, Canadanovic-Brunet JM, Velicanski AS, Cvetkovic DD, Tumbas VT (2013) Antiradical, antimicrobial and cytotoxic activities of commercial beetroot pomace. Food Funct 4:713–721. https://doi.org/10.1039/c3fo30315b
63. Sakihama Y, Maeda M, Hashimoto M, Tahara S, Hashidoko Y (2012) Beetroot betalain inhibits peroxynitrite-mediated tyrosine nitration and DNA strand cleavage. Free Radic Res 46:93–99. https://doi.org/10.3109/10715762.2011.641157
64. Taira J, Nanbu H, Ueda K (2009) Nitric oxide-scavenging compounds in *Agrimonia pilosa* Ledeb on LPS-induced RAW264.7 macrophages. Food Chem 115:1221–1227. https://doi.org/10.1016/j.foodchem.2009.01.030
65. Gentile C, Tesoriere L, Allegra M, Livrea MA, D'Alessio P (2004) Antioxidant betalains from cactus pear (*Opuntia ficus-indica*) inhibit endothelial ICAM-1 expression. Ann N Y Acad Sci 1028:481–486. https://doi.org/10.1196/annals.1322.057
66. Netzel M, Stintzing FC, Quaas D, Strass G, Carle R, Bitsch R, Bitsch I, Frank T (2005) Renal excretion of antioxidative constituents from red beet in humans. Food Res Int 38:1051–1058. https://doi.org/10.1016/j.foodres.2005.03.016
67. Lu X, Wang Y, Zhang Z (2009) Radioprotective activity of betalains from red beets in mice exposed to gamma irradiation. Eur J Pharmacol 615:223–227. https://doi.org/10.1016/j.ejphar.2009.04.064
68. Alimi H, Hfaeidh N, Bouoni Z, Sakly M, Ben Rhouma K (2012) Protective effect of *Opuntia ficus indica* f. inermis prickly pear juice upon ethanol-induced damages in rat erythrocytes. Alcohol 46:235–243. https://doi.org/10.1016/j.alcohol.2011.09.024
69. Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL (1996) Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environ Health Perspect 104:1296–1300
70. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K et al (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2095–2128. https://doi.org/10.1016/S0140-6736(12)61728-0
71. Miller S, Stagl J, Wallerstedt DB, Ryan M, Mansky PJ (2008) Botanicals used in complementary and alternative medicine treatment of cancer: clinical science and future perspectives. Expert Opin Investing Drugs 17:1353–1364. https://doi.org/10.1517/13543784.17.9.1353
72. Kumar SS, Manoj P, Giridhar P, Shrivastava R, Bharadwaj M (2015) Fruit extracts of *Basella rubra* that are rich in bioactives and betalains exhibit antioxidant activity and cytotoxicity against human cervical carcinoma cells. J Funct Foods 15:509–515. https://doi.org/10.1016/j.jff.2015.03.052

73. Reddy MK, Alexander-Lindo RL, Nair MG (2005) Relative inhibition of lipid peroxidation, cyclooxygenase enzymes, and human tumor cell proliferation by natural food colors. J Agric Food Chem 53:9268–9273. https://doi.org/10.1021/jf051399j
74. Nowacki L, Vigneron P, Rotellini L, Cazzola H, Merlier F, Prost E, Ralanairina R, Gadonna J-P, Rossi C, Vayssade M (2015) Betanin-enriched red beetroot (*Beta vulgaris* L.) extract induces apoptosis and autophagic cell death in MCF-7 cells. Phytother Res 29: 1964–1973. https://doi.org/10.1002/ptr.5491
75. Lee EJ, An D, Nguyen CTT, Patil BS, Kim J, Yoo KS (2014) Betalain and betaine composition of greenhouse- or field-produced beetroot (*Beta vulgaris* L.) and inhibition of HepG2 cell proliferation. J Agric Food Chem 62:1324–1331. https://doi.org/10.1021/jf404648u
76. Kapadia GJ, Tokuda H, Konoshima T, Nishino H (1996) Chemoprevention of lung and skin cancer by *Beta vulgaris* (beet) root extract. Cancer Lett 100:211–214. https://doi.org/10.1016/0304-3835(95)04087-0
77. Govind JK, Magnus AA, Rao GS, Takanari A, Akira I, Harukuni T (2011) Cytotoxic effect of the red beetroot (*Beta vulgaris* L.) extract compared to doxorubicin (adriamycin) in the human prostate (PC-3) and breast (MCF-7) cancer cell lines. Anti Cancer Agents Med Chem 11:280–284. https://doi.org/10.2174/187152011795347504
78. Szaefer H, Krajka-Kuzniak V, Ignatowicz E, Adamska T, Baer-Dubowska W (2014) Evaluation of the effect of beetroot juice on DMBA-induced damage in liver and mammary gland of female Sprague–Dawley rats. Phytother Res 28:55–61. https://doi.org/10.1002/ptr.4951
79. Vulic J, Canadanovic-Brunet J, Cetkovic G, Tumbas V, Djilas S, Cetojevic-Simin D, Canadanovic V (2012) Antioxidant and cell growth activities of beet root pomace extracts. J Funct Foods 4:670–678. https://doi.org/10.1016/j.jff.2012.04.008
80. Kapadia GJ, Kapadia GJ, Azuine MA, Sridhar R, Okuda Y, Tsuruta A et al (2003) Chemoprevention of DMBA-induced UV-B promoted, NOR-1-induced TPA promoted skin carcinogenesis, and DEN-induced phenobarbital promoted liver tumors in mice by extract of beetroot. Pharmacol Res 47:141–148. https://doi.org/10.1016/S1043-6618(02)00285-2
81. Lechner JF, Lechner JF, Wang LS, Rocha CM, Larue B, Henry C et al (2010) Drinking water with red beetroot food color antagonizes esophageal carcinogenesis in N- nitrosomethylbenzylamine-treated rats. J Med Food 13:733–739. https://doi.org/10.1089/jmf.2008.0280
82. Kapadia GJ, Rao GS, Ramachandran C, Iida A, Suzuki N, Tokuda H (2013) Synergistic cytotoxicity of red beetroot (*Beta vulgaris* L.) extract with doxorubicin in human pancreatic, breast and prostate cancer cell lines. J Complement Integr Med 10:113–122. https://doi.org/10.1515/jcim-2013-0007
83. D-m Z, Brewer M, Garcia F, Feugang JM, Wang J et al (2005) Cactus pear: a natural product in cancer chemoprevention. Nutr J 4:25. https://doi.org/10.1186/1475-2891-4-25
84. Sreekanth D, Arunasree MK, Roy KR, Chandramohan RT, Reddy GV, Reddanna P (2007) Betanin a betacyanin pigment purified from fruits of *Opuntia ficus-indica* induces apoptosis in human chronic myeloid leukemia cell line-K562. Phytomed 14:739–746. https://doi.org/10.1016/j.phymed.2007.03.017
85. Serra AT, Matias AA, Almeida APC, Bronze MR, Alves PM et al (2011) Processing cherries (*Prunus avium*) using supercritical fluid technology. Part 2. Evaluation of SCF extracts as promising natural chemotherapeutical agents. J Supercrit Fluids 55:1007–1013. https://doi.org/10.1016/j.supflu.2010.06.006
86. Serra AT, Poejo J, Matias AA, Bronze MR, Duarte CMM (2013) Evaluation of Opuntia spp. derived products as antiproliferative agents in human colon cancer cell line (HT29). Food Res Int 54:892–901. https://doi.org/10.1016/j.foodres.2013.08.043
87. Kim H, Choi H-K, Moon JY, Kim YS, Mosaddik AC, Somi K (2011) Comparative antioxidant and antiproliferative activities of red and white pitayas and their correlation with flavonoid and polyphenol content. J Food Sci 76:C38–C45. https://doi.org/10.1111/j.1750-3841.2010.01908.x

88. Arya V, Kashyap CP, Tikka B, Sharma S, Kumari S et al (2011) Human cancer cell lines-a brief communication. J Chem Pharm Res 3:514–520
89. HogenEsch H, Nikitin AY (2012) Challenges in pre-clinical testing of anti-cancer drugs in cell culture and in animal models. J Control Release 164:183–186. https://doi.org/10.1016/j.jconrel.2012.02.031
90. Allegra M, Tesoriere L, Livrea MA (2007) Betanin inhibits the myeloperoxidase/nitrite-induced oxidation of human low-density lipoproteins. Free Radic Res 41:335–341. https://doi.org/10.1080/10715760601038783
91. Tesoriere L, Allegra M, Butera D, Livrea MA (2004) Supplementation with cactus pear (*Opuntia ficus-indica*) fruit decreases oxidative stress in healthy humans: a comparative study with vitamin C. Am J Clin Nutr 80:391–395
92. Siriwardhana N, Jeon YJ (2004) Antioxidative effect of cactus pear fruit (*Opuntia ficus-indica*) extract on lipid peroxidation inhibition in oils and emulsion model systems. Eur Food Res Technol 219:369–376. https://doi.org/10.1007/s00217-004-0956-8
93. Tesoriere L, Butera D, D'Arpa D, Di Gaudio F, Allegra M et al (2003) Increased resistance to oxidation of betalain-enriched human low density lipoproteins. Free Radic Res 37:689–696. https://doi.org/10.1080/1071576031000097490
94. Clemente A, Desai PV (2011) Evaluation of the hematological, hypoglycemic, hypolipidemic and antioxidant properties of *Amaranthus tricolor* leaf extract in rat. Trop J Pharm Res 10:595–602
95. Sani HA, Baharoom A, Ahmad MA, Ismail II (2009) Effectiveness of *Hylocereus polyrhizus* extract in decreasing serum lipids and liver MDA-TBAR level in hypercholesterolemic rats. Sains Malays 38:271–279
96. Wroblewska M, Juskiewicz J, Wiczkowski W (2011) Physiological properties of beetroot crisps applied in standard and dyslipidaemic diets of rats. Lipids Health Dis 10:178. https://doi.org/10.1186/1476-511x-10-178
97. Rashed MM, Shallan M, Mohamed DA, Fouda K, Hanna LM (2010) Hypolipidemic effect of vegetable and cereal dietary mixtures from Egyptian sources. Grasas Aceites 61:261–270. https://doi.org/10.3989/gya.111709
98. Hilou A, Nacoulma OG, Guiguemde TR (2006) In vivo antimalarial activities of extracts from *Amaranthus spinosus* L. and *Boerhaavia erecta* L. in mice. J Ethnopharmacol 103:236–240. https://doi.org/10.1016/j.jep.2005.08.006
99. Velićanski AS, Cvetković DD, Markov SL, Vulić JJ, Dilas SM (2011) Antibacterial activity of *Beta vulgaris* L. pomace extract. Acta Period Technol 2011(42):263–269. https://doi.org/10.2298/APT1142263V
100. Canadanovic-Brunet JM, Savatovic SS, Cetkovic GS, Vulic JJ, Đjilas SM et al (2011) Antioxidant and antimicrobial activities of beet root pomace extracts. Czech J Food Sci 29:575–585
101. Rauha J-P, Remes S, Heinonen M, Hopia A, Kähkönen M et al (2000) Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol 56:3–12. https://doi.org/10.1016/S0168-1605(00)00218-X
102. Hayek SA, Ibrahim SA (2012) Antimicrobial activity of xoconostle pears (*Opuntia matudae*) against *Escherichia coli* O157: H7 in laboratory medium. Int J Microbiol. https://doi.org/10.1155/2012/368472
103. Tenore GC, Novellino E, Basile A (2012) Nutraceutical potential and antioxidant benefits of red pitaya (*Hylocereus polyrhizus*) extracts. J Funct Foods 4:129–136. https://doi.org/10.1016/j.jff.2011.09.003
104. Yong YY, Dykes G, Lee SM, Choo WS (2017) Comparative study of betacyanin profile and antimicrobial activity of red pitahaya (*Hylocereus polyrhizus*) and red spinach (*Amaranthus dubius*). Plant Foods Hum Nutr 72:41–47. https://doi.org/10.1007/s11130-016-0586-x
105. Sigurdson GT, Tang PP, Giusti MM (2017) Natural colorants: food colorants from natural sources. Annu Rev Food Sci Technol 8:261–280. https://doi.org/10.1146/annurev-food-030216-025923

106. Downham A, Collins P (2000) Colouring our foods in the last and next millennium. Int J Food Sci Technol 35:5–22. https://doi.org/10.1046/j.1365-2621.2000.00373.x
107. Francis FJ (2002) Food colorings. In: MacDougall DB (ed) Colour in food – improving quality. Woodhead Publishing, Cambridge
108. Stintzing FC, Carle R (2007) Betalains – emerging prospects for food scientists. Trends Food Sci Technol 18:514–525. https://doi.org/10.1016/j.tifs.2007.04.012
109. Shenoy VR (1993) Anthocyanins-prospective food colours. Curr Sci 64:575–579
110. Francis FJ (2000) Anthocyanins and betalains: composition and applications. Cereal Foods World 45:208–213
111. Driver MG, Francis FJ (1979) Purification of phytolaccanin (betanin) by removal of phytolaccatoxin from *Phytolacca americana*. J Food Sci 44:521–523. https://doi.org/10.1111/j.1365-2621.1979.tb03826.x
112. Driver MG, Francis FJ (1979) Stability of phytolaccanin, betanin and FD&C Red #2 in dessert gels. J Food Sci 44:518–520. https://doi.org/10.1111/j.1365-2621.1979.tb03825.x
113. Castellar MR, Obon JM, Fernandez-Lopez J (2006) The isolation and properties of a concentrated red-purple betacyanin food colourant from *Opuntia stricta* fruits. J Sci Food Agric 86:122–128. https://doi.org/10.1002/jsfa.2285
114. Fernandez-Lopez JA, Angosto JM, Gimenez PJ, Leon G (2013) Thermal stability of selected natural red extracts used as food colorants. Plant Foods Hum Nutr 68:11–17. https://doi.org/10.1007/s11130-013-0337-1
115. Esatbeyoglu T, Wagner AE, Schini-Kerth VB, Rimbach G (2015) Betanin-a food colorant with biological activity. Mol Nutr Food Res 59:36–47. https://doi.org/10.1002/mnfr.201400484
116. Counsell JN, Jeffries GS, Knewstubb CJ (1979) Natural colors for foods and other uses. In: Counsell JN, Dunastable JA (eds) Some other natural colors and their applications. Applied Science, London
117. von Elbe JH (1977) The betalaines. In: Furia ET (ed) Current aspects of food colorants. CRC Press, Boca Raton
118. Block JE, Amundson CH, von Elbe JH (1981) Energy requirements of beet colorant production. J Food Process Eng 5:67–75. https://doi.org/10.1111/j.1745-4530.1981.tb00262.x
119. Clydesdale FM (1993) Color as a factor in food choice. Crit Rev Food Sci Nutr 33:83–101. https://doi.org/10.1080/10408399309527614
120. Chattopadhyay P, Chatterjee S, Sen SK (2008) Biotechnological potential of natural food grade biocolorants. African. J Biotechnol 7:2972–2985
121. Moßhammer MR, Stintzing FC, Carle R (2005) Development of a process for the production of a betalain-based colouring foodstuff from cactus pear. Innovative Food Sci Emerg Technol 6:221–231. https://doi.org/10.1016/j.ifset.2005.02.001
122. Castellar R et al (2003) Color properties and stability of betacyanins from Opuntia fruits. J Agric Food Chem 51:2772–2776. https://doi.org/10.1016/j.ifset.2005.02.001
123. Obon JM et al (2009) Production of a red-purple food colorant from *Opuntia stricta* fruits by spray drying and its application in food model systems. J Food Eng 90:471–479. https://doi.org/10.1016/j.jfoodeng.2008.07.013
124. Cai M, Sun M, Corke H (2001) Identification and distribution of simple and acylated betacyanins in the Amaranthaceae. J Agric Food Chem 49:1971–1978. https://doi.org/10.1021/jf000963h
125. Cai Y, Sun M, Wu H, Huang R, Corke H (1998) Characterization and quantification of betacyanin pigments from diverse *Amaranthus species*. J Agric Food Chem 46:2063–2070. https://doi.org/10.1021/jf9709966
126. Cai YZ, Sun M, Corke H (2005) Characterization and application of betalain pigments from plants of the Amaranthaceae. Trends Food Sci Technol 16:370–376. https://doi.org/10.1016/j.tifs.2005.03.020
127. Cai Y, Corke H (1999) *Amaranthus* betacyanin pigments applied in model food systems. J Food Sci 64:869–873. https://doi.org/10.1111/j.1365-2621.1999.tb15930.x

128. Gengatharan A, Dykes GA, Choo WS (2016) Stability of betacyanin from red pitahaya (*Hylocereus polyrhizus*) and its potential application as a natural colourant in milk. Int J Food Sci Technol 51:427–434. https://doi.org/10.1111/ijfs.12999
129. Gengatharan A, Dykes GA, Choo WS (2017) The effect of pH treatment and refrigerated storage on natural colourant preparations (betacyanins) from red pitahaya and their potential application in yoghurt. LWT Food Sci Technol 80:437–445. https://doi.org/10.1016/j.lwt.2017.03.014
130. Saenz C (2000) Processing technologies: an alternative for cactus pear (*Opuntia spp*.) fruits and cladodes. J Arid Environ 46:209–225. https://doi.org/10.1006/jare.2000.0676
131. Bazaria B, Kumar P (2016) Compositional changes in functional attributes of vacuum concentrated beetroot juice. J Food Process Preserv 40:1215–1222. https://doi.org/10.1111/jfpp.12705
132. Herbach KM, Stintzing FC, Carle R (2004) Impact of thermal treatment on color and pigment pattern of red beet (*Beta vulgaris* L.) preparations. J Food Sci 69:C491–C498
133. Kathiravan T, Nadanasabapathi S, Kumar R (2014) Standardization of process condition in batch thermal pasteurization and its effect on antioxidant, pigment and microbial inactivation of ready to drink (RTD) beetroot (*Beta vulgaris* L.) juice. Int Food Res J 21:1305
134. Kumar S, Kumar P (2015) Rheological modeling of non-depectinized beetroot juice concentrates. J Food Meas Charact 9:487–494. https://doi.org/10.1007/s11694-015-9257-0
135. Vanajakshi V, Vijayendra SVN, Varadaraj MC, Venkateswaran G, Agrawal R (2015) Optimization of a probiotic beverage based on Moringa leaves and beetroot. LWT Food Sci Technol 63:1268–1273. https://doi.org/10.1016/j.lwt.2015.04.023
136. Slavov A, Karagyozov V, Denev P, Kratchanova M, Kratchanov C (2013) Antioxidant activity of red beet juices obtained after microwave and thermal pretreatments. Czech J Food Sci 31:139–147
137. Czyzowska A, Klewicka E, Libudzisz Z (2006) The influence of lactic acid fermentation process of red beet juice on the stability of biologically active colorants. Eur Food Res Technol 223:110–116. https://doi.org/10.1007/s00217-005-0159-y
138. Guldiken B, Toydemir G, Memis KN, Okur S, Boyacioglu D, Capanoglu E (2016) Home-processed red beetroot *(Beta vulgaris L.) products*: changes in antioxidant properties and bioaccessibility. Int J Mol Sci 17:858. https://doi.org/10.3390/ijms17060858
139. Moussa-Ayoub TE, Jaeger H, Youssef K, Knorr D, El-Samahy S (2016) Technological characteristics and selected bioactive compounds of *Opuntia dillenii* Cactus fruit juice following the impact of pulsed electric field pre-treatment. Food Chem 210:249–261. https://doi.org/10.1016/j.foodchem.2016.04.115
140. Moßhammer MR, Maier C, Stintzing FC, Carle R (2006) Impact of thermal treatment and storage on color of yellow-orange cactus pear (*Opuntia ficus-indica* L Mill. cv. 'Gialla') juices. J Food Sci 71:C400–C406. https://doi.org/10.1111/j.1750-3841.2006.00134.x
141. Jimenez-Aguilar DM, Escobedo-Avellaneda Z, Martin-Belloso O, Gutierrez-Uribe J, Valdez-Fragoso A (2015) Effect of high hydrostatic pressure on the content of phytochemical compounds and antioxidant activity of prickly pears (*Opuntia ficus-indica*) beverages. Food Eng Rev 7:198–208. https://doi.org/10.1007/s12393-015-9111-5
142. Stintzing FC, Schieber A, Carle R (2003) Evaluation of colour properties and chemical quality parameters of cactus juices. Eur Food Res Technol 216:303–311. https://doi.org/10.1007/s00217-002-0657-0
143. Castellar MR, Obon JM, Alacid M, Fernandez-Lopez JA (2008) Fermentation of *Opuntia stricta* (Haw.) fruits for betalains concentration. J Agric Food Chem 56:4253–4257. https://doi.org/10.1021/jf703699c
144. Castro-Munoz R, Barragan-Huerta BE, Yanez-Fernandez J (2015) Use of gelatin- maltodextrin composite as an encapsulation support for clarified juice from purple cactus pear (*Opuntia stricta*). LWT Food Sci Technol 62:242–248. https://doi.org/10.1016/j.lwt.2014.09.042
145. Herbach KM, Stintzing FC, Carle R (2004) Thermal degradation of betacyanins in juices from purple pitaya *Hylocereus polyrhizus* (Weber) Britton & Rose monitored by high performance

liquid chromatography-tandem mass spectometric analyses. Eur Food Res Technol 219:377–385. https://doi.org/10.1007/s00217-002-0657-0
146. Wong YM, Siow LF (2015) Effects of heat, pH, antioxidant, agitation and light on betacyanin stability using red-fleshed dragon fruit (*Hylocereus polyrhizus*) juice and concentrate as models. J Food Sci Technol-Mysore 52:3086–3092. https://doi.org/10.1007/s13197-014-1362-2
147. Gandia-Herrero F, Cabanes J, Escribano J, Garcia-Carmona F, Jimenez-Atienzar M (2013) Encapsulation of the most potent antioxidant betalains in edible matrixes as powders of different colors. J Agric Food Chem 61:4294–4302. https://doi.org/10.1021/jf400337g
148. Gandia-Herrero F, Jimenez-Atienzar M, Cabanes J, Garcia-Carmona F, Escribano J (2010) Stabilization of the bioactive pigment of *Opuntia fruits* through maltodextrin encapsulation. J Agric Food Chem 58:10646–10652. https://doi.org/10.1021/jf101695f
149. Gokhale SV, Lele SS (2011) Dehydration of red beet root (*Beta vulgaris*) by hot air drying: process optimization and mathematical modeling. Food Sci Biotechnol 20:955–964. https://doi.org/10.1007/s10068-011-0132-4
150. Kaimainen M, Laaksonen O, Järvenpää E, Sandell M, Huopalahti R (2015) Consumer acceptance and stability of spray dried betanin in model juices. Food Chem 187:398–406. https://doi.org/10.1016/j.foodchem.2015.04.064
151. Koul VK, Jain MP, Koul S, Sharma VK, Tikoo CL, Jain SM (2002) Spray drying of beet root juice using different carriers. Indian J Chem Technol 9:442–445
152. Nemzer B, Pietrzkowski Z, Sporna A, Stalica P, Thresher W et al (2011) Betalainic and nutritional profiles of pigment-enriched red beet root (*Beta vulgaris L.*) dried extracts. Food Chem 127:42–53. https://doi.org/10.1016/j.foodchem.2010.12.081
153. Ravichandran K, Palaniraj R, Saw N, Gabr AMM, Ahmed AR (2014) Effects of different encapsulation agents and drying process on stability of betalains extract. J Food Sci Technol Mysore 51:2216–2221. https://doi.org/10.1007/s13197-012-0728-6
154. Robert P, Torres V, Garcia P, Vergara C, Saenz C (2015) The encapsulation of purple cactus pear (*Opuntia ficus-indica*) pulp by using polysaccharide-proteins as encapsulating agents. LWT Food Sci Technol 60:1039–1045. https://doi.org/10.1016/j.lwt.2014.10.038
155. Saponjac VT, Canadanovic-Brunet J, Cetkovic G, Jakisic M, Djilas S (2016) Encapsulation of beetroot pomace extract: RSM optimization, storage and gastrointestinal stability. Molecules 21:584. https://doi.org/10.3390/molecules21050584
156. Vergara C, Saavedra J, Saenz C, Garcia P, Robert P (2014) Microencapsulation of pulp and ultrafiltered cactus pear (*Opuntia ficus-indica*) extracts and betanin stability during storage. Food Chem 157:246–251. https://doi.org/10.1016/j.foodchem.2014.02.037
157. Gutierrez TJ, Guzman R, Jaramillo CM, Fama L (2016) Effect of beet flour on films made from biological macromolecules: native and modified plantain flour. Int J Biol Macromol 82:395–403. https://doi.org/10.1016/j.ijbiomac.2015.10.020
158. Zamudio-Flores PB, Ochoa-Reyes E, Ornelas-Paz JD, Aparicio-Saguilan A, Vargas-Torres A et al (2015) Effect of storage time on physicochemical and textural properties of sausages covered with oxidized banana starch film with and without betalains. CyTA-J Food 13:456–463. https://doi.org/10.1080/19476337.2014.998713
159. Jin SK, Choi JS, Moon SS, Jeong JY, Kim GD (2014) The assessment of red beet as a natural colorant, and evaluation of quality properties of emulsified pork sausage containing red beet powder during cold storage. Korean J Food Sci Anim Resour 34:472–481. https://doi.org/10.5851/kosfa.2014.34.4.472
160. Prudencio ID, Prudencio ES, Gris EF, Romazi T, Bordignon-Luiz MT (2008) Petit suisse manufactured with cheese whey retentate and application of betalains and anthocyanins. LWT Food Sci Technol 41:905–910. https://doi.org/10.1016/j.lwt.2007.05.019
161. Mridula D, Gupta RK, Bhadwal S, Khaira H, Tyagi SK (2016) Optimization of food materials for development of nutritious pasta utilizing groundnut meal and beetroot. J Food Sci Technol Mysore 53:1834–1844. https://doi.org/10.1007/s13197-015-2067-x

162. Vareltzis K, Buck EM, Labbe RG (1984) Effectiveness of a betalains potassium sorbate system versus sodium nitrite for color development and control of total aerobes, *Clostridium perfringens and Clostridium sporogenes* in chicken frankfurters. J Food Prot 47:532–536
163. Juvvi P, Chakkaravarthi A, Debnath S (2016) Emerging technique for healthier frying for production of reduced-fat beetroot (*Beta vulgaris*) chips. J Food Sci Technol Mysore 53:3502–3511. https://doi.org/10.1007/s13197-016-2326-5
164. Moussa-Ayoub TE, Youssef K, El-Samahy SK, Kroh LW, Rohn S (2015) Flavonol profile of cactus fruits (*Opuntia ficus-indica*) enriched cereal-based extrudates: authenticity and impact of extrusion. Food Res Int 78:442–447. https://doi.org/10.1016/j.foodres.2015.08.019
165. Msaddak L, Abdelhedi O, Kridene A, Rateb M, Belbahri L (2017) *Opuntia ficus-indica* cladodes as a functional ingredient: bioactive compounds profile and their effect on antioxidant quality of bread. Lipids Health Dis 16:32. https://doi.org/10.1186/s12944-016-0397-y
166. Zhu F, Cai YZ, Sun M, Corke H (2008) Influence of *Amaranthus* betacyanin pigments on the physical properties and color of wheat flours. J Agric Food Chem 56:8212–8217. https://doi.org/10.1021/jf801579c

Nutraceutical Potential of Guava

50

Moni Gupta, Anshu Wali, Anjali, Sachin Gupta, and Sudheer K. Annepu

Contents

M. Gupta (✉) · A. Wali · Anjali
Division of Biochemistry, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu, Jammu and Kashmir, India
e-mail: moniguptaskuast@gmail.com

S. Gupta (✉)
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, India
e-mail: sachinmoni@gmail.com

S. K. Annepu
Divison of Crop Improvement, ICAR-Directorate of Mushroom Research, Solan, Himachal Pradesh, India
e-mail: sudheerannepu@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_85

Abstract

Psidium guajava commonly known as guava is one of the economical fruit crops belonging to the Myrtaceae family and grows in tropical and subtropical region. Largely grown as wild crop or selection variants, disease-free quality planting materials for establishment of guava orchard is necessary. The fruit is also labeled as super-fruit, and because of its unique flavor, taste, and health-promoting qualities, it is regarded as functional food or potent nutraceutical. It is rich in antioxidant compounds and contains a high level of ascorbic acid content, carotenoids, and phenolic compounds. It is acclaimed as the "poor man's apple of the tropic." Traditionally guava leaves and fruits are used in folk medicine for the treatment of various ailments like diarrhea, flatulence, gastric pain, wounds, rheumatism, ulcers, etc. Guavas possess antioxidant, antimicrobial, anticancer, antidiabetic, and anti-inflammatory activities, supporting a great therapeutic potential and a wide range of clinical applications. The important active biochemical compounds in guava are essential oils, phenolics, flavonoids, carotenoids, triterpenoids, esters, aldehydes, etc. Guava fruit is highly perishable, so to increase its shelf life, it may be processed into various value-added products like guava juices, squash, nectar, leather, jam, jellies, powder, etc. This chapter describes in detail about the guava plant, ecological requirements for its growth, various methods of its propagations, current knowledge about its nutraceutical properties and its application in preparing guava-based value-added products.

Keywords

Psidium guajava · Guava · Cultivation · Phytochemicals · Bioactive potential · Nutraceutical · Value-added products

1 Introduction

Guava (*Psidium guajava Linn.*) belonging to the family Myrtaceae, with about 133 genera and more than 3,800 species, is native to Mexico, Central America, the Caribbean, and the Northern part of South America [1]. It is also grown in all the tropical and subtropical areas of the world including India and adapts to different climatic conditions but prefers dry climates. It is widely grown in tropics with its fruit widely known for its exotic flavor and potent aroma. Guava is a traditionally used plant because of its immense food and nutrition value. World production of guava is estimated to be about 1.2 million tons; however India and Pakistan contributes to about 50 percent of the world production [2]. Guava is also known as poor man's fruit or apple of tropics. The common varieties of guava are the red

(*P. guajava* var. *pomifera*) and the white (*P. guajava* var. *pyrifera*) [3, 4]. Various parts of the guava plant, viz., root, bark, leaves, and fruits, are found to possess many pharmacological properties as it is used in the treatment of various disorders [5]. Guava is rich in minerals and functional components such as vitamins and phenolic compounds which makes beneficial contribution to the human diet and is well accepted by the consumers [6]. All parts of this tree, including fruits, leaves, seeds, bark, and roots, have been utilized traditionally in many countries for treatment of looseness of the bowels, menstrual disarranges, vertigo, anorexia, stomach-related issues, gastric deficiency, aroused mucous layer, laryngitis, skin issues, ulcers, vaginal discharge, cold, cough, cerebral ailments, nephritis, jaundice, diabetes, malaria, and rheumatism [7–9]. It additionally has anticancer properties. The leaves of the guava tree are full of antioxidants, anti-inflammatory agents, antibacterial, and even tannins that can have significant health benefits, from treating stomach troubles to chronic diseases like cancer [10]. The fruit is labeled as super-fruit, and because of its unique flavor, taste, and health-promoting qualities, it is regarded as functional food or potent nutraceutical [11]. The term nutraceutical was coined by Stephen DeFelice in 1989 from nutrition and pharmaceuticals. It is defined as a food that provides health benefits, including prevention and cure of a disease [12]. The term nutraceuticals as commonly used in marketing has no regulatory definition [13]. The concept of nutraceuticals, functional food, designer food, phytochemicals, bioactive compounds, etc. is misnomers and quite confusing and are often used interchangeably. The supplementary diets or dietary supplements however differ from nutraceutical in many ways. Nutraceuticals are not only supplementary food included in diets but also conventional food which is part of the daily diet having health benefitting potentials. Nutraceuticals are also referred to as natural functional food or bioactive phytochemicals that have health-promoting, disease-/disorder-preventing, or medicinal properties.

These nutraceutical contains the macro- and micronutrients like carbohydrates, proteins, fats, lipids, vitamins, minerals, antioxidants, etc.; however, bioactive compounds are minor components of food and are defined as having the following characteristics: they are present in low concentrations; they are not considered to be nutrients; and they have a proven health effect [14]. Interaction between functional food components such as prebiotics, probiotics, phytochemicals, and intestinal microflora has consequences on human health [2].

2 Ecological Requirements

Guava can be grown under a wide range of climatic conditions. Compared to other fruit crops, guava is highly resistant to drought. As compared to the tropical areas, those with distinct winter promote production of abundant crop with better quality. In the tropics and subtropics, guava is found from sea level to an altitude of 1500 m asl. Guava trees growing at lower elevation are generally vigorous with heavy fruit set. Sandy loamy soils with the pH range of 5.5–7.5 are highly suitable for growing guava. However, it can be grown with minimum care in marginal and soils affected

with salinity. A temperature range of 23–28 °C during flowering to fruiting is found to be optimum. Temperatures lower than 7–8 °C cease the plant growth, and the leaves turn to purple. Natural defoliation occurs during the low winter temperatures and the flowering induced with rise in temperature and increased soil moisture. A rainfall pattern with alternating dry and wet conditions is highly suitable for maximum fruit set and high yields.

3 Cultivation

3.1 Cultivars

Majority of the cultivars of guava have been evolved after selection from seedling variants. Several promising genotypes from other countries have also been introduced into India by researchers. Even though many varieties are available in India, Allahabad Safeda and Sardar (L-49) occupied maximum share owing to its yield and market acceptability. Recently ICAR-IIHR, Bengaluru, released a pink-fleshed variety named as Lalit that has attained a wide popularity across the guava-growing regions of the country. Apart from these prominent cultivars, some of the cultivars grown in India and their important varietal characters are described in below table.

S. no	Cultivar	Developed/ released by	Characters
1	Allahabad Safeda	CISH, Lucknow	The fruits are relatively soft and contain less number of seeds. Suitable for both table and processing purposes
2	Sardar	CISH, Lucknow	Fruits with white flesh and more number seeds. Skin is harder than Allahabad Safeda
3	Lalit	CISH, Lucknow	Suitable for high-density planting. Epicarp is in saffron yellow color, and the flesh is in red blush color. High yielder compared to the existing commercial varieties in India
4	Shweta	CISH, Lucknow	Fruits are very attractive with white creamy epicarp and red spots of blush; flesh is snow white in color
5	Allahabad Surkha	CISH, Lucknow	Large and uniform pink color fruits with deep pink color flesh. The fruit contains strong flavor with less number of seeds
6	Pant Prabhat	GBPUA&T, Pant Nagar	Fruits with smooth peel and light yellow in color. White color pulp with small seeds and high flavor
7	Arka Mridula	ICAR-IIHR, Bengaluru	Pulp is white in color with sweet taste. Keeping quality is good
8	Arka Amulya	ICAR-IIHR, Bengaluru	Medium-sized fruits with white and sweet pulp. Pulp contains round-shaped small number of seeds. Keeping quality is good
9	Arka Kiran	ICAR-IIHR, Bengaluru	Fruits with firm pulp and pink in color

(*continued*)

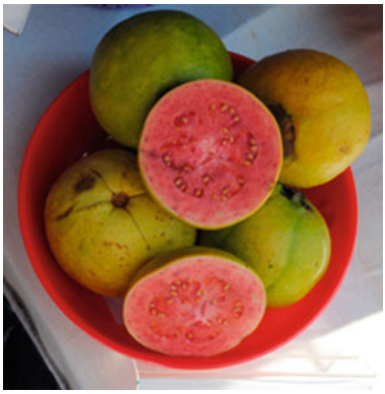
S. no	Cultivar	Developed/ released by	Characters
10	Arka Rashmi	ICAR-IIHR, Bengaluru	Medium-sized fruits with deep pink color pulp and soft seeds
11	Hisar Safeda	CCSHAU, Hisar	Medium-sized round-shaped fruits with smooth and yellowish green surface. Pulp is creamy white in color with few number of seeds
12	Hisar Surkha	CCSHAU, Hisar	Medium-sized round-shaped fruits with smooth and yellowish green surface. Pulp is pink in color with harder seeds

Allahabad Safeda

Sardar

Lalit

Arka Amulya



Arka Rashmi

Arka Kiran

Photos courtesy: ICAR-Indian Institute of Horticultural Research, Bengaluru

Apple color, Anakapalli, Banarasi Surkha, Chittidar, Dholka, Dharwar, Habsi, Karela, Punjab smooth, Sangam, Mizapuri seedling, Nasik smooth white, Thailand guava, Philippine guava, Florida seedling, etc. are some of the locally grown cultivars available in India.

3.2 Propagation

In spite of large number of private and public sector nurseries, still there is a shortage of disease-free quality planting materials for establishment of guava orchard. Mass multiplication through vegetative propagation results in uniform crop with relatively short pre-bearing period in guava compared to the seed propagation. Out of the several methods of vegetative propagation, air layering, wedge grafting, budding, and stooling are found better for raising the productive seedlings in guava [15]. Healthy, disease-free, and vigorous mother trees should be selected for raising the nursery stock.

3.2.1 Air Layering

In this method, the healthy branches of 1.2 cm or more diameter are selected and then girdled by removing a strip of bark with the width of about 2 cm. The girdled area is then covered with the sphagnum and wrapped with the polythene film. Within 3–4 weeks, the roots start developing from the layered portion. The rooted layers are detached from the mother plant and can be used for planting. Rainy season is the best season for air layering.

3.2.2 Wedge Grafting

The rootstock selected for grafting is split to about 4–4.5 cm with a knife, and wedge-shaped cut slanting from both the sides is made on the lower side of the scion material. The scion material is inserted in to the rootstock split and pressed properly to bring both the materials into contact with each other. The union is then tied with the polythene strip. The scion starts sprouting after 9–12 days and should be transferred for hardening after 1 month.

3.2.3 Budding

Patch budding is considered to be the efficient method of propagation in guava with high success rate. In this method, healthy seedlings of 1 year old are selected, and 1–1.5 cm long patch is removed from the rootstock. Unsprouted, dormant buds selected from the leaf axils of scion variety is fitted into the rootstock patch and tied with the polythene strip. After 2–3 weeks, the strips should be removed to examine the success of the budding.

3.2.4 Stooling

In this method, self-rooted plants are planted 0.5 m apart in the stooling bed and then allowed to grow for about 3 years. Then these are cut down to the ground level, and new shoots emerges on the stumps. All these shoots are mounted with the soil to a

height of 30 cm. On the onset of monsoon, the shoots are detached from the mother plant and can be used for direct planting.

3.3 Layout and Planting Systems

This refers to the planting of trees in an orderly manner to ensure the maximum number of trees per unit area and to facilitate the smooth operation of intercultural activities. Planting systems such as square, rectangular, triangular, hexagonal, and quincuncial systems can be adopted based on the availability of land. After completion of layout, the pits of 75 cm × 75 cm × 75 cm are dug and left open for 15 days. The grafts are to be placed in the center of the pits and pressed tightly all around. The graft/bud union should remain well above the soil surface. A planting distance of 5 m × 5 m is recommended for healthy and vigorous growth of the plants. With this spacing 400 trees can be planted in one ha of land. High-density planting with the spacing of 3.0 m × 3.0 m and 1.5 m × 3.0 m is also recommended for specific varieties. Rows should be planted in North-South direction to facilitate maximum sunlight exposure. Pits should be watered copiously immediately after planting. Mulching can be done at the basins to conserve the moisture during the initial stages of establishment.

3.4 Crop Regulation

Flowering and fruiting in guava follow specific pattern and occur in two major seasons, once during March to May and the later in July–August [16]. The fruits from March to May flowering are harvested in rainy season, and the fruits from second flowering are harvested during winter, i.e., late October to mid-February. The July flowering gives more number of flowers and good quality of the fruits compared to the summer flowering. Orchard losses can be avoided by adopting effective crop regulation practices to manage the flowering in guava and for harvesting the good quality fruits. Spraying urea at 10–15% twice during bloom (April–May) eliminates the rainy season fruiting [17].

3.5 Crop Management

3.5.1 Orchard Management

Guava, being a perennial crop, needs utmost care while selecting the varieties and the quality planting material. Any mistakes committed in the early stages of the orchard management cannot be rectified at a later stage.

3.5.2 Nutrient and Water Management

Amount of manures and fertilizers to be applied to guava tress depends upon the age of tree and the soil characteristics. At the time of planting, each pit should be filled

with well rotten farm yard manure at 15–20 kg and 1.5 kg of single superphosphate. A recommended dose of 260 g urea + 375 g SSP + 100 g of MOP per plant should be supplied in the first year. The dose increases with each passing year.

The fertilizers are applied in two split doses preferably in June and September. Guava is highly prone to micronutrient deficiency and remedial measures should be taken immediately with foliar sprays of micronutrients. Furrow and basin method of irrigation are most popular in guava orchards [18]. In the initial stages of plant growth, it is imperative to maintain the optimum soil moisture by watering every alternate day. After 2 years of age, watering may be done once in every 7 to 10 days interval during the summers, and irrigation should be avoided during the rainy season. During the dry season, the flowering and fruiting will be greatly influenced by the water availability [19]. Sprinkler and drip irrigations can be adopted for increasing the productivity and quality of fruits [20].

3.6 Harvesting and Handling

The quality of guava fruit depends on the stage of maturity. The fruits are usually ready to harvest 4–5 months after the flowering. Guava fruits are generally handpicked. Color change, specific gravity, total soluble solids, acidity, etc. are the useful criteria to judge the maturity levels in guava [21]. At the time of maturity, the specific gravity becomes <1.0, and it starts floating on the water surface. The fruits with the specific gravity of 1.00–1.02 have better shelf life and suitable for long-distance transport. It is desirable to pick the fruits along with 5–7 cm stalk and two to three leaves. Proper grading and sorting increase the postharvest shelf and fetch high prices to the growers. At room temperature, the shelf life is for a few days only. The fruits picked during the winter season can be stored for 7–8 days at ambient temperatures whereas rainy season picked fruits can be stored only for 2–3 days, depending on the variety. At low temperatures of 10–15 °C, the shelf life can be extended up to 3–4 weeks [22]. For low temperature storage, fruits must be picked at hard, green, and immature stage without color break.

4 Morphology

Guava trees are little shrubby evergreen trees with a considerable measure of wide-spreading branches and square fleece twigs. The branches are screwy bringing inverse clear out. The blooms are white borne independently, or in little gatherings in axils of leaves of late development, petals are incurved; they are fragrant with four to six petals. Each blossom bears various white needlelike stamens which oblige smooth anthers. Self-fertilization is conceivable, yet cross-pollination by creepy crawlies brings about higher yields [23]. The organic product is little, 3 to 6 cm long in different shapes going from ovoid, round, to pear-formed contingent on species. The external skin might be unpleasant having a severe taste or delicate and sweet.

The skin shading is typically green before development however winds up noticeably yellow, maroon, or green when ready with tissue running from white, yellow, and pink to red differing with species. Natural product might be thin-shelled with many seeds installed in a firm mash or might be thick-shelled with fewer seeds. Kind of natural product shifts from sweet to profoundly corrosive. The distinctive fragrance ranges from solid and penetrating to mild and pleasant [24].

5 Nutritional Attributes

Guava is reported to possess rich nutritional attributes ranging from carbohydrates, minerals, vitamins, to antioxidants. The nutritional composition of guava has been reported to vary with cultivars, season, and environment. The main carbohydrate in the form of simple sugars has been identified as glucose and xylose [25]. Every 100 gram of fresh guava fruit servings in general contributes to 75–85% of moisture, 3–7% of total dietary fiber, 1–6% of protein, and 0.7 to 0.11% of lipids. Among mineral contents, potassium content (352.7 mg/100gft) is reported to be highest in fresh guava, followed by phosphorus (17.8–30 mg/100gft), calcium (9.1–17 mg/100gft), and iron (0.4–0.7 mg/100gft) [2, 26, 27]. It is also reported to contain many of the vitamins required by human body of which the vitamin C content (50–300 mg/100gft) is reported to be highest in guava [28].

In a study [29], it was observed that with the advancement of fruit maturity at different stages, the total soluble solid (TSS), sugar (total, reducing, and non-reducing), and ascorbic acid contents increased significantly, while during fruit ripening stage both acidity and pectin decreased. Of all the guava cultivars studied, L-49 showed highest TSS (12.250B), total sugar (8.50%), and ascorbic acid (265.09%) followed by Allahabad Safeda, while L-49 showed minimum acidity (0.26%) and maximum pectin content (0.77%). Pectin methyl esterase (PME) activity increased progressively in all the cultivars up to half ripe stage (HRS) and subsequently decreased at full ripe stage (FRS). Maximum PME activity was found in L-49 (56.25 units/g.f.wt) at HRS, whereas it showed a decrease at FRS (52.25 units/g fresh weight (FW) followed by Allahabad Safeda and Lalit. Thus, it was concluded that L-49 was superior among all the commercial cultivars of guava grown under subtropical condition followed by Allahabad Safeda and Lalit.

5.1 Phytochemicals

Phytochemicals (phyto, plant) are biologically active chemical compounds found in plants offering health advantages for humans in addition to those attributed to macronutrients and micronutrients [30]. Phenolic compounds are secondary metabolites which are produced in the shikimic acid of plants and pentose phosphate through phenylpropanoid pathway [31]. They contain benzene rings with one or more hydroxyl substituent and range from simple phenolic molecules to highly polymerized compounds [32]. The phytochemical analysis of guava leaves revealed

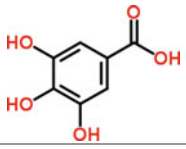
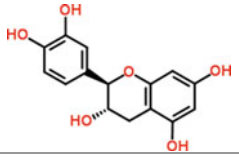
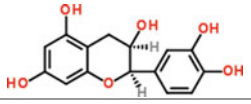
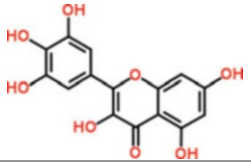
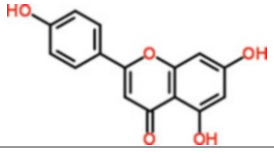
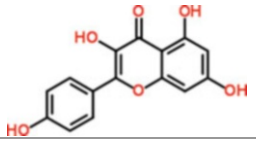
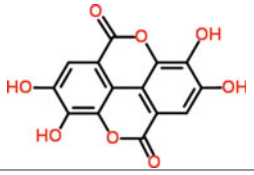
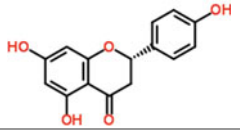
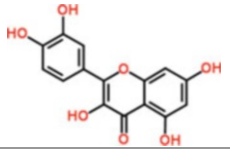
the presence of flavonoids, glycosides, alkaloids, steroids, and many other metabolites and absence of tannins and saponins [33].

Guava is one of the promising fruits rich in lectins, saponins, tannins, phenols, triterpenes, and flavonoids. High levels of vitamins, dietary fiber, and carotenoids, altogether make guava therapeutically an important fruit [34]. The fruit pulp is rich in ascorbic acid and carotenoids (lycopene, β-carotene, and β-cryptoxanthin). The seeds, skin, and bark have glycosides, carotenoids, and phenolic compounds.

5.1.1 Polyphenols

Polyphenols are synthesized by numerous plants as secondary metabolites. Polyphenolic compounds serve as both functional components to the plant and the consumer. For instance, some polyphenols serve as pigment (anthocyanins) compounds to ward off insects and other herbivores such as astringent, tannins, and UV light protectants such as carotenoids [35].These compounds are also important in foods for their sensory attributes such as color, astringency, and bitterness, as well as their possible nutritional properties. Polyphenols are products of three major plant metabolic pathways. Phenolic compounds consist of a phenol and an aromatic ring with at least one hydroxyl group attached. Phenolic compounds can be classified into several categories based on their structures. Phenolic acids are secondary metabolites of the shikimate pathway; the phenylpropenoid pathway produces the cinnamic acid derivatives which are precursors of flavonoids and ligans, and the "flavonoid route" produces the numerous and diverse flavonoid compounds [36]. Polyphenols, which include flavonoids, have at least two phenol groups. The largest polyphenols are the tannins which can be classified into two subgroups, the hydrolyzable and the condensed tannins. Hydrolyzable tannins are those which are readily hydrolyzed by acids or enzymes into gallic or ellagic acid [37]. Hydrolyzable tannins are commonly found in foods such as guava, grapes, and wine. Both condensed and hydrolyzable tannins have been shown to have antioxidant, enzyme-inhibiting, and antimicrobial properties [36]. Condensed tannins, also called "vegetable tannins" or proanthocyanidins, are flavonoid polymers which can be degraded in the presence of acid and heat to form either cyanidin or delphinidin and are relatively stable as compared to the hydrolyzable tannins [37]. Common condensed tannins include polymers of catechin and epicatechin which can be found in guava, teas, and numerous fruits and vegetables. The third phenolic group is the phenolic acids that consist of a benzene ring and at least one carboxylic acid group. Examples of phenolic acids are caffeic, chlorogenic, p-coumaric, gallic, and ellagic acids (Table 1). Many phenolic acids are linked through ester, ether, and acetal bonds to either structural components of the plant such as cellulose, proteins, or lignin; to larger polyphenols (tannins); or to smaller organic molecules such as glucose [38]. This leads to considerable diversity among the various classes of polyphenolics and therefore inherent difficulty in analysis and identification. Limited information exists on the guava phenolics; however, previous studies have shown guava to contain a variety of polyphenolics including flavonols, phenolic acids, flavan-3-ols, and condensed tannins [39, 40].

Table 1 Phenolics in guava

S. no	Chemical name	Structure[a]	Reference
1	Gallic acid		[41]
2	Catechin		[41]
3	Epicatechin		[39]
4	Myricetin		[39]
5	Apigenin		[39]
6	Kaempferol		[41]
7	Ellagic acid		[42]
8	Naringenin		[43]
9	Quercetin		[42]

[a]Structure taken from ChemSpider structure draw online software

5.1.2 Ascorbic Acid

Fruits are the major source of ascorbic acid, a nutrient required for humans and one of the most abundant antioxidants consumed. L-ascorbic acid is an excellent reducing agent, and large quantities may help stabilize phenolics and other antioxidants during processing by the donation of hydrogen atoms. This reducing ability is due to its 2,3-enediol moiety. Ascorbic acid is often considered as an index of nutrient quality during processing and storage of foods because of its stabilizing nature [44, 45]. Guava contains approximately 230 mg of total ascorbic acid/100 g of edible portion of fruit, five times more than a serving of orange. Among all fruits, it is second to acerola cherry in vitamin C content [46].

5.1.3 Carotenoids

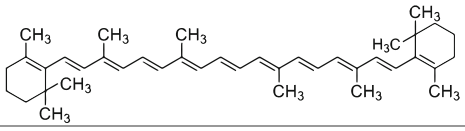
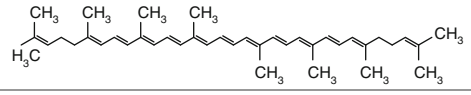
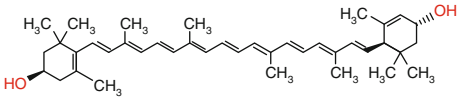
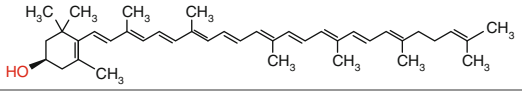
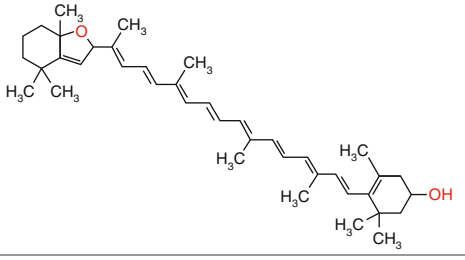
Carotenoids are abundant in red-, yellow-, orange-, and green-colored vegetables and fruits. After chlorophyll, they are the second most widely occurring plant pigment found in nature. Carotenoids are tetraterpenes that can be classified into two major groups including carotenes (hydrocarbons) and xanthophylls (oxygenated hydrocarbons). Carotenoids may be straight chained, such as lycopene, or contain a five or six carbon ring on one or both ends, such as β-carotene [47]. The high degree of hydration and long carbon chain length of these molecules make them hydrophobic and therefore fat-soluble molecules. The major purpose of carotenoids in the human diet is to serve as precursors to provitamin A. In order to serve this purpose, the carotenoid must contain a β-ionone ring [45]. Carotenoids containing this structure include β- and α-carotene and ß-cryptoxanthin. Carotenoids without this structure, such as lycopene, do not possess provitamin A activity yet serve as dietary antioxidants. As an antioxidant, carotenoids are known to quench singlet oxygen and protect against cellular oxidative damage [48]. A wide variety of carotenoids have been identified in guava including phytofluene, β-carotene, lycopene, cryptoflavin, cryptoxanthin, and lutein (Table 2) [28]. Lycopene is a fat-soluble carotenoid responsible for the red or pink pigment in several fruits and vegetables such as tomatoes, watermelon, pink grapefruit, and guava. Structurally, lycopene is a linear, 40 carbon hydrocarbon containing 11 conjugated and 2 non-conjugated double bonds [49]. Of all the dietary carotenoids, lycopene has the highest singlet oxygen quenching ability in the body. However, since lycopene lacks a β-ionone ring, it does not provide vitamin A activity.

5.2 Chemical Composition of Leaves

5.2.1 Leaf Phenolics

All parts of guava have been used for various purposes such as hepatoprotection, antioxidant, anti-inflammatory, antispasmodic, anticancer, antimicrobial, antihyperglycemic, analgesic, endothelial progenitor cells, anti-stomachache, and antidiarrhea. The primary constituents of guava leaves are phenolic, isoflavonoids, gallic acid, catechin, epicathechin, rutin, naringenin, and kaempferol having hepatoprotective, antioxidant, anti-inflammatory, antispasmodic, anticancer, antimicrobial, antihyperglycemic, and analgesic actions [51].

Table 2 Structure of major carotenoids reported in guava

S. no	Name	Structure	References
1	Beta-carotene		[28, 50]
2	Lycopene		[28, 50]
3	Lutein		[28]
4	Rubixanthin		[28]
5	Cryptoflavin		[28]

Gallic acid, catechin, and epicatechin have been found to inhibit pancreatic cholesterol esterase thereby decreasing cholesterol levels. Catechins are important as a preventive treatment for diabetes type 2 and obesity. Guava leaves contain two important flavonoids quercetin known for its spasmolytic, antioxidant, antimicrobial, anti-inflammatory actions and guaijaverin known for its antibacterial action [43, 52]. Quercetin has been associated with decreased mortality from heart disease and decreased incidence of stroke. Rutin is effective in the inhibition of triglyceride accumulation in adipocytes. Naringenin and kaempferol can promote moderate cytostatic activity against all cell lines, and kaempferol can be useful as anticancer [53, 54]. Leaf extract of guava has been reported for their antibacterial activity because of the presence of flavonoid glycosides, morin-3-O-alpha-L-lyxopyranoside, and morin-3-O-alpha-L-arabopyranoside [55]. Phenolic contents are key players of antimicrobial property of fruit leaves, certifying the importance of organic product of leaves as a solid nutritious items as well as multiresistant bacterial drug.

5.2.2 Leaf Oil

The leaves contain various constituents such as fixed oil (6%), volatile oil (0.36%), resin (3.15%), tannin (8.5%), fat, cellulose, chlorophyll, mineral salts, and a number of other substances [56]. In addition, the leaves contain an essential oil rich in cineol

and four triterpenic acids as well as three flavonoids, quercetin, its 3-L-4-4-arabinofuranoside (avicularin), and its 3-L-4-pyranoside with strong antibacterial action [52]. Guava leaves contain essential oil with the primary components being α-pinene, β-pinene, limonene, menthol, terpenyl acetate derivation, isopropyl liquor, longicyclene, caryophyllene, β-bisabolene, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cadinene, and curcumene [23].

Guava is known for its efficacy as a potent antimicrobial agent. The guava leaf crude extract showed minimum inhibitory concentration of 3.75 mg/ml for *Bacillus subtilis* and *Pseudomonas aeruginosa*. The phytochemical analysis of the extract revealed the presence of bioactive compounds such as saponins, alkaloids, flavonoids, terpenoids, carbohydrates, and tannins [57]. The methanolic extract has showed toxicity against clinically important gastrointestinal pathogens, viz., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholera* with *S. typhi* being highly susceptible with a zone of inhibition of 2 mm at 4 mg/ml [33].

5.3 Chemical Composition of Guava Fruit

5.3.1 Fruit Nutrients and Antioxidants

The main constituents of guava fruit are vitamins, tannins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols, and triterpenoid acids. These and other compounds are related to many health effects of guava [4].

Guava is a rich source of dietary fibers; vitamins A, C, and folic acid; and various dietary minerals like potassium, copper, and manganese. Pulp contains ascorbic acid and carotenoids (lycopenes, β-carotene) possessing antioxidant, antihyperglycemic, and antineoplastic properties [56]. Reports indicate that a single guava (*Psidium guajava*) fruit contains about four times the amount of vitamin C as an orange. Ascorbic acid is recognized for its important antioxidant effects [51, 58]. It has been reported that strawberry guava (*P. littorale* var. *cattleianum*) notably containing 90 mg of vitamin C per serving has about 25% more of the amount found in more common varieties, with its total vitamin C content in one serving still providing 100% of the dietary intake [59]. Further, guava also contains both carotenoids and polyphenols like allocatechin, guaijaverin, leucocyanidin, and amritoside [5, 60] which are reported as major classes of antioxidant pigments giving them relatively high potential antioxidant value among plant foods [61]. Some authors have found high concentrations of carotenoids (beta-carotene, lycopene, and beta-cryptoxanthin), vitamin C, and polyphenols in guava pulp [50]. Lycopene has been correlated with the prevention of cardiovascular damage because of its positive effects on dyslipidemia. The pulp and peel of the guava are a remarkable source of antioxidants and antioxidant dietary fiber (AODF) [62].

Guava fruits contain carotenoids and polyphenol pigments responsible for production of fruit skin and flesh color. Therefore, guavas that are red-orange in color have more pigment content (polyphenol, carotenoid, and provitamin A) than yellow-green ones [63]. Ojezele et al. (2013) found the highest concentrations of

the bioactive principles in ethanolic extracts of the plants and reported the quantity of different bioactive components, i.e., tannin (11.5 mg/g), total polyphenol (1.67 mg/g), alkaloid (59.85%), and oxalate (6.66%) in guava [64]. In white and red guava, the ascorbic acid contents reported were 130 and 112 mg/100 g fw; total phenolic content, 145.52 and 163.36 mg gallic acid equivalents (GAE)/100 gfw; and total flavonoids contents, 19.06 and 35.85 mg catechin equivalents (CE)/100 gfw, respectively. The solid-phase microextraction (SPME)/gas chromatography (GC)/mass spectrometry (MS) analysis revealed the presence of cinnamyl alcohol, ethyl benzoate, ß-caryophyllene, (E)-3-hexenyl acetate, and α-bisabolene as the major constituents in white and red guavas [65].

5.4 Chemical Composition of Guava Seeds

The guava seeds have varying amounts of macronutrients and micronutrients with a high content of total dietary fiber, protein, iron, zinc, and reduced calorie content. The lipid profile of guava seeds has shown a predominance of unsaturated fatty acids (87.06%) particularly linoleic acid and oleic acid as well as significant amounts of bioactive compounds such as ascorbic acid (87.44 mg/100 g), total carotenoids (1.25 mg/100 g), and insoluble dietary fiber (63.55 g/100 g) [66].

The seeds also contain glycosides, carotenoids, and phenolic compounds having antimicrobial properties [56]. Pelegrini et al. (2008) isolated and purified the peptide Pg-AMP1 from guava seeds. Pg-AMP1 showed clear growth reduction in *Klebsiella* sp. and *Proteus* sp., the principal pathogens involved in urinary and gastrointestinal hospital infections. SDS-PAGE and mass spectrometry (MALDI-TOF) characterized Pg-AMP1, a monomer with a molecular mass of 6029.34 Da. Amino acid sequencing revealed clear identity to the plant glycine-rich protein family with Pg-AMP1, the first such protein with antimicrobial activity against gram-negative bacteria. Thus, Pg-AMP1 shows potential in the near future to contribute toward development of novel antibiotics from natural sources [67].

6 Bioactive Potential of Guava

6.1 Antioxidant Activity

Antioxidants play a crucial role in both enzymatic and nonenzymatic browning reactions and help to prevent lipid oxidation in foods as well. Dietary antioxidants include vitamins A, C, and E as well as numerous non-nutritive compounds such as polyphenolics, flavonoids, carotenoids, and thiol-containing compounds. Biologically antioxidants may be defined as compounds that prevent free radicals generated during various metabolic reactions occurring in living cells from destroying host cells. Free radicals result from reactive oxygen species (ROS) that contain an unpaired electron rather than the paired electrons present in stable functioning molecules. These free radicals can interfere in the body and cause destructive

damage that could lead to many chronic health issues including cardiovascular diseases, stroke, atherosclerosis, and cancer. Antioxidants that are chemically reducing agents donate electrons and cause a substance to be reduced and thus help to reduce the number of free radicals and in the process may reduce the risks of such diseases [68].

Almost all the parts of guava are reported to have antioxidant properties. *Psidium guajava* fruit peel aqueous extract has the ability to reduce the oxidative stress of the pancreas in streptozotocin-induced (45 mg/kg) diabetic rats by lowering malondialdehyde (MDA) and protein carbonyl level and the increased activity of superoxide dismutase (SOD) and glutathione (GSH) level [69]. Besides, the antihyperglycemic effect of guava is also associated with its antioxidative activity [70]. Pink guava puree supplementation can decrease lipid peroxidation and increase antioxidant enzyme activity such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase in spontaneous hypertensive rat's blood [71]. The antioxidant activity values of guava leaves determined by 2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) assays were 10.28 μg fresh weight (fw)/μg and 78.56 μg Trolox equivalent (TE)/g fw for white guava and 7.82 μg/μg DPPH fw and 111.06 μg TE/g fw for red guava [65].

The comparative study of antioxidant activity and free radical-scavenging effects of extracts from guava leaves and dried fruit indicated that 94.4–96.2% of linoleic acid oxidation was inhibited by the addition of guava leaf and guava tea extracts at a concentration of 100 μg/ml. The guava dried fruit extracts exhibited weaker antioxidant effects than the leaf extracts. The results also demonstrated that the scavenging effects of guava leaf extracts on $ABTS^{+}$ radicals and superoxide anion increased with increasing concentrations. The guava leaf extracts displayed a significant scavenging ability on the peroxyl radicals. The extracts from leaves of various guava cultivars exhibited more scavenging effects on free radicals than did commercial guava tea extracts and dried fruit extracts. The chromatogram data indicated that guava extracts contained phenolic acids such as ferulic acid which appeared to be responsible for their antioxidant activity. The studies have shown that there exists a linear relationship between free radical-scavenging ability and the content of phenolic compounds of guava leaf extracts [72].

Mile et al. (2011) explored the possibility of obtaining phenolic extracts with antioxidant activity (AA) from Colombian guava seeds (*Psidium guajava* L.) using supercritical carbon dioxide adding ethanol as cosolvent (SC CO2/EtOH). The crude extracts were obtained by using block extraction designs step by step (four steps) as a function of pressure (10, 15, and 20 MPa) and temperature (313, 323, and 333 K). In each one of the extracts, the total phenolic content (TPC) and AA were determined through β-carotene bleaching and scavenging DPPH (2, 2-diphenyl-1-picrylhydrazyl) methods. Fraction IV showed a good performance in preventing the formation of peroxides, while the best crude extract inhibited the degradation of conjugated dienes suggesting that the guava seeds are a promising source of antioxidants which can be extracted using SC CO2/EtOH by special extraction step design and these compounds can thus be used as preservatives in foods such as edible oils [73].

6.2 Anti-Inflammatory Activity

Inflammation is a normal response to infection which involves the innate and adaptive immune systems. However, when allowed to continue unchecked, inflammation may result in autoimmune or autoinflammatory disorders, neurodegenerative disease, or even cancer [74]. A decoction of *P. guajava* leaves is used for the treatment of various inflammatory ailments including rheumatism. The presence of polyphenolic compounds and triterpenoids in the leaf of *P. guajava* contribute to its anti-inflammatory and analgesic effects. The aqueous extract of *P. guajava* at a dose of 50–800 mg/kg, i.p. (intraperitoneal), produced dose-dependent and significant inhibition of fresh egg albumin-induced acute inflammation (edema) in rats. Further, leaf extract (50–800 mg/kg, i.p.) also produced dose-dependent and significant analgesic effects against thermally and chemically induced nociceptive pain (pain caused by damage to body tissue) in mice [75].

Jang et al. 2014 investigated the in vitro and in vivo anti-inflammatory activity of ethanolic leaf extract of guava and demonstrated the significant inhibition of lipopolysaccharide (LPS)-induced production of nitric oxide and prostaglandin E2 by guava leaf extract (GLE) in a dose-dependent manner. GLE suppressed the expression and activity of both inducible nitric oxide synthase and cyclooxygenase-2 in part by the downregulation of ERK1/2 (extracellular signal-regulated kinase-1) activation in RAW 264.7 macrophages [76]. Studies on the anti-inflammatory activity of the aqueous extract of guava leaves (*P. guajava* L.) on white male rats through carrageenan-induced paw edema method have shown that the percentage of inflammation or edema (% E) is optimal at the 4th hour. Guava extract at 125, 250, and 500 mg/kg BW (body weight) reduced inhibitory percentage activities by 40.81, 55.45, and 43.61% ($p < 0.05$), respectively, suggesting that guava extract acts as anti-inflammatory properties by decreasing edema level [77].

6.3 Antidiabetic Activity

Guavas have high fiber content and low glycemic index thus playing an important role in preventing the development of diabetes. The fiber content ensures that the sugar levels are well regulated and the low glycemic index inhibits a sudden spike in sugar levels. The formation of advanced glycation end products (AGEs) are the major factors responsible for the complications associated with diabetes. In vitro studies support the anti-glycative potential of guava leaves. The investigation of the antihyperglycemic efficacy and mechanisms of action of *P. guajava* in streptozotocin (STZ)-induced diabetic rats revealed that oral administration of *P. guajava* leaf extract (300 mg/kg body weight/day) for 30 days to streptozotocin-induced diabetes rats significantly decreased the levels of blood glucose and glycosylated hemoglobin and improved the levels of plasma insulin and hemoglobin [78, 79]. The possible mechanism for the antihyperglycemic activity of *P. guajava* leaf involves the protection of pancreatic tissues as well as islet β-cells by guava leaf extracts against lipid peroxidation and the DNA strand breaks induced by STZ thus reducing the loss of insulin-positive β-cells and

insulin secretion, therefore strengthening the possibility of antihyperglycemic potential [70]. There are studies supporting that guava fruit could protect kidney against diabetic progression via its anti-oxidative, anti-inflammatory, and anti-glycative effects [80].

The tannins, polyphenolic compounds, flavonoids, pentacyclic triterpenoids, guiajaverin, quercetin, etc. were speculated to account for the hypoglycemic effects of the plant's leaf extract [81]. The aqueous guava leaf extract enhanced glucose uptake in rat clone 9 hepatocytes which revealed that phenolics are the principal component of the extract and high polarity fractions of the guava leaf extract are enhancers to glucose uptake in rat clone 9 hepatocytes and quercetin is the major active compound which promotes glucose uptake in liver cells.

Water-soluble solids showed higher superoxide dismutase-like activity and lipid peroxidation inhibition ability than ethanol-soluble solids in vitro, suggesting that anti-peroxidation of lipids is a possible mechanism for guava leaves to retard the progress of type 2 diabetes [82, 83]. In addition, normal, mild, and severely diabetic rat models had shown hypoglycemic as well as antidiabetic effect of the unripe guava fruit peel aqueous extract [84].

Long-term administration of guava leaf extracts increases the plasma insulin level and glucose utilization in diabetic rats. The activities of hepatic hexokinase, phosphofructokinase, and glucose-6-phosphate dehydrogenase in diabetic rats fed with aqueous extracts were higher than in the normal diabetic group, which provided evidence to support the antihyperglycemic effect of guava leaf extract and the health function of guava leaves against type 2 diabetes [85, 86]. The comparative study of the hydroalcoholic extracts of the fresh and dry leaves of guava plant for antihyperglycemic potential against alloxan-induced diabetes in rats revealed that the animals administered with doses of 500 mg/kg body weight of extract orally continuously for 30 days caused significant reduction in the fasting serum blood glucose levels. Among the two extracts, fresh leaf extract showed significant antihyperglycemic activity than the dry leaf extract which nearly produced equal reduction in serum blood glucose levels to that of standard glibenclamide 10 mg/kg body weight [87]. The evaluation of antidiabetic effect of *P. guajava* leaves on $Lepr^{db}/Lepr^{db}$ mice (*mice* that develop type 2 diabetes due to a recessive, autosomal mutation in the leptin receptor) showed significant blood glucose lowering effects after intraperitoneal injection of the extract at a dose of 10 mg/kg in both 1- and 3-month-old $Lepr^{db}/Lepr^{db}$ mice suggesting that the extract from *P. guajava* leaves possesses antidiabetic effect in type 2 diabetic mice model [85].

6.4 Antidiarrheal Activity

Diarrhea is one of the major problems in the world. The ripe fruit of guava has been reported as laxative which is used to treat constipation. The studies indicate that guava fruit is more effective antidiarrheal when it is used with the peel, but if taken unripe fruits in large quantity cause indigestion and vomiting [26, 88]. The leaf decoction of guava has been proved useful in case of gastroenteritis and chronic diarrhea, while the young leaves and shoots have been reported for dysentery and

diarrhea [89]. The binding of certain chemicals present in guava such as lectin to *E. coli* (a common diarrhea-causing organism) prevents its adhesion to the intestinal wall and thus preventing infection resulting diarrhea [90]. Guava leaf extract has also shown to have tranquilizing effect on intestinal smooth muscle, inhibits chemical processes found in diarrhea, and aids in the reabsorption of water in intestines. In another research, an alcoholic leaf extract was reported to have a morphine-like effect by inhibiting the gastrointestinal release of chemicals in acute diarrheal disease. This morphine-like effect was thought to be related to a chemical, quercetin.

In a study carried out with leaf extract of the plant, inhibition of gastrointestinal release of acetylcholine by quercetin present in extract was suggested as a possible mode of action in the treatment of acute diarrheal disease [91, 92, 93].

6.5 Antimicrobial Activity

Biswas et al. (2013) determined the antimicrobial potential of guava leaf extracts against foodborne and spoilage bacteria, viz., *E.coli* and *Salmonella enteritidis* (gram-negative) and *S. aureus* and *Bacillus cereus* (gram-positive). The findings suggested that the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3 mm, and the ethanol extract had a mean zone of inhibition of 6.11 and 11.0 mm against *B. cereus* and *S. aureus*, respectively. This demonstrates that guava leaf extract might be a good candidate in the search for a natural antimicrobial agent. The mechanism by which they can inhibit the microorganisms can involve different modes of action. It has been reported that guava oils and extracts penetrate the lipid bilayer of the cell membrane, rendering it more permeable, leading to the leakage of vital cell contents [94, 95].

A study conducted to screen the antimicrobial effect of essential oils and methanol, hexane, and ethyl acetate extracts from guava leaves against bacterial strains isolated from seabob shrimp and laboratory culture strains revealed the inhibitory activity of essential oil and extract against *S. aureus* and *Salmonella* spp. The researchers concluded that guava leaf extracts and essential oil are very active against *S. aureus*, thus making up important potential sources of new antimicrobial compounds [96]. Choudhary et al. (2012) provided phytochemical and antimicrobial details of the methanolic leaf extract of *P. guajava* against clinically important gastrointestinal pathogens, viz., *S. aureus*, *P. aeruginosa*, *E. coli*, *S. typhi*, and *Vibrio cholerae*. The methanolic extract showed toxicity against all the bacteria, *S. typhi* being highly susceptible with a zone of inhibition of 2 mm at 4 mg/ml [33]. The aqueous and methanolic extracts of *P. guajava* leaves showed antimicrobial activity against bacterial elastase from *P. aeruginosa* and human neutrophil elastase (HNE), but the methanolic extract of the leaves showed more inhibitory capacity than that of the aqueous extract against both enzymes. The good inhibitory capacity of methanolic leaf extract as compared to water extract was due to the extraction of many active compounds present in leaves by methanol as a solvent stronger than that of water [97].

The comparative studies to investigate the antibacterial activity of extracts of the leaves, bark, and root of *P. guajava* as well as the leaves *of Moringa oleifera* against *Staphylococcus aureus*, *Streptococcus* spp., *Klebsiella* spp., *Proteus* spp., and *Pseudomonas* spp. revealed that the inhibitory effect of *P. guajava* bark at 280 mg/ml against *Klebsiella* spp. competed favorably with reflacine. Extracts of *P. guajava* leaves, root, and the synthetic drug reflacine gave equal antimicrobial effect against *Pseudomonas* spp. These results indicate that extracts of *P. guajava* can be used for the treatment of pathogenic infections caused by some bacteria [98].

6.6 Anticancer Activity

The dry extract of guava leaves has promising activity to be applied topically in the oral cavity or in the development of antitumor formulation or even be used as a functional food [99]. The antiproliferative capacities of guava peel, flesh, and seed on four cancer cell lines, A549 (human lung cancer cells), MCF-7 (human breast cancer cells), HepG2 (human hepatoma cells), and HT-29 (human colon cancer cells) evaluated by the MTT (3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide) assay revealed that guava possesses strong antioxidant and anticancer actions. The active components of guava were identified as catechin, galangin, homogentisic acid, gallic acid, kaempferol, and cyanidin 3-glucoside, and the content of these in guava peel and seed were higher than that in guava flesh suggesting that guava could be developed to functional food for prevention of some diseases [41]. The acetone extracts of guava branch (GBA) is reported to have cytotoxic effects on HT-29 cells. The GBA showed high cytotoxic effects via the MTT reduction assay, LDH release assay (Lactate dehydrogenase), and colony formation assay. GBA of 250 μg/ml concentration showed 35.5% inhibition against HT-29 cells. As expected, GBA-induced characteristic apoptotic effects in HT-29 cells, including chromatin condensation and sharking that occurred 24 h after the cells, were treated [100].

Guava leaves have been reported to interfere with multiple signaling cascades linked with tumor genesis and provide a source of potential therapeutic compounds for both the prevention and treatment of cancer. The molecular mechanisms of guava leaf hexane (GHF) fraction in apoptotic potential are found to be correlated with the suppression of AKT (phosphatidylinositol 3-kinase) and *Akt* (protein kinase B)/mTOR (mammalian target of rapamycin)/S6 K1 (ribosomal protein S6 kinase beta-1) and MAPK(mitogen-activated protein kinase) signaling pathways in human prostate cancer cells. This effect of GHF is correlated with downregulation of various proteins that mediate cell proliferation, cell survival, metastasis, and angiogenesis [101]. The budding leaves of *Psidium guajava* contain huge amounts of soluble polyphenolics including gallic acid (348 mg/g), catechin (102 mg/g), epicatechin (60 mg/g), rutin (100 mg/g), quercetin (102 mg/g), and rutin (100 mg/g) and exhibit potent anticancer activity [61]. It has been reported that essential oil of *P. guajava* has the potent ant proliferative activity.

It could be used as an antitumor chemopreventive in view of anti-angiogenesis and anti-migration. The IC_{50} of *P. guajava oil* for DU145 cells was 0.57 mg ml^{-1} [102].

7 Value-Added Products and Nutraceuticals

Fruits are directly consumed by health conscious people but the changing era, lifestyle, and urbanization have drastically modified food habits of people like frequent use of ready-to-eat food, ready-to-serve fruit juices, jams, jelly, leather, powder, etc. Guavas also soften quickly during ripening being a climacteric fruit and therefore have a relatively short shelf life that limits the distribution of fresh guava fruit in market. Due to these fragile conditions, most guavas are processed into juice, puree, jam, jelly, syrup, nectar, fruit paste, or canned as halves [25]. The study conducted to determine the antioxidant activity, nutritional composition (sugar, protein, and fat,), and the bioactive phytochemicals (total phenolic compounds, flavonoid phenolic, condensed and hydrolyzable tannin, ascorbic acid, pigments such as anthocyanin, and carotenoids) as well as fiber content present in fresh fruits and flour cultivated in Argentina showed that the flour preserved flavor, aroma, and color of pulp from fresh fruits. The flour contained around 30% of sugar, 20% of total protein, and 0.5% of fat and high level of crude fiber. Carotenoids and ascorbic acid were the dominant phytochemicals in flour as well as in fresh fruits. The guava flour showed antioxidant activity with SC50 values similar to fresh fruits. The flour showed nutraceutical characteristics that are demanded by functional food and could be used as a dietary supplement [103].

Lanier (2005) studied phytochemical, antioxidant, and storage stability of thermally processed guava to assess the thermal stability and shelf life properties in guava nectar. He reported that possible degradation of the numerous phytochemicals in guava may occur during thermal processing and storage. This includes loss of ascorbic acid, isomerization of lycopene and other carotenoids, and decreases in overall polyphenolics and antioxidant activity [103, 104].

Verma et al. (2015) explored the antioxidant potential and functional value of guava (*Psidium guajava* L.) powder in muscle foods. They reported that guava powder which is rich in dietary fiber (43.21%) and phenolics (44.04 mg GAE/g) possesses good radical-scavenging activity which resulted in significant decrease ($p < 0.05$) in pH of emulsion and nuggets, emulsion stability, cooking yield, and moisture content of nuggets while ash and moisture content of emulsion were increased. Total phenolics, total dietary fiber (TDF), and ash content significantly increased ($p < 0.05$) in nuggets with added guava powder. Guava powder was found to retard lipid peroxidation of cooked sheep meat nuggets as measured by TBARS (2-thiobarbituric acid reactive substances) number during refrigerated storage which also did not affect sensory characteristics of the products and can be used as a source of antioxidant dietary fiber in meat foods [105].

Factors influencing the nutraceutical activity of guava fruits in fruit juices were reported, and it was observed that the levels of vitamin C and DPPH (2,2-diphenyl-1-picrylhydrazyl)-scavenging activity showed a sharp decrease with complete destruction after storage for 4 weeks at 5–10 °C. Levels of polyphenols and TEAC (trolox equivalent antioxidant capacity) showed a slower decrease in the levels [34].

Freeze-drying produced the best quality guava powder in terms of ascorbic acid, polyphenolic content, antioxidant activity, and flavor retention, though it was quite

hygroscopic in nature. It can thus be inferred that guava powder is a good source of natural antioxidants which can be an alternative to synthetic antioxidants. From the above study, it can be concluded that freeze-dried guava powder can be utilized as an ingredient for the development of value-added food product as it contains high total phenolic content with other nutrients. It was also found high in the mineral content and thus can be used in preparing a functional food [106].

In a study, guava powder (GP) was used as source of aroma and phenolic compounds to fortify wheat bread 10% (GB10) and 20% (GB20), as a substitute for wheat flour. Phenolic compounds, antioxidant capacity, volatile compounds profile, and sensory acceptability of control bread (CB; without GP) and guava breads (GB) were evaluated, and it was observed that incorporation of GP increased the phenolic compounds contents of bread two- to threefold. Ten phenolic compounds were identified in GB20, and quercetin-3-orutinoside was the major compound, while in CB, ferulic acid was the major among the six phenolic compounds in CB. Bread making seemed to promote the release of phenolic compounds from structural components. Breads incorporated with GP presented a richer volatile profile than CB, especially due to the presence of terpenes. GB improved aroma profile of bread. GP added aroma compounds and phenolic antioxidants and seemed to be an interesting approach to enhance bread bioactivity and acceptability [107].

8 Conclusion

Today, major concern of any individual is achieving better health and quality life either through use of synthetic chemical drugs like multivitamins or through the supplementary diets or dietary supplements. However the pharmaceutical industry is more focused toward development of new indigenous plant-based drugs through investigation of leads from traditional system of medicine and traditional uses of natural compounds. Thorough screening of literature available on *Psidium guajava* depicted various importance of guava leaf, fruit, and seed in treating large number of diseases and their use in making many value-added products. But these products are yet to be introduced in market as no branded products is available in market. The major drawback is the peculiar smell at ripening stage which needs biotechnological intervention to make this crop a superfood. Although guava possesses enormous health benefits, it can be an efficient nutraceutical in combating malnutrition and food in security.

References

1. Stone B (1970) The flora of Guam. Micronesica 82:373–378
2. Yahia EM (2018) Fruits and vegetable phytochemicals: chemistry and human health. Wiley, Hoboken, pp 1067–1076
3. Kaneria M, Chanda S (2011) Phytochemical and Pharmacognostic evaluation of leaves of *Psidium guajava* L. (Myrtaceae). Pharmacogn 23:32–41

4. Haida KS, Baron A, Haida KS (2011) Phenolic compounds and antioxidant activity of two varieties of guava and rue. Rev Bras Ciênc Saúde 28:11–19
5. Begum S, Hassan SI, Siddiqui BS (2002) Two new triterpenoids from the fresh leaves of *Psidium guajava*. Planta Med 68:1149–1152
6. Luiz CC, Carlos AF, Santos FV, Lima GPP (2011) Antioxidant content in guava (*Psidium guajava*) and araca (*Psidium* spp.) germplasm from different Brazilian regions. Plant Genet Resour Charact Util 9:384–391
7. Kumar A (2012) Importance for life *'Psidium guajava'*. Int J Res Pharm Biomed Sci 3:137–133
8. Sanda KA, Grema HA, Geidam YA, Bukar-Kolo YM (2011) Pharmacological aspects of *Psidium guajava*: an update. Int J Pharmacol 7:316–324
9. Kamath JV, Rahul N, Ashok Kumar CK, Lakshmi SM (2008) *Psidium guajava* L.: a review. Int J Green Pharm 2:9–12
10. Joseph B, Priya M (2011) Review on nutritional, medicinal, and pharmacological properties of guava (*Psidium Guajava* Linn.). Int J Pharma Bio Sci 2:53–69
11. Ojewole JA, Awe EO, Chiwororo WD (2008) Antidiarrhoeal activity of *Psidium guajava* Linn. (Myrtaceae) leaf aqueousextract in rodents. J Smooth Muscle Res 44(6):195–207
12. Keservani RK, Vyas N, Jani S, Raghuvanshi R, Sharma AK (2010) Nutraceutical and functional food as future food: a review. Der Pharmacia Lett 2:106–116
13. Gupta S, Chauhan D, Mehla K, Sood P, Nair A (2010) An overview of nutraceuticals: current scenario. J Basic Clin Pharm 1:55–62
14. Ajila CM, Prasada Rao UJS (2013) Mango peel dietary fiber: composition and associated bound phenolics. J Funct Foods 5:444–450
15. Singh G, Gupta S, Mishra R, Singh GP (2005) Wedge grafting for rapid multiplication of guava. ICAR News 11:2–3
16. Singh G (2004) Techniques for producing multiple crops in hi-density planting in guava. ICAR News 10:1–2
17. Singh G (2007) Recent developments in production of guava. Acta Hort 735:161–176
18. Deshmukh MM, Sen NL (2001) Evaluation of drip irrigation it's evaporation based irrigation scheduling distribution patterns on performance of guava. Advan Horti Forestry 8:25–31
19. Dubey AK, Pathak RA, Pathak RK (2002) Effects of drip irrigation on guava on plant growth and nutrients status of leaves. Progress Hortic 34:56–59
20. Sharma S, Patra SKR, Roy GB, Bera S (2013) Influence of drip irrigation and nitrogen fertigation on yield and water productivity of guava. The Bioscan 8:783–786
21. Mercado-Silvaa E, Pedro B, Ma De Los A (1998) Fruit development, harvest index and ripening changes of guava as produced in Central Mexico. Postharvest Biol Technol 13:143–150
22. Reyes MU, Paul RE (1995) Effect of storage and ethylene treatment on guava (*Psidium guajava* L.) fruit ripening. Postharvest Biol Technol 6:357–365
23. Morton J (1987) Fruits of warm climates. Florida Flair Books, Miami, pp 356–363
24. Mao SE, Campbell CW (1994) The guava horticultural sciences department fact sheet HS-4. Florida cooperative extension service, Institute of food and agricultural sciences University of Florida
25. Jiménez-Escrig A, Rincón M, Pulido R, Saura-Calixto F (2001) Guava fruit (*Psidium guajava* L) as a new source of antioxidant dietary fiber. J Agric Food Chem 49:5489–5493
26. Conway P (2001) Tree medicine: A comprehensive guide to the healing power of over 170 trees. Judy Piatkus Ltd, London, pp 2173–2177
27. Osorio DV, Acosta LMV, Hincapié GA (2014) Analysis of nutritional and functional properties of dry *guava*. Ing Univ Bogotá (Colombia) 18(1):159–175
28. Mercadante AZ, Steck A, Pfander H (1999) Carotenoids from guava: isolation and structure elucidation. J Agric Food Chem 47:145–151
29. Dolkar D, Bakshi P, Gupta M, Wali VK, Kumar R, Hajarika TK, Kher D (2017) Biochemical changes in guava (*Psidium guajava*) fruits during different stages of ripening. Indian J Agric Sci 87:257–260

30. Saxena M, Saxena J, Nema R, Singh D, Gupta A (2013) Phytochemistry of medicinal plants. J Pharmacogn Phytochem 1:168–182
31. Randhir R, Lin YT, Shetty K (2004) Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. Process Biochem 39:637–646
32. Velderrain-Rodríguez GR, Palafox-Carlos H, Wall-Medrano A, AyalaZavala JF, Chen CYO, Robles-Sanchez M, Astiazaran-García H, Alvarez-Parrilla E, González-Aguilar GA (2014) Phenolic compounds: their journey after intake. Food Funct 5:189–197
33. Choudhary S, Sharan L, Sinha MP (2012) Phytochemical and antimicrobial screening of *Psidium Guajava L.* leaf extracts against clinically important gastrointestinal pathogens. J Nat Prod Plant Resour 2:524–529
34. Pathare P, Nilegaonkar S, Agte V (2017) Factors influencing the nutraceutical activity of guava fruits. Adv Food Sci Eng 1:107–111
35. Herrmann KM (1995) The shikimate pathway as an entry to aromatic secondary metabolism. Plant Physiol 107:7–12
36. De Bruyne T, Pieters L, Deelstra H, Vlietinck A (1999) Condensed vegetable tannins: biodiversity in structure and biological activities. Biochem Syst Ecol 27:445–449
37. Hagerman A, Riedl K, Jones A, Sovik K, Ritchard N, Hartzfeld R (1998) High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 46:1887–1892
38. Robbins R (2003) Phenolic acids in food: an overview of analytical methodology. J Agric Food Chem 51:2866–2887
39. Miean KH, Mohamed S (2001) Flavonoid (Myricetin, Quercetin, Kaempferol, Luteolin, and Apigenin) content of edible tropical plants. J Agric Food Chem 49:3106–3112
40. Misra K, Seshadri TR (1967) Chemical components of the fruits of Psidium guava. Phytochemistry 7:641–645
41. Chen YH, Zhou T, Zhang YJ, Zou ZF, Wang F, Xu DP (2015) Evaluation of antioxidant and anticancer activities of guava. Int J Food Nutr Saf 6(1):1–9
42. Mahattanatawee K, Manthey JA, Luzio G, Talcott ST, Goodner KL, Baldwin EA (2006) Total antioxidant activity and fiber content of select Florida grown tropical fruits. J Agric Food Chem 54(19):7355–7363
43. Rishika D, Sharma R (2012) An update of pharmacological activity of *Psidium guajava* in the management of various disorders. Int J Pharm Sci Res 3:3577–3584
44. Fennema OR (1996) Food chemistry, 3rd edn. Marcel Dekker, New York
45. Fennema OR (1977) Loss of vitamins in fresh and frozen foods. Food Technol 31(12):32
46. Uddin MS, Hawlader MNA, Ding L, Mujumdar AS (2002) Degradation of ascorbic acid in dried guava during storage. J Food Eng 51:21–26
47. Macdougall D (2002) Colour in food. Woodhead publishing, Abington/Cambridge, pp 190–211; 278–286
48. Deshpande SS, Deshpande US, Salunkhe DK (1995) Nutritional and health aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK (eds) Food antioxidants – technological, toxicological and health perspectives. Marcel Dekker, New York, pp 361–382
49. Rao AV, Agarwal S (1999) Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: a review. Nutr Res 19:305–323
50. Oliveira Dda S, Lobato AL, Ribeiro SM, Santana AM, Chaves JB et al (2010) Carotenoids and vitamin C during handling and distribution of guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), and papaya (*Carica papaya* L.) at commercial restaurants. J Agric Food Chem 58:6166–6172
51. Barbalho SM, Farinazzi-Machado FM, de Alvares Goulart R, Brunnati AC, Ottoboni AM et al (2012) *Psidium guajava* (guava): a plant of multipurpose medicinal plants. Med Aromat Plants 1:104
52. Oliver-Bever (1986) Bep: medicinal plants in tropical West Africa. Cambridge University Press, Cambridge

53. Ngamukote S, Mäkynen K, Thilawech T, Adisakwattana S (2011) Cholesterol lowering activity of the major polyphenols in grape seed. Molecules 16:5054–5061
54. Gosmann G, Barlette AG, Dhamer T, Arçari DP, Santos JC (2012) Phenolic compounds from Maté (Ilex paraguariensis) inhibit Adipogenesis in 3T3-L1 Preadipocytes. Plant Foods Hum Nutr 67:156–161
55. Arima H, Danno G (2002) Isolation of antimicrobial compounds from guava (*Psidium guajava* L.) and their structural elucidation. Biosci Biotech Bioch 66:1727–1730
56. Nadkarni KM, Nadkarni AK (1999) Indian materia medica – with ayurvedic, unani-tibbi, siddha, allopathic, homeopathic, naturopathic and home remedies, vol 1. Popular Prakashan Private Ltd., Bombay
57. Bisht R, Chanyal S, Agrawal PK (2016) Antimicrobial and phytochemical analysis of leaf extract of medicinal fruit plants. Asian J Pharm Clin Res 9:131–136
58. Hassimotto NM, Genovese MI (2005) Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. J Agric Food Chem 53:2928–2935
59. Healthaliciousness.com (2008) Nutrient facts comparison for common guava, strawberry guava, and oranges (http://www.healthaliciousness.com/nutritionfacts/sbsl.php?one=9139&two=9140&three=9200). Retrieved. 2008-DEC-21.
60. Ghosh P, Mandal A, Chakraborty P et al (2010) Triterpenoids from *Psidium guajava* with biocidal activity. Indian J Pharm Sci 2:504–507
61. Chen KC, Peng CC, Chiu WT, Cheng YT, Huang GT, Hsieh CL (2010) Action mechanism and signal pathways of *Psidium guajava* L. aqueous extract in killing prostate cancer LNCaP cells. Nutr Cancer 62:260–270
62. Kaljee ML, Dinh TV, Lorenz VS, Becky GL, Gia CD, Huu TL, Tan MT, Kim TLT, Clemens JD, Duc TD (2004) Healthcare use for diarrhoea and dysenter in actual and hypothetical cases, Nha Trang, Viet Nam. J Health Popul Nutr 22:139–149
63. Metwally AM, Omar AA, Harraz FM, El Sohafy SM (2010) Phytochemical investigation and antimicrobial activity of *Psidium guajava* L leaves. Pharmacogn Mag 6:212–218
64. Ojezele MO, Agunbiade S (2013) Phytochemical constituents and medicinal properties of different extracts of *Anacardium occidentale* and *Psidium guajava*. Asian J Biomed Pharm Sci 3:20–23
65. Thuaytong W, Anprung P (2011) Bioactive compounds and prebiotic activity in Thailand-grown red and white guava fruit (*Psidium guajava* L.). Food Sci Technol Int 17:205–212
66. Uchôa-thomaz AMA, Sousa EC, Carioca JOB, Morais SMD, Lima AD, Martins CG, Alexandrino CD, Ferreira PAT, Rodrigues ALM, Rodrigues SP, Thomaz JCDA, Silva JDR, Rodrigues LL (2014) Chemical composition, fatty acid profile and bioactive compounds of guava seeds (*Psidium guajava* L.). Food Sci Technol 34:485–492
67. Pelegrini PB, Murad AM, Silva LP, Dos Santos RC, Costa FT, Tagliari PD, Bloch C Jr, Noronha EF, Miller RN, Franco OL (2008) Identification of a novel storage glycine-rich peptide from guava (*Psidium guajava*) seeds with activity against gram-negative bacteria. Pub Med 29:1271–1279
68. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G, Mainland CM (1998) Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J Agr Food Chem 46:2686–2693
69. Budin SB, Ismail H, Chong PL (2013) *Psidium guajava* fruit peel extract reduces oxidative stress of pancreas in streptozotocin-induced diabetic rats. Sains Malays 42:707–713
70. Huang CS, Yin MC, Chiu LC (2011) Antihyperglycemic and antioxidative potential of *Psidium guajava* fruit in streptozotocin-induced diabetic rats. Food Chem Toxicol 49:2189–2195
71. Nor NM, Yatim AM (2011) Effects of pink guava (*Psidium guajava*) puree supplementation on antioxidant enzyme activities and organ function of spontaneous hypertensive rat. Sains Malays 40:369–372
72. Chen HY, Yen GC (2007) Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. Food Chem 101:686–694

73. Hernández-Acosta MA, Castro-Vargas HI, Parada-Alfonso F (2011) Integrated utilization of guava (*Psidium guajava* L.): antioxidant activity of phenolic extracts obtained from guava seeds with supercritical Co_2-ethanol. J Braz Chem Soc 22:2383–2390
74. Dinarello (2010) Anti-inflammatory agents: present and future. Cell 140:935–950
75. Ojewole JA (2010) Anti inflammatory and analgesic effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract in rats and mice. Methods Find Exp Clin Pharmacol 28:441–446
76. Jang M, Jeong SW, Cho SK, Ahn KS, Lee JH, Yang DC, Kim JC (2014) Anti-inflammatory effects of an ethanolic extract of guava (*Psidium guajava* L.) leaves in vitro and in vivo. J Med Food 17:678–685
77. Weni L, Harliansyah W (2011) Anti-inflammatory activity of the extract of guava leaves (*Psidium guajava* L) in the rat (Rattus norvegicus L). Indones J Cancer Chemopre 2:169–172
78. Soman S, Rajamanickam C, Rauf AA, Indira M (2011) Beneficial effects of *Psidium guajava* leaf extract on diabetic myocardium. Exp Toxicol Pathol 65:91–95
79. Wu JW, Hsieh CL, Wang HY, Chen HY (2009) Inhibitory effects of guava (*Psidium guajava* L) leaf extracts and its active compounds on the glycation process of protein. Food Chem 113:78–84
80. Lin CY, Yin MC (2012) Renal protective effects of extracts from guava fruit (*Psidium guajava* L) in diabetic mice. Plant Food Hum Nutr 67:303–308
81. Ojewole J (2005) Hypoglycemic and hypotensive effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract. Method Find Exp Clin 27:689–695
82. Chuang PT, Shen SC, Wu NJ, Wu JSB (2008) Anti-peroxidation effect of guava (*Psidium guajava* Linn.) leaf soluble solids in vitro and in streptozotocin/nicotinamide-induced diabetic rats. J Sci Food Agric 88:2173–2179
83. Cheng FC, Shen SC, Wu JSB (2009) Effect of guava (*Psidium guajava* L) leaf extract on glucose uptake in rat hepatocytes. J Food Sci 74:H132–H138
84. Rai PK, Jaiswal D, Mehta S, Wathal G (2009) Anti-hyperglycaemic potential of *Psidium guajava* raw fruit peel. Indian J Med Res 129:561–565
85. Oh WK, Lee CH, Lee MS, Bae EY, Sohn CB, Oh H, Kim BY, Ahn JS (2005) Antidiabetic effects of extracts from *Psidium guajava*. J Ethnopharmacol 96:411–415
86. Shen SC, Cheng FC, Wu NJ (2008) Effect of guava (*Psidium guajava* Linn) leaf soluble solids on glucose metabolism in type 2 diabetic rats. Phytother Res 22:1458–1464
87. Rapaka R, Vennam SR (2012) Evaluation and comparison of anti-diabetic activity of hydroalcoholic extracts of fresh and dry leaves of *Psidium guajava* in type-ii diabetes mellitus Int. Res J Pharm App Sci 2:62–65
88. Burkill HM (1997) The useful plants of West Tropical Africa, families M-R. Royal Bot Gard Kew 4:89–93
89. Lutterodt GD, Ismail A, Basheer RH, Baharudin HM (1999) Antimicrobial effects of *Psidium guajava* extract as one mechanism of its Antidiarrhoeal action. Malays J Med Sci 6:17–20
90. Rodriguez RC, Cruz PH, Rios HG (2001) Lectins in fruits having gastrointestinal activity their participation in hemagglunating property of *Escherichia coli* O157. Arch Med Res 32:251–257
91. Lutterodt GD (1992) Inhibition of microlax-induced experimental diarrhea with narcotic-like extracts of *Psidium guajava* leaf in rats. J Ethnopharmacol 37:151–157
92. Lutterodt GD, Maleque A (1998) Effects on mice locomotor activity of a narcotic-like principle from *Psidium guajava* leaves. J Ethnopharmacol 24:219–231
93. Lin J, Puckree T, Mvelase TP (2002) Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. J Ethnopharmacol 79:53–56
94. Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A (2013) Antimicrobial activities of leaf extracts of Guava (*Psidium guajava* L) on two gram-negative and gram-positive bacteria. Int J Microbiol:1–7. Hindawi Publishing Corporation 2013:746165
95. Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol 94:223–253

96. Goncalves FA, Andrade Neto M, Bezerra JNS et al (2008) Antibacterial activity of guava, *Psidium guajava* Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from seabob shrimp, *Xiphopenaeus kroyeri* (Heller). Rev Inst Med Trop Sao Paulo 50:11–15
97. Deena M, Moideen KAV, Prasad SR (2016) Preferential inhibition of bacterial elastase over human neutrophil elastase by leaf extracts of *Psidium guajava*: an in vitro study. Natl J Physiol Pharm 6:123–127
98. Okechukwu RI, Ujowundu CO, Okika WO, Ukaoma AA, Anuforo HU, Ezea CO (2015) Studies on the phytochemical and antibacterial activities of aqueous and ethanol extracts of *Psidium guajava* and *Moringa oleifera*. Sch Acad J Biosci 3:320–324
99. Braga T, Dores R, Ramos C, Evangelista F, Tinoco L, Varotti F, Carvalho M, Sabino A (2014) Antioxidant, antibacterial and antitumor activity of ethanolic extract of the *Psidium guajava* leaves. Am J Plant Sci 5:3492–3500
100. Ashrafa A, Sarfraza RD, Muhammad AR, Adeel M, Muhammad SNN (2016) Chemical composition, antioxidant, antitumor, anticancer and cytotoxic effects of *Psidium guajava* leaf extracts. Pharm Biol 54:1971–1981
101. Ryu NH, Park KR, Kim SM, Yun HM, Nam D, Lee SG, Jang HJ, Ahn KS, Kim SH, Shim BS, Choi SH, Mosaddik A, Cho SK, Ahn KS (2012) A hexane fraction of guava leaves (*Psidium guajava* L) induces anticancer activity by suppressing AKT/mammalian target of rapamycin/ribosomal p 70 S6 kinase in human prostate cancer cells. J Med Food 15:231–241
102. Salib JY, Michael HN (2004) Cytotoxic phenylethanol glycosides from *Psidium guajava* seeds. Phytochemical 65:2091–2093
103. Moreno MA, Zampini Catiana I, Costamagna M, Sayago JE, Ordoñez RM, Isla MI (2014) Phytochemical composition and antioxidant capacity of *Psidium guajava* fresh fruits and flour. Nutr Sci 5:725–732
104. Lanier F (2005) Phytochemical, antioxidant, and storage stability of thermally processed guava (*Psidium guajava*) and guava juice blends. M.Sc University of Florida, Gainesville
105. Verma AK, Rajkumar V, Banerjee R, Biswas S, Das AK (2013) Guava (*Psidium guajava* L.) powder as an antioxidant dietary fibre in sheep meat nuggets. Asian Australas J Anim Sci 26 (6):886–895
106. Verma M, Singh J, Kaur D, Mishra V, Rai GK (2015) Effect of various dehydration methods and storage on physicochemical properties of guava powder. J Food Sci Technol 52:528–534
107. Castelo-Branco VN, Lago MG, Minuzzo DA, Moura-Nunes N, Torres AG, Nunes JC, Monteiro M (2016) Bread formulated with guava powder was enriched in phenolic and aroma compounds, and was highly acceptable by consumers. J Food Sci Technol 53:4168–4178

Cereal-Based Fermented Foods of Africa as Functional Foods

51

Ome Kalu Achi and Naomi U. Asamudo

Contents

Abstract

The demand for consumption of health promoting foods is growing worldwide due to the increased awareness of consumers on the impact of food on health. Traditional fermented foods prepared from cereals such as maize, rice, millet, or sorghum are common in Africa. Fermentation of these cereal grains by traditional methods exploit mixed cultures of various beneficial microorganisms, referred to

O. K. Achi (✉)
Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Nigeria
e-mail: omekachi@yahoo.com

N. U. Asamudo
Department of Microbiology, University of Uyo, Uyo, Nigeria
e-mail: naomiasamudo@yahoo.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_31

as probiotics. The improved knowledge of functional aspects of these foods are related to the interactions of bioactive living cells with the host or indirectly as the result of the ingestion of bioactive molecules released during fermentation such as dietary fiber, minerals, vitamins, and antioxidants. Lactic acid bacteria (LAB), yeasts, and fungi are the major microorganisms often encountered together in the production of beverages and fermented foods. The beneficial effects of probiotic consumption include improvement of intestinal health by the regulation of microbiota, stimulation and development of the immune system, synthesizing and enhancing the bioavailability of nutrients, alleviation of lactose intolerance symptoms, and reducing the risk of certain other diseases. African cereal fermented foods could provide an abundant opportunity available for it to be made more functional by incorporating probiotic LAB strains with disease-specific functions and could also facilitate the understanding of when to use probiotics for specific pathological states.

Keywords
Traditional fermented foods · Cereal-based foods and beverages · Fermentation · Functional foods · Probiotic organisms · Lactic acid bacteria

1 Introduction

The health-beneficial concept of fermented foods has developed through rapid expansion of scientific investigations over the years thereby arousing consumer awareness of the functional basis for consuming such traditional foods in relation to health promotion and disease prevention [1, 2]. The realization that a healthy lifestyle, including nutrition, reduces the risk of disease and increases health and well-being has received a huge amount of publicity. Fermented foods are an important part of the diet in many cultures. Fermentation primarily has been used to preserve foods, enhance shelf life, and improve flavor. The beneficial effect of fermented foods on health is due to their excellent functional and nutritional properties assisted by fermenting microorganisms, and novel bioactive compounds released. The fermentation process and the resulting fermented products have recently attracted scientific interest as consumers are becoming aware of the possible positive role diet can play in disease risk management and perhaps because of their increasing interest in the relations between food and quality of life. In addition, microorganisms contributing to the fermentation process have recently been associated with many health benefits, and so these microorganisms have become another focus of attention. The most studied microorganisms in food fermentations are the lactic acid bacteria (LAB). During fermentation, these bacteria synthesize vitamins and minerals, produce biologically active components with enzymes such as proteinase and peptidase, and remove some non-nutrients [3, 4]. The bioactive compounds which are produced by the bacteria responsible for fermentation are also well known for their health benefits. Among these are conjugated linoleic acids (CLA), which have a blood pressure lowering effect; exopolysaccharides, which exhibit

prebiotic properties [5]; bacteriocins, which show antimicrobial effects; and bioactive peptides, which exhibit antioxidant, antimicrobial, antiallergenic, and blood pressure lowering effects [6]. As a result, fermented foods provide many health benefits such as antioxidant, antimicrobial, antifungal, anti-inflammatory, antidiabetic, and antiatherosclerotic activities [7].

Functional food development in the African context is associated with an increased interest in foods such as fruits, vegetables, and wholegrain products or in industrially manufactured products that offer additional health benefits. Most modern diets of the western world are very different than the traditional ones in the sense that they contain elevated amounts of processed foods and ready-to-eat products that contain numerous chemicals or refined additives that are used to increase shelf life, flavor, and physical properties. The westernization of urban African diets has contributed to some of the leading causes of death and increases in the risk of numerous diseases [4]. All of these unhealthy foods and bad eating habits, as it seems, are important causes of the increased incidence of obesity, high cholesterol levels in blood, high blood pressure, diabetes, and many other health problems including certain types of cancers [8]. According to Waters [9], this has led to an increase in demand for the nondairy-based functional beverage substitutes with high acceptance and functionality [10].

The traditional African fermented cereal-based foods have potential to fill this gap because they are potential sources of novel probiotics and may serve as a veritable delivery vehicle for functional food components [11–13].

Cereal-based beverages have been tested as functional and probiotic foods because of their nutritious and health-promoting properties [14]. They can be considered as belonging to the functional food category due to their beneficial effect on health [15]. In particular, the consumption of functional foods containing probiotics would be one avenue to reduce the risk of specific diseases such as diarrhea and malnutrition [16]. Normally, in the western dietary culture, the typical delivery vehicles for probiotics are predominantly associated with milk in yoghurt-type products. This would be impracticable in the African setting because of the unfamiliarity and relative high cost of such products [12]. Traditional fermented cereal products may be more easily accepted, as noted by Franz [12], by African rural populations with its high infant mortality. According to Corbo et al. [17], an attractive approach to improve the nutritional value of fermented functional foods relies upon the activity of functional bacteria [18]; for example, many LAB and *Bifidobacterium* spp. have been reported to produce vitamins such as folate, cobalamin, menaquinone (vitamin K), riboflavin, and thiamine [19]. The use of these cultures in food fermentation potentially provides routes not only to enhance the nutritional profile of the food but also to deliver microorganisms to the gut, where they can synthesize such vitamins *in vivo* [20]. Cereal fermentation leads to the decrease of the level of carbohydrates as well as some nondigestible poly- and oligosaccharides, while the availability of certain amino acids and B vitamins is improved [18]. Indeed, the selection of appropriate starter cultures for each variant of cereal beverage is an industrial need to drive, accelerate, and standardize the fermentation [21]. This chapter reviews the probiotic and functional attributes of African cereal fermented food products.

2 Major Factors Hampering the Development of Fermented Foods

The production of fermented foods, no doubt, is still largely a traditional family art carried out in homes in a crude manner. Consequently, production has not increased substantially to more than a cottage industry. Many of these foods gradually acquired the label of food for the poor population or were associated with low incomes. Among the various factors working against traditional fermented foods, the following appear valid [22]:

- Inadequate raw material, grading, and cleaning contributing to the presence of foreign matter (such as insects, stones) in the final product
- Crude handling and processing techniques employed
- Lack of durability (shelf life)
- Lack of homogeneity
- Unattractive presentation, which inhibits consumers from developing regular purchasing attitudes. However, plastic containers have replaced banana leaves as packaging material for food: a step in overcoming this hindrance [23]

Despite a long history of a large variety of fermented foods across Africa, these are not necessarily being passed down to the next generation or incorporated in the food-based dietary guidelines. The negative perceptions about fermented foods conveyed by especially among young urban people exist. A range of factors contributes to these perceptions. Traditional fermented foods are considered inferior because of their taste and quality (as a result of nonstandardized artisanal practices). According to Reid [16], the uneasiness with traditional fermented foods exhibited by young urban people is that their generation does not have time to wait for traditional fermented foods that take a while to prepare or cost too much. They opined that westernization/urbanization of African society, at least in cities, was seen as a barrier to embracing most fermented foods unless they could be made available as an affordable, easy, and tasty option. Many fermented cereals are multipurpose. A single product may be prepared in varying thicknesses and used as a fermented gruel for both adults and children, or it may be watered down and used as a fermented thirst-quenching beverage. As Wood [24] remarked, the latter type of product makes a meaningful contribution to nutrition. But, one among the factors that may work against fermented foods is the increasing popularity of junk foods introduced by western countries [25]. The potential of their replacement by cola-type beverages would result in a serious negative impact on the nutrition of people in developing countries.

Given the reported health benefits of fermented foods [26, 27], the general view is that consumption of these foods should be encouraged, especially since they are part of the traditional healthy diet. More importantly, the development of a traditional probiotic cereal fermented food in a locally sustainable way can reduce the adverse effect of malnutrition. Standardized starter cultures for consistent product development and innovation according to Reid [16], are seen as important for a new

approach to fermented foods The factors outlined here should continue to serve as a general guideline to some major goals in the exercise of improving the present status of fermented foods.

3 Cereal Fermentation

Fermentation of cereals is an ancient and inexpensive food preservation method and a cultural and traditional practice within indigenous communities in Africa and in most developing countries [28]. Examples of beneficial aspects of fermented foods [29–31], some of which are yet to be realized by industrialized settings, are listed in Fig. 1 emphasizing the broad nature and diversity of the benefits.

The microbiological and chemical compositions of cereal fermented beverages provide a complex probiotic effect owing to the presence of lactic acid bacteria and yeast. The metabolic substances produced during fermentation have proven

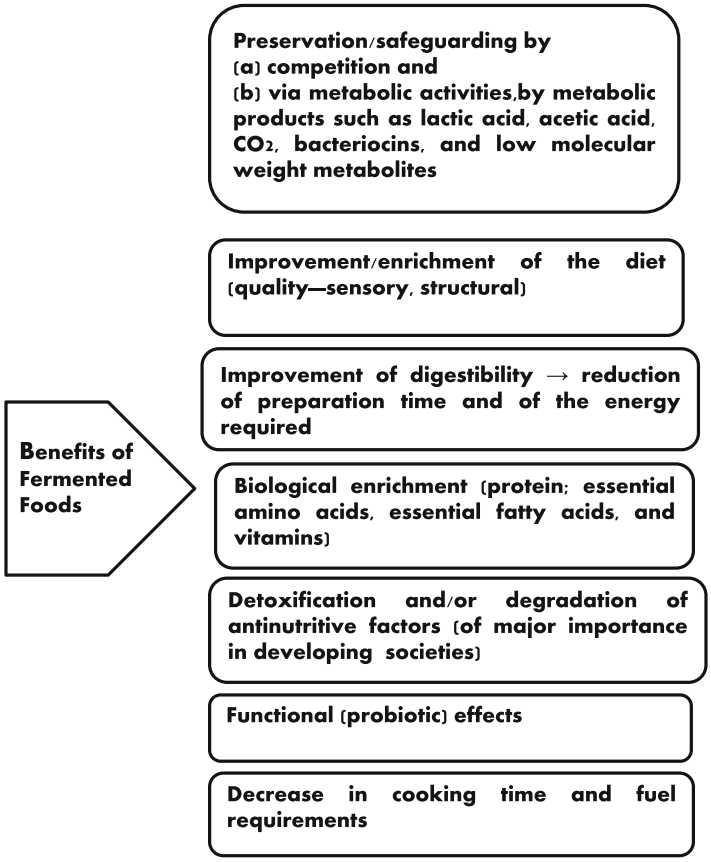


Fig. 1 Benefits of fermented foods

nutraceutical activities [32]. Probiotic microorganisms participate in the fermentation of various foods, generating bioactive components that enhance the functionality of food products [33]. These foods also serve as delivery vehicles of probiotic strains to the desired target sites in the human or animal body. To achieve health benefits, functional foods are expected to support the growth and maintain strains' viable count in the range of about 10^6–10^7 cfu/g of the product [34]. Naturally fermented cereals account for up to 80% of total calorie consumption in many African countries [14]. Owing to their potential for nutritional enhancement and the fact that their consumption substantially lowers the risk of significant diet-related diseases, cereals assume a basic role in the diet of industrialized countries [13]. Although cereals are deficient in certain basic nutritional components (e.g., essential amino acids such as lysine and group B vitamins) and also contain established antinutrients (phytic acid, tannins, and polyphenols), fermentation, also through probiotics, might be the most simple and economical way of improving their nutritional value, sensory properties, and functional qualities [35]. Fermentation of cereals leads to the decrease in the level of carbohydrates as well as some non-digestible poly- and oligosaccharides, while the availability of certain amino acids and B vitamins is improved.

Fermentation also provides optimum pH for enzymatic degradation of phytic acid which in turn increases the levels of soluble iron, zinc, and calcium [36]. According to Charalampopoulos et al. [37], multiple beneficial effects of cereals are exploited as:

(i) Fermentable substrates for growth of probiotic microorganisms, especially lactobacilli and bifidobacteria
(ii) Dietary fiber promoting several beneficial physiological effects
(iii) Prebiotics due to their content of specific nondigestible carbohydrates
(iv) Encapsulation materials for probiotic in order to enhance their stability

Cereals can be used as sources of nondigestible carbohydrates not only for promoting several beneficial physiological effects but also for selectively stimulating the growth of lactobacilli and bifidobacteria that are present in the colon and act as prebiotics [37, 38]. β-glucan and arabinoxylan, oligosaccharides such as galacto- and fructo-oligosaccharides, and resistant starch are the most important cereal-based water-soluble fibers, which have been utilized to fulfill the prebiotic concept during fermentation and product development.

Traditionally, many of the cereals are used in the production of gruels which are fermented into alcoholic beverages such as "*burukutu*" and "*pito*" or nonalcoholic beverage and meals such as "*kunu*" and "*ogi"* or "*agidi*" and represent a major dietary component in African countries [40–43]. These foods are used as weaning food for infants and children [44–46] and also for adults. The microorganisms may be indigenous to the food, or may be added as a starter culture after pretreating or cooking the product [35].

Lactic acid bacteria are a major contributor to fermentation process that takes place in the production of cereal-based fermented foods of Africa [47]. Fermenting

cereals with lactic acid bacteria provides a final product that contains lactic acid as a hallmark among other metabolites that may contribute to product characteristics [48, 49]. These organisms are generally regarded as safe (GRAS). Lactic acid bacteria can produce antimicrobial agents that exert strong antagonistic activity against many spoilage and pathogenic microorganisms. Metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde, acetoine, carbon dioxide, reuterin, reutericyclin, and bacteriocins [50] are examples of antimicrobial agents produced by LAB. The potential of antimicrobials from lactic acid bacteria is illustrated in Fig. 2. Organic acid produced by LAB leads to a reduction in pH levels and increases the production of hydrogen peroxide. These products exhibit antibacterial activity against various pathogenic microorganisms, including Gram-positive and Gram-negative bacteria [51, 52].

Fermentation can be applied to designing and manufacturing of functional foods. Since some of the major categories of cereal-based functional foods contain live microorganisms, they can serve as fermentable medium for the growth of probiotic

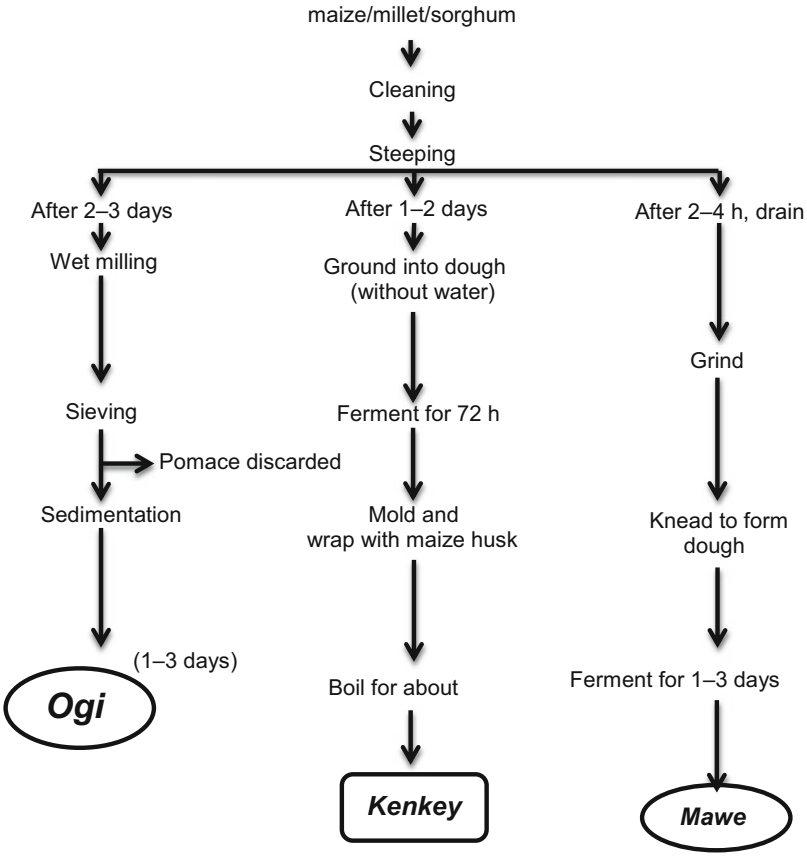


Fig. 2 Flowchart for the preparation of *ogi*, *kenkey*, and *mawe*

microorganisms and may be considered as vehicles for probiotic foods [22, 35, 52]. As noted by Kort et al. [53], the use of probiotic cultures for cereal fermentations can contribute to improving health and nutritional properties of the fermented foods by the delivery of beneficial bacteria, bioactive compounds such as vitamins, and sequestering toxic components.

3.1 Cereal Fermented Foods as Functional Foods

Cereal-based fermented food products are consumed in almost all parts of the world. Cereals contribute over half of the global food produced, and they are grown in over 73% of the world [54]. They are comprised of carbohydrates (60–70%), proteins (7–11%), fat (1.5–5%), crude fiber (2–7%), minerals, and vitamins [33, 55]. Proteins found in cereal are generally low quality compared to animal-based proteins, as a result of a lower amount of some essential amino acids, such as threonine, lysine, and tryptophan. The presence of antinutrients such as phytic acid, tannins, and polyphenols, can also bind to proteins, leading to a reduction in digestibility [42]. Fermentation by LAB has been shown to reduce phytic acids and tannins, therefore enhancing protein availability and digestion in various cereals such as maize, sorghum, and finger millet [54, 56]. Moreover, such fermented cereals have a higher composition of riboflavin, thiamine, niacin, and lysine [57]. Within the last decade, traditional fermented foods are increasingly considered healthy and wholesome, and as a result, public interest in their nutritional and health impact has increased, as has their demand. There is increasing scientific evidence to support the hypothesis that some foods and food components besides their provision of basic nutrition needs may modulate various physiological functions and may play detrimental or beneficial roles in some diseases [58]. These beneficial effects, which some food components exert beyond basic nutrition, have led to the concept of functional foods and nutraceuticals [59, 60]. "Let food be thy medicine and medicine be thy food," the age-old quote by Hippocrates, is certainly the opinion of today [61]. From a natural health perspective, functional food refers to nutritionally superior food products that have been enhanced through processing, fermentation, or growth technologies.

Due to lack of refrigeration facilities in less developed environments, many African traditional fermented foods rely heavily on lactic acid fermentation as a means of preservation and shelf-life extension. The low pH and high acidity encountered in cereal fermented beverages contribute to their bacteriostatic and antimicrobial properties. The need to develop cereal-based probiotic yoghurt-like beverages will go a long way in stimulating the concept of food as a health-promoting substance beyond basic nutrition [62].

3.1.1 Functional Foods

Functional foods are defined as the food or dietary components that may provide a health benefit beyond basic nutrition, do not contain synthetic compounds, and reduce illnesses with their bioactive nutrient. In this way, they help optimize physical and mental well-being [63].

The concept of functional foods is not new, but what does this term really mean? Functional foods, first introduced in Japan in the mid-1980s, contain bioactive food compounds or physiologically active nutrients and non-nutrients According to the definition, functional food is a part of an everyday diet and is demonstrated to offer health benefits and to reduce the risk of chronic disease beyond the widely accepted nutritional effects. This type of food is known in the Japanese market as "Foods for Specified Health Use" (FOSHU). The functional foods comprise: (i) conventional foods containing naturally occurring bioactive substances (e.g., dietary fiber), (ii) foods enriched with bioactive substances (e.g., probiotics, antioxidants), and (iii) synthesized food ingredients introduced to traditional foods (e.g., prebiotics).

Due to developments in ingredient technology, advances in scientific understanding of nutrients to promote well-being, and better management of health conditions, health and wellness trends are driving the commercialization of new ingredients for food and beverages. Functional components in functional foods include probiotic microorganisms, prebiotics, and bioactive compounds such as dietary fiber, polyunsaturated fatty acids, essential amino acids, antioxidants, vitamins, and minerals [64, 65].

Several methods are available for the production of functional foods which may rely on:

- Cereal compounds such as dietary fiber components, enrichment with an incorporated external ingredient, e.g., vitamin, minerals
- Modification of the functional compound enzymatically for release of bioactives, minerals or
- The synthesis of a functional compound by fermentation
- Addition of a probiotic strain such as *Lactobacillus* or *Bifidobacterium* [64]

In the light of this, a food can be made functional by applying any technological or biotechnological means to increase the concentration of, add, remove, or modify a particular component as well as to improve its bioavailability, provided that component has been demonstrated to have functional effect [66].

3.1.2 Fermented Probiotic Cereal Foods

Probiotics are living microorganisms which when ingested in certain numbers may exert health benefits beyond inherent general nutrition [67]. A probiotic food, on the other hand, is a live bacterial food supplement which, when ingested, may improve the well-being of the host in a variety of ways by influencing the balance of the host's intestinal flora. However, there is no general consensus as to whether probiotics should be viable in all cases to exert a beneficial effect, with some studies demonstrating that nonviable probiotic bacteria can have a beneficial effect on the host [68, 69].

Many traditional fermented cereal food products have been found to contain components with potential health benefits [45, 70]. Some may contain live microorganisms and are consumed in an active state of fermentation. Some African fermented products such as *Obiolor* [83] and *kunu zaki* are consumed unknowingly

as probiotic drinks, by local communities. The fermenting microorganisms might also indirectly impart health-promoting characteristics in food through the microbial production of bioactive metabolites (referred to as biogenics), such as certain vitamins, bioactive peptides, organic acids, or fatty acids during fermentation. Given that fermented food products can contain probiotics, prebiotics, or both, it is not surprising that their consumption has long been associated with good health [71]. While the potential benefit of probiotic bacteria has been generally accepted for decades, it is only recently that research has been able to document the beneficial health effect due to some specific strains [72]. The applications of probiotics have been well established throughout generations. The interest in the microorganisms in the recent years emanated from the discovery of their health effect in lowering plasma cholesterol. Probiotics are essentially nonexistent within poorer communities of Africa but some fermented probiotic cereal products are now been manufactured and may have an appeal for those communities. An initiative to introduce probiotic yogurt to Uganda, Tanzania, and Kenya has led to the creation of one gram sachets [25, 73] that contain dried starter culture of *Streptococcus thermophilus* and probiotic strains *Lactobacillus rhamnosus* GR-1 (Fiti), or a generic version of *L. rhamnosus* GG (Yoba) [26, 38, 53]. Each sachet can produce 100 L of probiotic yogurt, with a colony-forming unit (CFU)/mL of at least 1 billion, and is currently consumed by over 100,000 people per day in these countries [74]. Similarly, the effect of potentially human-derived probiotic bacteria on the physicochemical composition and acceptance of fermented cereal beverages showed good acceptance [75]. In a similar manner, an improved ogi *dogik*, which has been developed by Okagbue [70] using lactic acid starter with antimicrobial activities against diarrheagenic bacteria, were easily accepted.

The millet porridge *koko i*s a natural lactic fermented food product consumed daily by many people in Northern Ghana as lunch or an in-between meal. The fermented liquid top-layer called *koko sour water* (KSW) is also used fresh by the local population as a treatment for up-set stomachs or as a refreshing drink. As reported by Lei and Jakobsen, [45], the predominant LAB isolated from the fermentation of the cereal showed pronounced antimicrobial activity together with acid and bile tolerance. KSW with a low pH 3.6 and a content in the order of 10^8 live lactobacilli per milliliter is assumed to have a probiotic effect.

Regarding the functionality of probiotics, it is thought that in order to exert beneficial effects, they must be viable and available at a high concentration, typically at least 10^8–10^9 CFU per gram of product and should survive the human gastric juice in the stomach and reach the small intestine and the colon. It is generally agreed that best effect is achieved when the microorganisms colonize the intestinal epithelium since they can affect the intestinal immune system, displace enteric pathogens, and provide antimutagens and antioxidants, and possibly other effects by cell signaling.

3.1.3 Mechanism of Probiotic Action

The selection of a probiotic strain for human health and nutritional benefits requires that several aspects of functionality have to be considered: (1) acid tolerance and tolerance to human gastric juice; (2) bile tolerance (an important property for

survival in the small bowel); (3) adherence to epithelial surfaces and persistence in the human GI-tract; (4) immunostimulation, but no proinflammatory effect; (5) antagonistic activity against pathogens such as *Helicobacter pylori, Salmonella* sp., *Listeria monocytogenes*, and *Clostridium difficile*; and (6) antimutagenic and anticarcinogenic properties. The adhesion to mucosal surfaces by probiotic microorganisms is an important ability for the colonization of the human gastrointestinal tract, prevents their elimination by peristalsis, and provides a competitive advantage over pathogens.

Adhesion provides an interaction with the mucosal surface facilitating the contact with gut-associated lymphoid tissue mediating local and systemic immune effects. Thus, only adherent probiotics have been thought to effectively induce immune effects and to stabilize the intestinal mucosal barrier. Autoaggregation appeared to be necessary for the adhesion of probiotic strains to intestinal epithelial cells and coaggregation abilities may form a barrier that prevents colonization by pathogens [147].

The adhesion process can be divided into two steps: i.e., reversible adhesion due to long-range forces and subsequent interaction mediating a direct contact between microorganisms and support surfaces such as the hydrophobic interaction of microorganism and support. Biopsy sampling probably gives the most accurate information on the adhesion ability of probiotic strains. However, there are severe limitations in the technique: first and most importantly, ethical considerations limit the use of the technique. Secondly, the technique is very laborious and therefore only few individuals can be included in trials. Thirdly, the evacuation of the colon prior to colonoscopy probably leads to a loss of large number of adhering bacteria, leaving only the bacteria with the strongest adhering ability attached [148]. It could be argued that strong adhesion ability may increase the risk of infection in the host. Also, some probiotic strains are poorly adhering in vitro and/or in vivo and still they can show positive effects in the hosts.

The antimicrobial properties of probiotics can be attributed to both the competition for nutrients and the production of inhibitory compounds such as organic acids, hydrogen peroxide, and bacteriocins. The lowering of pH due to organic acids (especially lactic and acetic acids) produced by these bacteria in the gut has a bactericidal or bacteriostatic effect [49, 111].

The capacity to produce different antimicrobial compounds may be one of the critical characteristics for effective competitive exclusion of pathogen survival in the intestine and expression of a probiotic effect for the host. Although probiotic strains may produce bacteriocins, their role in the pathogen inhibition in vivo can only be limited, since traditional bacteriocins have an inhibitory effect only against closely related species such as other *Lactobacillus* or on spore-formers such as *Bacillus or Clostridium*. However, low-molecular-weight metabolites (such as hydrogen peroxide, lactic and acetic acids, and other aroma compounds) and secondary metabolites may be more important since they show wide inhibitory spectrum against many harmful organism like *Salmonella, Escherichia coli, Clostridium*, and *Helicobacter.* Lactic acid bacteria of the genera *Lactococcus*, *Pediococcus*, and *Lactobacillus* produce diacetyl which is rarely present in food fermentations at sufficient levels

to make a major contribution to antibacterial activity [111]. However, diacetyl production in sufficient amount inhibits the proliferation of food pathogens. The efficacy of bacteriocins particularly nisin to inhibit target organisms in food is determined by the chemical composition and physical conditions of the food system. The inhibition of such bacterial cells is caused by destabilization of the function of the cytoplasmic membrane.

Lactic acid fermentation not only inhibit pathogenic and spoilage organisms but also enhance flavor and texture by several mechanisms, such as the production of organic acids, hydrogen peroxide, and antimicrobial substances, as well as by lowering pH and oxidation/reduction potential [76, 77]. The development of functional fermented cereal food products has been the subject of several publications and reviews [11, 12, 35, 56, 61, 62, 78–80].

4 Production Methods for Fermented Cereal Foods and Beverages

Generally, African traditional cereal fermented foods are produced using various manufacturing techniques, raw materials, and microorganisms. On the basis of the use of microorganism(s), there are two types of food fermentation, namely, spontaneous fermentations and directed or controlled fermentations [44, 81].

4.1 Natural or Spontaneous Fermentations

Majority of the traditional cereal fermented foods and beverages from Africa are being produced spontaneously without inoculation or natural fermentations are carried out by the native microorganisms occurring on the raw material and in the environment of the production site [35, 82].

Spontaneous fermentation is a complex process carried out by the sequential action of different microorganisms. Contamination from the environment and equipment associated with the fermentation could be assumed to serve as natural inoculum of microorganisms in these foods [83, 84].

Most traditional African fermented food products are artisanal and are closely related to the local natural microbiota, which makes them a pertinent source of beneficial indigenous microorganisms [11]. As pointed out by Navarette-Bolanos [85], these native microorganisms, which have remained for a long time in the production areas, seemingly are the result of a natural selection associated to adaptation to the local environmental conditions. However, spontaneous fermentation is neither predictable nor controllable and occasionally presents low yield and poor quality outcomes.

The development of spontaneous food fermentation was primarily governed by climatic conditions, the availability of typical raw materials, the sociocultural ethos, and ethnic preferences. The growth and activity of microorganisms play an essential role in biochemical changes in the substrates during fermentation [32]. A large

number of fermented foods and beverages are made by this process, such as *ogi*, *uji*, *obiolor*, alcoholic beverages, etc. The quality of spontaneously fermented products is dependent on the microbial load and spectrum of the raw material, but it is always unpredictable because of the diverse microbiota initially present [86].

The spontaneous traditional fermentation is a complex microbial process operated by both cultivable and noncultivable microbial species, growing in succession or in combination throughout the fermentation process, exhibiting different metabolic patterns that can be either beneficial or detrimental to the product quality. In such conditions, the substrates and coexisting strains often interact through trophic or nutritional relations via multiple mechanisms [85].

4.2 Backslopping

Optimization of spontaneous fermentation processes has been made possible through inoculation of the raw material with a small quantity of a previously performed successful fermentation. After a few refreshments, repeated rebuilding or "backslopping" leads to a stable microbiota, giving rise to a selective enrichment of the best adapted strains [56, 87]. The dominant strain can be seen as a starter that shorten the fermentation process and reduce the risk of fermentation failure [31]. Based on spontaneous methods, the back-slopping technique is used in the preparation of several traditional fermented products [29, 88]. Back-slopping is also practiced in the production of *bushera*, but this has been considered to lead to fast production of acid and hence excessive sourness. Therefore, back-slopping is practiced in households where they prefer sour *bushera* over sweet one [89].

4.3 Controlled Fermentation

As the variation in the microbial community on such raw materials is considerable, such wild fermentations are difficult to reproduce and standardize. The need for consistent food quality culminated in the much better defined fermentation processes of today. In short, control is achieved through the pasteurization of the raw materials and the subsequent use of starter cultures. This results in the development and improvement of inoculants containing high concentrations of live microorganisms, and referred to as starter cultures. The development of pure cultures allowed the application of starter cultures for controlled food fermentation. A starter culture is a microbiological culture that actually performs fermentation or assists the beginning of the fermentation process in preparation of various foods and fermented beverages. Such cultures can consist of single strains or of (un)defined mixtures of strains and they are a major determinant for the organoleptic properties of the final product [90]. The modern large-scale production of fermented cereal-based foods is almost entirely dependent on the use of defined strains of these microorganisms, which could replace the undefined strain mixtures traditionally used for the manufacture of these products. A number of studies have been carried out to characterize yeast and

lactic acid bacteria diversity in cereal fermented foods and beverages in order to select suitable starter cultures or consortia for improving safety, quality, sensory and sometimes probiotic properties [91–93].

Many of the studies focus on the characterization of the microorganisms that are commonly associated with the processing of these products [42, 52, 94–99, 101, 103]. Salient information found in many African fermented cereal food products is shown in Table 1. The isolation of indigenous strains from the local production area and their selection and use as starter could ensure the adequate control of lactic fermentation and preserve some positive organoleptic contributions [14, 103].

4.4 Fermented Cereal-Based Products

Important class of fermented foods and beverages are made from cereals, which are popular in the continent of Africa. In particular, the natural microbial component is used to ferment grains including maize, millet, rice, or sorghum. The grains are often malted, heated, mashed, and sometimes filtered. Back-slopping is quite common, but

Table 1 Traditional lactic acid cereal-based fermented foods and beverages consumed in various countries in Africa

Product	Country	Cereal malt	Nature of fermented product	Product use
Banku	Ghana	Maize, cassava	Dough	Cooked dough
Ben-saalga, *koko*	Burkina Faso, Ghana	Pearl millet	Slurry	Gruel
Bushera	Uganda	Sorghum, millet + (S, Mi)	Slurry	Beverage
Gowé (*Sifanu*)	Benin	Sorghum + (S)	Cooked slurry	Beverage
Hussuwa	Sudan	Sorghum + (S)	Dough	Dough-like food
Injera	Ethiopia	Tef, sorghum, corn, finger millet barley	Batter	Flat bread
Kenkey	Ghana	Maize	Dough	Cooked/steamed dough
Kisra	Sudan, South Africa	Sorghum, pearl millet	Dough to thick batter	Flat bread
Mahewu	Zimbabwe	Maize + (S, Mi)	Slurry	Beverage
Mawè	Benin, Togo	Maize	Dough	Basis for ready-to-serve foods
Ogi	West Africa	Maize, millet, sorghum	Slurry	Basis for ready-to-serve foods
Togwa	East Africa	Maize + (Mi)	Cooked slurry	Beverage

the microbial populations responsible for the fermentation are now been characterized in an effort to source for starter cultures.

A wide range of cereal-based products is known, often with specific local differences in composition and method of preparation. A few principal categories of African cereal products could be distinguished. They include (semi) solid cooked doughs and porridges and liquid beverages, which may be nonalcoholic gruels [1, 76]. Table 1 presents these categories, showing the cereal-based food products that will be discussed in more detail in this paper. In this part of the chapter, these foods will be discussed with special reference to production methods, functional microbiota, and their impact on their nutritional contribution to the diet.

4.4.1 Ben-Saalga

Ben-saalga, a thin porridge, is prepared by cooking the fermented sediment of pearl millet (*Pennisetum glaucum*) in water, and is typical of Burkina Faso [76]. *L. fermentum*, *L. plantarum*, and *Ped. pentosaceus* typically dominate the natural fermentation. Some *L. plantarum* strains are able to degrade starch [96, 103] which can be beneficial as they can positively contribute to increase the energy density of cereal-based fermented gruels through starch hydrolysis [85, 96]. Although considerable losses of millet nutrients are suggested to occur as a result of discarding the coarse particles during preparation and by leaching into the water fraction, antinutritional components of pearl millet such as phytate are reported to be degraded by more than 50% in commercial *ben-saalga*, thereby facilitating the dietary uptake of proteins and minerals [76, 103].

4.4.2 Ogi

Fermented maize product, ogi, is a popular weaning and breakfast cereal in sub-Saharan Africa. *Ogi* is traditionally prepared by natural fermentation of maize grains to produce popular breakfast gruel and a complementary food for children. It is also made from sorghum or millet grains with the specific composition affecting the viscosity, fermentability, and content of the final product [104]. The stages of traditional *ogi* production include steeping (washing the grains, soaking in water for 24–72 h, wet milling, and wet sieving) and souring (sedimentation of the filtrate for 12–28 h) to obtain sour *ogi* [89, 105–107]. The periods of fermentation and souring determine the degree of sourness (measured by titratable acidity) and, to a large extent, the nutrient status of *ogi*.

The predominant microorganism in the fermentation responsible for the production of lactic acid is *L. plantarum.* A number of studies point to the ogi fermentative organisms as having probiotic qualities [91, 108]. *Ogi* is normally prepared as a water suspension and cooked before consumption. The cooked product is usually a gel of variable degree of stiffness. The fluid or semisolid cooked *ogi* is called by different names such as "*eko*," "*akamu*," or "*kafa*" in different localities, while the stiff gel is called "*agidi*" in Nigeria. *Agidi* is prepared by cooking, wrapping in leaves, and then allowed to set to form a stiff jelly. Ogi is usually consumed after heat treatment which destroys the lactic acid bacteria present, and therefore its probiotic effects. It must be pointed out that the functional aspects of these foods are related

not only to the interactions of bioactive living cells with the host but also indirectly as a result of the ingestion of bioactive molecules released during fermentation. Such bioactive components include dietary fiber, minerals, vitamins, and antioxidants. LAB fermentation directly affects nutrient availability by hydrolyzing carbohydrates and nondigestible oligosaccharides into functional compounds. On the other hand, the souring water obtained from ogi fermentation is normally used as a sweetened rich probiotic beverage and encouraged by nutritionists as a health-promoting drink and antidiarrheal tonic, thus further justifying their significance as functional foods [92, 108].

Fermented ogi liquor is considered as a preventative food in common diseases connected with diarrhea and abdominal discomfort. It is reported that many nursing mothers in many parts of Nigeria do give their babies *ogi* liquor (water from the fermented cereal pulp) and this causes the termination of such illness as diarrhea and abdominal discomfort [109]. Adebolu et al. [110] evaluated the antibacterial activities of ogi liquor from different grains against some common diarrheal bacteria in southwest Nigeria and discovered the inhibition of the pathogens by the ogi liquor which contains a variety of organisms including *Lactobacillus* species.

4.4.3 Obiolor

Obiolor is a nonalcoholic beverage produced from fermented sorghum and millet malts in Nigeria. *Obiolor* is consumed daily by the Igala tribe in Nigeria and highly associated with good health [83]. It is a thin gruel with sweet taste. The sweet taste is attributed to sorghum and millet malt. It is produced by steeping sorghum and millet grains in water overnight, after what, the grains are wrapped in fresh banana leaves and allowed to germinate for 3 days. The germinated grains (80% sorghum +20% millet) are wet-milled and prepared into slurry. The slurry is mixed with boiled water (ratio 1:4 v/v). The mash is cooled, filtered, and the residue discarded, while the filtrate is concentrated by boiling for 30 min with continuous stirring. The resulting gruel is cooled rapidly and allowed to spontaneously ferment for 24 h at ambient temperature, after which it is ready for consumption [83]. Chemical studies on the nutritional and antioxidant disposition of *obiolor* show it to contain 96% moisture, 7.8% crude protein, 8.9% available carbohydrate, 0.39% crude fat, 0.3% crude fiber, 2.4% ash, and 459.3 kJ/g energy value [112]. The beverage also was found to reduce ferric ion and Aflatoxin B_1-mediated increase in lipid peroxidation products (conjugated dienes, lipid hydroperoxides, and malondialdehydes) and protein carbonyl in animal models were reduced by the beverage. As evident from its antioxidant scavenging activity in addition to the gross energy content, the beverage can serve as functional food.

In another study, the effect of obiolor beverage on dyslipidemia, protein oxidation, lipid peroxidation, and DNA fragmentation in the liver of rats fed a high-fat diet was investigated. Results indicate that high-fat-diet-mediated alterations in liver and serum total cholesterol, triacylglycerol, high-density lipoprotein cholesterol, low-density cholesterol, and very low-density lipoprotein cholesterol were reportedly reversed by Obiolor [112]. The chemical study was able to show that

obiolor extenuated high-fat-diet-mediated dyslipidemia, protein oxidation, lipid peroxidation, and DNA fragmentation in rat [113].

4.4.4 Gowé

Gowé is an indigenous fermented sorghum-based sour beverage, which is widely consumed in urban areas of Benin Republic. It is made from a blend of malted and nonmalted sorghum flour that is produced by spontaneous fermentation involving mixed cultures of lactic acid bacteria (LAB) and yeasts. The fermentation process takes place in an environment with a moisture content varying between 52% and 87%. The sweet and sour dough obtained by decantation during fermentation needs to be cooked and further diluted in water to obtain the beverage. The dominant microorganisms of gowé fermentation were the lactic acid bacteria *Lactobacillus fermentum*, *Weissella confusa*, *Lactobacillus mucosae*, and *Pediococcus acidilactici*, and the yeasts *Kluyveromyces marxianus* and *Pichia anomala* [93]. Some of the lactic acid bacteria mentioned above have been proven to exhibit probiotic properties [114, 116].

Current efforts are geared toward the utilization of the sweet and sour dough without the hydrothermal treatment in order to preserve the probiotic qualities. A significant decrease in pH from 6.1 to 3.3, with a concomitant increase in titratable acidity (11–60 g/kg as lactic acid, dry weight), was observed after 24 h of fermentation when LAB was used either alone or in combination with yeasts in controlled conditions [93, 98, 99]. The LAB count increased significantly from 6.1 to 9.4 log CFU/ml, while the yeast count remained constant throughout fermentation.

4.4.5 Bushera

Bushera is the most common traditional fermented beverage produced in southwestern Uganda. It is mainly prepared from sorghum grains which may be germinated or nongerminated. *Bushera* is produced at household level by spontaneous fermentation. It is consumed by all age groups, and is used both as a weaning food and a thirst-quenching drink in the households and in B*ushera* bars [115]. The product is a common delight in both urban and rural areas of western Uganda. The sorghum or millet flour from the germinated sorghum and millet grains is mixed with the boiling water and left to cool to ambient temperature [116] Germinated millet or sorghum flour is then added and the mixture is left to ferment at ambient temperature for 1–6 days. The lactic acid bacteria isolated from Bushera comprised of five genera, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, and *Streptococcus*. *Lb. brevis* was more frequently isolated than other species [115]. Studies on the probiotic potentials of the fermentative organisms are still inconclusive, but evidence of antidiarrheal properties is established.

4.4.6 Borde

Borde is a cereal-based traditional fermented beverage and is widely consumed in the southern and western parts of Ethiopia. It is produced by spontaneous fermentation using rudimentary equipment [117]. Borde is an opaque, effervescent, whitish-gray to brown cultured beverage, with a dark consistency and a sweet-sour taste. It is

an important product because both adults and children often consume it as a low-cost meal replacement. Maize is more frequently used than other cereals (wheat, tef, sorghum, finger millet, and barley) for borde fermentation because of its low price [18] in rural Ethiopian villages. A combination of lactobacilli and yeasts are known to be involved in the traditional fermentation of a mixture of malted cereals (75%) and unmalted cereals (25%) for borde production. It usually takes about 4 days to produce a batch of borde. An average worker consumes about 1–2 liters of *borde* a day, which could sustain the individual without additional food for most of the day [117]. Consumers believe that borde enhances lactation and mothers are encouraged to drink substantial amounts of it after giving birth. Borde is also considered to alleviate malaria, diarrhea, constipation, and abscesses [107].

4.4.7 Mahewu

Mahewu, as reported by Gadaga et al. [118], is a sour beverage made from corn meal and sorghum/millet malt, and which is commonly fermented *by Lactococcus lactis* subsp. *lactis*. It is prepared from the maize porridge, which is mixed with the water. The sorghum, millet malt, or wheat flour is then added and left to ferment. The spontaneous fermentation process is carried out by the natural flora of the malt at the ambient temperature. *Lactobacillus bulgaricus* and *Lactobacillus brevis* have also been isolated from *mahewu*. *Mahewu*, inoculated with a *Lactobacillus* starter, is commercially produced in South Africa [119, 120].

4.4.8 Kwete

Kwete, on the other hand, is another Ugandan fermented cereal-based beverage mainly produced from a mixture of maize and malted millet flour. The millet grains are the only raw materials which are soaked (24–48 h), germinated (2–3 days), and sun-dried (1–2 days) during *kwete* production. The process of souring to produce sourdough is uncontrolled and carried out at ambient temperatures (24–30 °C) for 24 h. Kwete is ready for consumption within 24 h of fermentation and after being filtered using a cheese cloth or a bag woven from grass [121]. Filtering gives the kwete a smooth mouth feel as well as contributes to its uniform color distribution. Good quality *kwete* is creamish to light brown in color, with a thick consistency and a sweet-sour taste. The *Lactobacillus* and *Lactococcus* counts ranged from 5.40 to 7.36 log CFU/ml during fermentation. The greatest increase in lactic acid bacteria was noted between 24 and 48 h. Higher numbers of *Lactobacillus* than *Lactococcus* were observed, though by end of 72 h fermentation both species attained the same microbial population [123]. Fermentation of *kwete* is a spontaneous process initiated by the lactic acid bacteria and yeasts from both the malt and roasted sourdough. Malt also contributes the fermentable sugars and enzymes that initiate the fermentation process [123].

4.4.9 Kunun-Zaki

Kunun-zaki, an indigenous nonalcoholic beverage, is produced and widely consumed by adults and infants in the savannah region of Nigeria as a refreshing drink, an appetizer, a food complement, and to quench thirst. It is also used as a

substitute for or to complement soft drinks and wines at social gatherings. *Kunun-zaki* is prepared from either guinea corn (*Sorghum bicolor*), millet (*Pennisetum typhoides*), maize (*Zea mays*), or rice (*Oryza sativa*). Traditionally, the production involves steeping of whole millet or sorghum grains for 6–24 h, wet-milling with spices (ginger and pepper) and sweet potato, gelling of about 3/4 of the mixture in hot water pitching with about 1/4 fresh (ungelled) part of the mixture and allowing for overnight fermentation. The supernatant is ready for consumption after sieving.

The popularity of *Kunun-zaki* in Nigeria is due to its characteristic sweet-sour taste typical of other lactic acid bacterial fermented foods of African origin such as *mahewu* and *baganiya* [124]. The traditional processing of *kunun-zaki* involves the steeping of, wet-milling with spices, wet-sieving, and partial gelatinization of the slurry, followed by the addition of sugar and bottling. A brief fermentation usually occurs during *kunun-zaki* processing.

This short fermentation which usually takes place during steeping of the grains in water over a period of 8–48 h is known to involve both lactic acid bacteria and yeasts. Three species of lactic acid bacteria (*Lactobacillus plantarum*, *Lb. fermentum*, and *Lactococcus lacti*s) were isolated from fermenting kunun-zaki and characterized by [125].

4.4.10 Togwa

Another probiotic food togwa is a starch-saccharified beverage made from maize flour and finger millet malt [126] and usually consumed in the southern part of Tanzania. It is attractive to low-income groups due to its microbial stability, nutritional and organoleptic properties, as well as its probiotic potential [125]. *Togwa* is a sweet and sour nonalcoholic beverage and is one of the better studied African cereal beverages. It is produced from the flour of maize, sorghum, and finger millet and sometimes, cassava root; the chosen substrates are boiled, cooled, and fermented for approximately 12 h to form a porridge, which is then diluted to drink [127].

Its improvement in terms of flavor quality is essential for the development of the product for greater consumption. It is consumed by the working people and also used as refreshment as well as a weaning food. In the production of *togwa*, cereal flour is cooked in the water. After cooling at 35 °C, seed culture (old togwa) and cereal flour from the germinated grains are added. The fermentation process finishes at pH 4.0–3.2 [107, 128]. In a study on the microbiological and fermentation characteristics of *togwa*, Ref. [123] observed that the process was predominated by lactic acid bacteria (LAB) and yeasts. The isolated microorganisms were identified as *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus cellobiosus*, *Pediococcus pentosaceus*, *Weissella confusa*, *Issatchenkia orientalis*, *Saccharomyces cerevisiae*, *Candida pelliculosa*, and *Candida tropicalis*. The pH decreased from 5.24–5.52 to 3.10–3.34. Maltose increased initially and then decreased, fructose decreased, and glucose levels increased during the first 12 h of fermentation. The organic acids detected during fermentation included DL-lactic, succinic, formic, pyruvic, citric, pyroglutamic, and uric acid. Lactate was the predominant acid and increased significantly with time. The volatile organic compounds (VOC) detected included acetaldehyde, 2-methyl-propanal, 2-methyl-butanal,

3-methyl-butanal, ethanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, diacetyl, and acetoin lactic-fermented cereal foods like togwa, used in Tanzania as a weaning food or as a beverage can serve as a vehicle for strains with probiotic properties. Since togwa is not heat-treated after fermentation, it contains live lactic acid bacteria such as *L. plantarum* [123]. Kingamkono [51] found that fermenting *togwa* inhibited the growth of some enterotoxin-producing bacteria and reported that a significant reduction in the enteropathogen occurrence in rectal swabs of children under 5 years old was achieved when they were fed togwa.

5 Functional Properties of Lactic Acid Bacteria in Fermented Foods

LAB has two main advantages; the health-promoting benefits induced by the interaction of ingested probiotics with the host (probiotic function properties) and enhancement of the nutritional value of raw materials by producing bioactive microbial metabolites during the lactic acid fermentation. These metabolites include certain vitamins, bioactive peptides, organic acids, or fatty acids [128]. In this part of the chapter, the functionalities of lactic acid bacteria in fermented cereal foods will be highlighted (Fig. 3).

5.1 Functionality for Improved Nutritional Quality of Foods

The microorganisms used as starter cultures in cereal fermentations have been shown to improve the nutritional quality of food products by increasing the nutritional value and digestibility, and by reducing the effect of antinutritional compounds [134]. Fermentation of cereal-based beverages, as well as other food substrates, by LAB has been shown to improve protein digestibility [29], increase nutritional bioavailability of minerals and other micronutrients [123], prolong shelf life, and finally enhance organoleptic qualities [14, 21].

As pointed out earlier, LAB fermentation directly affects nutrient availability by hydrolyzing carbohydrates and nondigestible oligosaccharides into functional compounds. In addition, fermentation with probiotic bacteria of cereal-based extracts produces fermented product which combine health-promoting properties of both probiotics as well as prebiotics included in cereals, such as dietary fibers β-glucans and arabinoxylans [129]. The LAB hydrolysis of the cereal substrates allow for fermented foods to have an improved nutritional value, since they are often more easily digestible than unfermented food [9].

LAB can also be an important source of vitamins in some fermented food products. Cereal grains include many bioactive functional ingredients such as antioxidants, vitamins, and dietary fibers. Dietary fibers are considered among functional food components associated with promoting human health. LAB have the capability to accumulate water-soluble vitamins such as those included in the B group (B2, B7, B9, and B12) during fermentation, a key processes for the production

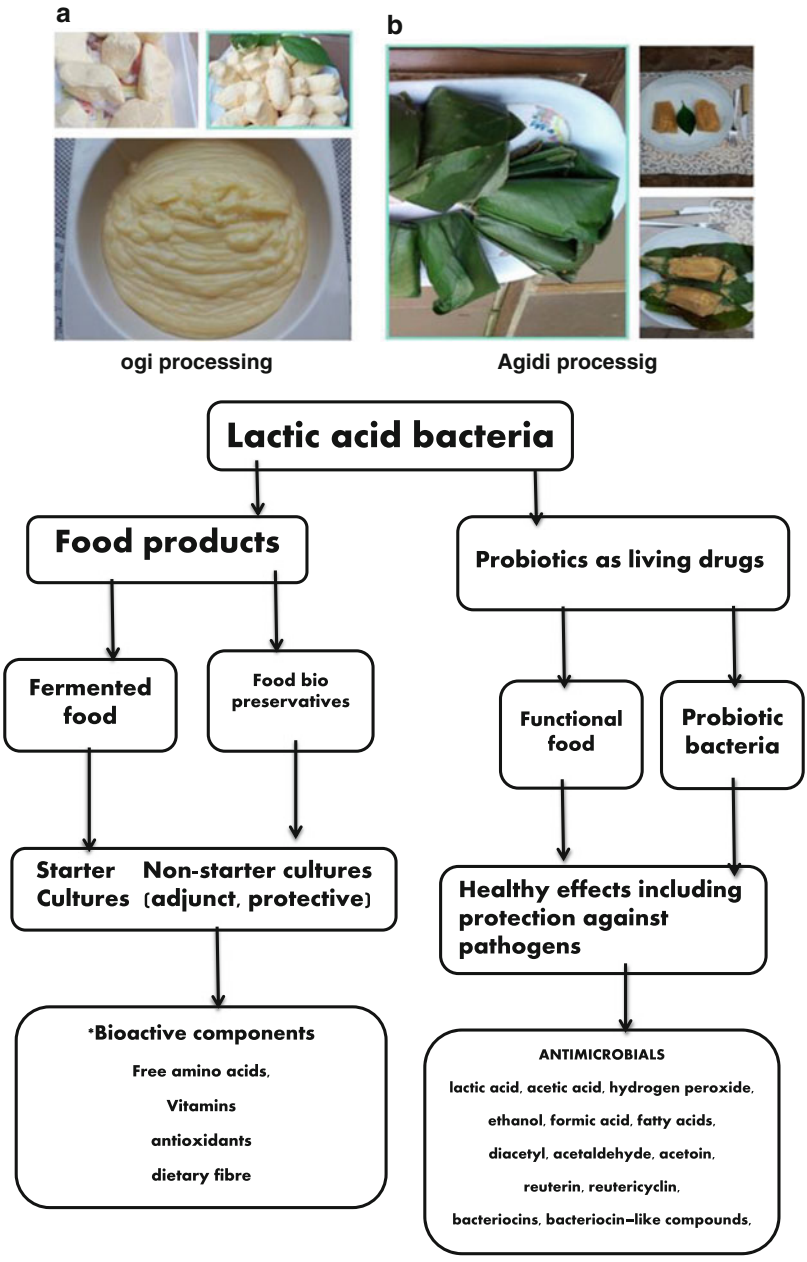


Fig. 3 Functional properties of lactic acid bacteria

of cereal-based beverages [130, 131]. The ability for lactobacilli to increase B-group vitamin production in cereal-based products offers novel opportunities for functional foods development. The possible strategies may lead to the elaboration of novel functional fermented foods [130, 149].

The nutritional quality of cereal grains and sensory properties of their products are inferior due to lower protein content, deficiency of certain essential amino acids, lower protein and starch availabilities, and the coarse nature of the grains, and is often counteracted by the presence of antinutrient components such as phytates, tannins, protease inhibitors, and polyphenols [33, 134]. In a sense, the antinutritive factors from cereals, including maize, sorghum, and millet, reduce the bioavailability of minerals such as calcium, iron, potassium, magnesium, manganese, and zinc, thus affecting the utilization of carbohydrates and proteins and causing deficiencies in the essential amino acids lysine, tryptophan, and methionine [9, 132]. LAB fermentation can overcome these problems by conferring to the final product, a higher protein quality and nutritional value, by inactivating protein inhibitors, or improving mineral solubility. In the case of cereal fermentation, the steps before fermentation, soaking, and germination causes increased activities of hydrolytic enzymes, improvement in the contents of certain essential amino acids, total sugars, and B-group vitamins, and a decrease in dry matter, starch, and antinutrients [129]. Phytic acid is present in cereals in the form of complexes with metal cations, viz. iron, zinc, calcium, and proteins. The enzymatic degradation of phytic acid requires an optimum pH which can be provided by natural fermentation. Such a degradation of phytic acid can increase the amount of soluble iron, zinc, and calcium a number of folds [36]. It has been reported that fermentation of millet grain for 12 and 24 h could reduce the food inhibitors, phytic acid and tannins [136].

5.2 Functionality to Improve the Health Benefits of the Host

Besides the quality improvements due to the prevention of spoilage and safety enhancement by the inhibition of pathogenic microorganisms, LAB fermented foods have numerous physiological effects on the host. The use of LAB as probiotics, living microorganisms that confer a beneficial effect on the host when administered in proper amounts, is the main health-related impact of this group of bacteria.

The dominance of *Lactobacillus* species during cereal fermentation and the presence of other bacteria with safety challenges were identified [99, 101, 146]. Therefore, harnessing many functional properties of these beneficial bacteria may significantly contribute to strategies that can improve nutritional quality and safety and promote health status of consumers of traditional fermented cereals, particularly in developing countries.

Starch is the major storage polysaccharide in cereal grains. Starch is composed of amylose and amylopectin. Amylase activity is an essential prerequisite during starchy food fermentation. Amylolytic bacterial strains are therefore desirable and could be readily applied as starter cultures during traditional cereal fermentation.

Amylase activity is an essential prerequisite during starchy food fermentation; it enhances hydrolysis of amylose and amylopectin to release fermentable maltose. Amylolytic bacterial strains are therefore desirable and could be readily applied as starter cultures during traditional cereal fermentation [137]. *L. plantarum* A6, an amylolytic strain, has previously been isolated from fermented cereals in Africa, and its α-amylase gene was cloned and sequenced [137], and the expression of this gene was also identified during in situ fermentation [101, 138]. Efforts have also been made to produce recombinant *L. plantarum* strains with amylolytic activity, but these clones are not particularly attractive to large-scale fermentation due to the requirement for the associated technologies that are not always available in developing countries.

5.3 Antimicrobial Activity of Probiotic Lactobacilli

Probiotic bacteria are able to change the population of the gut microbiota by influencing the metabolic and nutritional functions of commensal bacteria. They colonize the intestinal tract and exhibit protective properties by exerting antipathogenic effects based on competitive exclusion (for substrates and places of adhesion) and by inhibiting fermentation metabolites (organic acids and other antimicrobial compounds). They beneficially affect the host's physiology and health by improving microbial balance in the intestinal tract. Strong competitive exclusion of LAB toward pathogenic microbes has been observed in some studies. A *Lactobacillus* starter culture was used to produce an improved ogi called DogiK, which exhibited antimicrobial properties against some diarrheagenic bacteria [70, 139]. Application of probiotic starter cultures for control of infantile diarrhea showed promise, with some studies justifying their relevance in functional foods [140].

The antimicrobial properties of lactobacilli are also of special interest in developing strongly competitive starter cultures for food fermentation. Lactobacilli exert strong antagonistic activity against many microorganisms, including food spoilage organisms and pathogens. Production of the primary metabolite, lactic acid, and the resulting pH decrease is the main preserving factor in food fermentation. In addition, some strains may contribute to the preservation of fermented foods by producing other inhibitory substances, such as bacteriocins [141, 145]. Bacteriocins are antimicrobial proteinaceous compounds that are inhibitory toward sensitive strains and are produced by both Gram-positive and Gram-negative bacteria [142]. Research on bacteriocins from lactic acid bacteria has expanded during the last decades, to include the use of bacteriocins or the producer organisms as natural food preservatives.

Sanni et al. [141] reported the antimicrobial activity of partially purified bacteriocin produced during the natural lactic acid fermentation of *ogi* – a cereal fermented gruel by eight strains of lactic acid bacteria. The bacteriocin produced inhibited the growth of various target organisms with the inhibition strongly noticed using *Enterococcus faecalis* as indicator. While catalase treatment, pH changes and heat

treatment up to 80 °C had no effect on the activity of bacteriocin from these isolates, treatment with trypsin and proteinase K resulted in complete loss of inhibitory activity of the bacteriocins. A reduction in the inhibitory activity of the bacteriocins was also found to occur with increasing concentrations of glucose or peptone in the cultivation medium. There is no doubt that such microorganisms when used in cereal fermentations will provide a nutritious as well as a safe food product.

5.4 Fermented Food Functionality and Safety

The production of organic acids like lactic, acetic, and formic acids is the cornerstone of LAB's ability to impact on the growth of undesirable microflora from the early stages of fermentation, and therefore to preserve food. Carbon source, nitrogen source, and molecular oxygen play critical roles in supporting cell function and proliferation through providing of ATP reducing power and building blocks. Food preservation can also be achieved by LAB-triggered nutrient depletion, as well as by strain-specific production of inhibitors like ethanol, carbon dioxide, acetoin, diacetyl, acetaldehyde, or hydrogen peroxide (in aerobic growth). The balance between their presence and the potential negative changes in sensory properties of the products has, nevertheless, to be carefully considered. LAB has also the ability to produce different types of specific antimicrobial compounds to inhibit the activity of potential spoilage and pathogenic organisms. The selection of LAB capable of bacteriocin production, antimicrobial peptides, and proteins ribosomally synthesized has been the target of much research. Some of these bacteriocins, namely nisin, are already approved for use as food additive as they are very attractive alternatives to conventional antimicrobials.

Indigenous lactic acid fermented foods may have potential as probiotic treatment for diarrhea, due to high levels of lactic acid bacteria. Some fermented cereal-based foods available in West Africa may have probiotic potential.

Traditionally, uncooked Ogi is normally administered to diarrhea patients to reduce the frequency of stooling. Based on laboratory trials, Lei and Jacobsen [45] found that LAB isolated from *koko* can withstand the physiological challenges posed by the gastrointestinal tract (GIT) and may be able to colonize the GIT. In controlled human trials, Lei et al. [143] demonstrated that *koko sour water* (KSW) reduces diarrhea in children. Fecal enteric bacteria, such as *Salmonella*, *Shigella*, and *E. coli*, were found to be significantly less prevalent in children fed fermented maize gruel than in children who were not [144]. Similarly, lactobacilli strains isolated from uncooked ogi effectively inhibited the growth of *Salmonella* spp., either when inoculated after 8 and 24 h of growth of pathogen or when cultured overnight and then incubated with the pathogens [142–144]. Earlier, a *Lactobacillus* starter culture was used to produce an improved *ogi* called DogiK, which exhibited antimicrobial properties against some diarrheagenic bacteria [139]. Enhancement of lactation in nursing mothers is widely reported for the consumption of Kunun zaki, a fermented nonalcoholic cereal-based beverage in Northern Nigeria [124].

6 Conclusion

Fermentation is an age-old form of biopreservation in many parts of the world. Cereal fermentation provide an important source of energy and are represented by carbohydrates, proteins, and fats, but also fiber, vitamins, minerals, and other bioactive components essential to human health. Fermentation of cereals brings multiple improvements to nutritional composition, digestibility, shelf life, as well as flavor enhancement. Traditional cereal fermented food and beverages are widely consumed all over the continent of Africa. There is also a promising trend in the production of novel fermented cereal products aimed at the prevention of common diseases.

Naturally fermented foods may contain an abundant array of potential probiotic bacteria. Some of the cereal beverages are consumed while in an active state of fermentation. The number of live cultures in these foods may have enormous potential as candidates for the production of probiotic foods. Careful selection of these bacteria will provide an opportunity to develop foods that not only fulfill the commercial requirements, but also give the food functionality.

References

1. Das A, Raychaudhuri U, Chakraborty R (2012) Cereal based functional food of Indian subcontinent: a review. J Food Sci Technol 49:66–72
2. Ray MK, Singh GS, Mondal KC (2016) Folk to functional: an explorative overview of rice-based fermented foods and beverages in India. J Ethn Foods 3:5–18
3. Marsh AJ, Hill CR, P Cotter PD (2014) Fermented beverages with health-promoting potential: past and future perspectives. Trends Food Sci Tech 38:113–112
4. Todorov SD, LeBlanc JG (2014) Traditional fermented products – a good source for beneficial lactic acid bacteria. J Nutr Health Food Eng 1(4):00024. https://doi.org/10.15406/jnhfe.2014.01.00024
5. Wang CY, Wu SJ, Shyu YT (2014) Antioxidant properties of certain cereals as affected by food-grade bacteria fermentation. J Biosci Bioeng 117:449–456
6. Zannini E, Pontonio E, Waters DM, Arendt EK (2012) Applications of microbial fermentations for production of gluten-free products and perspectives. Appl Microbiol Biotechnol 93(2):473–485
7. Arena MP, Russo P, Capozzi V, López P, Fiocco D, Spano G (2014) Probiotic abilities of riboflavin-overproducing lactobacillus strains: a novel promising application of probiotics. Appl Microbiol Biotechnol 98(17):7569–7581
8. Myles IA (2014) Fast food fever: reviewing the impacts of the western diet on immunity. Nutr J 13:61–78
9. Waters DM, Mauch A, Cofey A, Arendt EK, Zannini E (2015) Lactic acid bacteria as a cell factory for the delivery of functional biomolecules and ingredients in cereal-based beverages: a review. Crit Rev in Food Sc Nutri 55:503–552
10. Zannini E, Mauch A, Galle S, Gänzle M, Coffey A, Arendt EK, Taylor JP, Waters DM (2013) Barley malt wort fermentation by exopolysaccharide-forming *Weissella cibaria* MG1 for the production of a novel beverage. J Appl Microbiol 115:1379–1387
11. Todorov SD, D Holzapfel WH (2015) Traditional cereal fermented foods as sources of functional microorganisms. In: Holzapfel WH (ed) Advances in fermented foods and beverages. Woodhead Publishing, Sawston, pp 123–153

12. Franz CMAP, Huch M, Mathara JM, Abriouel H, Benomar N, Reid G, Galvez A, Holzapfel WH (2014) African fermented foods and probiotics. Int J Food Microbiol 190:84–96
13. Singh U, Kochhar A, Singh S (2010) Complex carbohydrates: their effect in human health. Proc Indian Nat Sci Acad 76:81–87
14. Peyer LC, Zannini E, Arendt EK (2016) Lactic acid bacteria as sensory biomodulators for fermented cereal beverages. Trends Food Sci Technol 54:17–25
15. Coda R, Montemurro M, Rizzello CG (2017) Yogurt-like beverages made with cereals. In: Yogurt in health and disease prevention. Academic Press, London, pp 183–201
16. Reid G (2014) Harnessing microbiome and probiotic research in Sub-Saharan Africa: recommendations from an African workshop. Biology Publications. Paper 24. http://ir.lib.uwo.ca/biologypub/24
17. Corbo MRA, Bevilacqua L, Petruzzi F, Casanova P, Sinigaglia M (2014) Functional beverages: the emerging side of functional foods commercial trends, research, and health implications. Compr Rev Food Sci Food Saf 13:1192–1206
18. Gobbetti M, Di Cagno R, De Angeles M (2010) Functional microorganisms for functional food quality. Comprehensive Reviews in Food Science and Nutrition 50:716–721
19. LeBlanc JG, Laino JE, Juarez del Valle M, Vannini V, van Sinderen D, Taranto MP, Font de Valdez G, Savoy de Giori G, Sesma F (2011) B-group vitamin production by lactic acid bacteria – current knowledge and potential applications. J Appl Microbiol 111:1297–1309
20. O'Connor EB, Barrett E, Fitzgerald G, Hill C, Stanton C, Ross RP (2005) Production of vitamins, exopolysaccharides and bacteriocins by probiotic bacteria. In: Tamime A (ed) Probiotic dairy products. Blackwell Publishing Ltd, Oxford
21. Nionelli L, Coda R, Curiel JA, Poutanen K, Gobbetti M, Rizzello CG (2014) Manufacture and characterization of a yogurt-like beverage made with oat flakes fermented by selected lactic acid bacteria. Int J Food Microbiol 185:17–26
22. Achi OK (2005) The potential for upgrading traditional fermented foods through biotechnology. Afr J Biotechnol 4(5):375–380
23. Nath AK, Gupta A, Niopany B, Vyas G, Maneesri J, Thakur N, Sharma N, Achi OK, Hanpal P, Schillinger U (2016) Biotechnology and traditional fermented foods. In: Joshi VK (ed) Indigenous fermented foods of South East Asia. CRC Press, Boca Raton
24. Wood BJB (1994) Technology transfer and indigenous fermented foods. Food Res Int 27:269–283
25. Hesseltine CW (1983) Microbiology of oriental fermented foods. Ann Rev Microbiol 37:575–601. https://doi.org/10.1146/annurev.mi.37.100183.003043
26. Farnworth ER (2005) The beneficial health effects of fermented foods-potential probiotics around the world. Journal of Nutraceuticals, Functional & Medical Foods 4(3–4):93–117
27. Nagpal R, Kumar A, Kumar M, Behare PV, Jain S, Yadav H (2012) Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiol Lett 334:1–15
28. Odunfa SA, Oyewole OB (1998) African fermented foods. In: Wood BJB (ed) Microbiology of fermented foods, vol 2, 2nd edn. Blackie Academic and Professional, London. ISBN-13: 9780751402162
29. Holzapfel W (1997) Use of starter cultures in fermentation on a household scale. Food Control 8(5–6):241–258
30. Steinkraus KH (1996) Handbook of indigenous fermented foods. Marcel Decker Inc, New York
31. Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol 15:67–78
32. Tamang JP, Ki W, Holzapfel WH (2016) Review: diversity of microorganisms in global fermented foods and beverages. Front Microbiol 7:377. https://doi.org/10.3389/fmicb.2016.00377
33. Yadav H, Jain S, Rastamanesh R, Bomba A, Catanzaro R, Marotta F (2011) Fermentation technology in the development of functional foods for human health: where we should head. Ferment Technol 1:1–2

34. Lamsal B, Faubion JM (2009) The beneficial use of cereal components in probiotic foods. Food Rev Intl 25(2):103–114
35. Blandino A, Al-Aseeri ME, Pandiella SS, Cantero D, Webb C (2003) Cereal-based fermented foods and beverages. Food Res Int 36:527–543
36. Haard NF, Odunfa SA, Lee C–H, Quintero-Ramırez R, Lorence-Quinones A, Wacher-Radarte C (1999) Fermented cereals. A global perspective. In: FAO Agricultural Services Bulletin, vol 138. Food and Agriculture Organization, Rome
37. Charalampopulos D, Wang R, Pandiella SS, Webb C (2002) Application of cereals and cereal components in functional foods: a review. Int J Food Microbiol 79:131–141
38. Siró I, Kapolna E, Kapolna B, Lugasi A (2008) Functional food product development marketing and consumer acceptance – a review. Appetite 51:456–467
39. Steinkraus KH (2002) Fermentations in world food processing. Com Rev Food Sci Food Safety 1(1):23–32
40. Borresen EC, Henderson AJ, Kumar A, Weir TL, Ryan EP (2012) Fermented foods: patented approaches and formulations for nutritional supplementation and health promotion. Recent Pat Food Nutr Agric 4:134–140
41. Steinkraus KH (1997) Classification of fermented foods: worldwide review of household fermentation techniques. Food Control 8:331–317. https://doi.org/10.1016/S0956-7135(97)00050-9
42. Hounhouigan DJ, Nout MJR, Nago CM, Houben JH, Rombouts FM (1994) Microbiological changes in mawè during natural fermentation. World J Microbiol Biotechnol 10:410–413
43. Oyewole OB (1997) Lactic fermented foods in African their benefits. Food Control 8:289–297
44. Odunfa SA, Oyewole OB (1998) African fermented foods. In: Wood BJB (ed) Microbiology of fermented foods, vol 2, 2nd edn. Blackie Academic and Professional, London., ISBN-13: 9780751402162, pp 155–191
45. Lei V, Jakobsen M (2004) Microbiological characterization and probiotic potential of koko and koko sour water, African spontaneously fermented millet porridge and drink. J Appl Microbiol 96:384–397
46. Kalui CM, Mathara JM, Kutina PM (2010) Probiotic potential of spontaneously fermented cereal based foods: a review. Afr J Biotechnol 9(17):2490–2498
47. Franz CMAP, Holzapfel WH (2014) Examples of lactic fermented foods of the African continent. In: Lahtinen S, Guwehand AC, Salminen S, von Wright A (eds) Lactic acid bacteria; microbiological and functional aspects, 4th edn. CRC Press, Boca Raton, pp 265–284
48. Holzapfel WH, Schillinger U (2002) Introduction to pre- and probiotics. Food Res Int 35:109–116
49. Shah NP (2007) Functional cultures and health benefits. Int Dairy J 17:1262–1277
50. Sukovic JB, Kos J, Beganovic J, Pavunc AL, Habjanic K, Matocic S (2010) Antimicrobial activity of lactic acid bacteria. Food Technol Biotechnol 48(3):296–307
51. Kingamkono R, Sjögren E, Svanberg U (1999) Enteropathogenic bacteria in faecal swabs of young children fed on lactic acid-fermented cereal gruels. Epidemiol Infect 122(1):23–32
52. Nwachukwu E, Achi OK, Ijeoma IO (2010) Lactic acid bacteria in fermentation of cereals for the production of indigenous Nigerian foods. AfrJ Food Sci Technol 1(2):021–026
53. Kort R, Westerik N, Serrano LM, Douillard FP, Gottstein W, Mukisa IM, Tuijn CJ, Basten L, Hafkamp B, Meijer C et al (2015) A novel consortium of lactobacillus rhamnosus and Streptococcus Thermophilus for increased access to functional fermented foods. Microb Cell Factories 14:195–209
54. Hoseney RC (1992) Principles of cereal science and technology. American Association of Cereal Chemists, St. Paul. pp. 69–109, 185–189
55. Gull A, Prasad K, Kumar P (2016) Evaluation of functional, antinutritional, pasting and microstructural properties of millet flours. J Food MeasCharact 10:96–102
56. Salovaara H, Gänzle M (2011) Lactic acid bacteria in cereal-based products. In: Salminen S, von Wright A, Ouwehand A (eds) Lactic acid bacteria microbiological and functional aspects, 4th edn. Marcel Dekker, New York, pp 431–452

57. Shortt C (1999) The probiotic century: historical and current perspectives. Trends Food Sci Technol 10:411–417
58. Koletzko B, Aggett PJ, Bindels JG, Bung P, Ferré P, Gil A, Lentze MJ, Roberfroid M, Strobel S (1998) Growth, development and differentiation: a functional food science approach. Br J Nutr 80(Suppl 1):S5–45
59. Laparra JM, Sanz Y (2010) Interactions of gut microbiota with functional food components and nutraceuticals. Pharmacol Res 61:219–225
60. Ribiero H (2000) Diarrheal diseases in a developing nation. Amer J Gastroenterol 95:S14–S15
61. Vasiljevic T, Shah NP (2008) Probiotics—from Metchnikoff to bioactives. Int Dairy J 18(17):714–728
62. Nyanzi R, Jooste PJ (2012) Cereal based functional foods. In: Rigobelo EC (ed) Probiotics. Intech, Rijeka
63. Farnsworth ER (2008) The evidence to support health claims for probiotics. J Nutr 138:1250S
64. Salovaara H, Simonson L (2004) Fermented cereal-based functional foods. In: Hui YH, Goddik LM, Hansen AS et al (eds) Handbook of food and beverage fermentation technology. Marcel Dekker, New York, pp 721–727
65. Grajek WA, Olejnik Sip A (2005) Probiotics, prebiotics and antioxidants as functional foods. Acta Biochemica Polonia 52(3):665–671
66. Roberfroid MB (1998) Prebiotics and synbiotics: concepts and nutritional properties. Br J Nutr 80(4):S197–S202
67. Fuller R (1992) History and development of probiotics. In: Probiotics. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-2364-8_1
68. Ouwehand AC, Salminen S, Isolauri E (2002) Probiotics: an overview of beneficial effects. In: Siezen RJ, Kok J, Abee T, Schasfsma G (eds) Lactic acid bacteria: genetics, metabolism and applications. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-2029-8_1
69. Salminen S, Ouwehand A, Benno Y, Lee YK (1999) Probiotics: how should they be defined? Trends Food Sci Technol 10:107–110
70. Okagbue RN (1995) Microbial biotechnology in Zimbabwe: current status and proposals for research and development. J Appl Sci Southern Africa 1:148–158
71. Stanton C, Ross RP, Fitzgerald GF, Van Sinderen D (2005) Fermented functional foods based on probiotics and their biogenic metabolites. Curr Opin Biotechnol 16(2):198–203
72. Gorbach SL (2002) Probiotics in the third millenium. Digest Liver Dis 34:S2–S7
73. Reid G (2010) The potential role of probiotic yogurt for people living with HIV/AIDS. Gut Microbes 1:411–414
74. Stefano E, White J, Seney S, Hekmat S, McDowell T, Sumarah M, Reid G (2017) A novel millet-based probiotic fermented food for the developing world. Forum Nutr 9:529–547. https://doi.org/10.3390/nu9050529
75. Salmerón I, Thomas K, Pandiella SS (2015) Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. J Functional Foods 15:106–115
76. Nout MJR (2009) Rich nutrition from the poorest – cereal fermentations in Africa and Asia. Food Microbiol 26:685–692
77. Mbugua SK, Ahrens RH, Kigutha HN, Subramanian V (1992) Effect of fermentation, malted flour treatment and drum drying on nutritional quality of uji. Ecol Food Nutr 28:271–277
78. Guyot JP (2010) Fermented cereal products. In: Tamang JP, Kailasapathy K (eds) Fermented foods and beverages of the world. CRC Press, New York
79. Kohajdova Z (2014) Fermented cereal products. In: Montet D, Ray RC (eds) Microorganisms and fermentation of traditional foods. CRC Press, London
80. Mokoena MP, Mutanda T, Olaniran AO (2016) Perspectives on the probiotic potential of lactic acid bacteria from African traditional fermented foods and beverages. Food Nutr Res 8:29630
81. Divya JB, Varsha KKK, Nampoothiri M, Ismail B, Pandey A (2012) Probiotic fermented foods for health benefits. Eng Life Sci 12(4):377–390

82. Giraffa G (2004) Studying the dynamics of microbial populations during food fermentation. FEMS Microbiol Rev 28(2):251–260
83. Achi OK (1990) Microbiology of Obiolor: a Nigerian fermented non-alcoholic beverage. J Appl Microbiol 69(3):321–325. https://doi.org/10.1111/j.1365-2672.1990.tb01522.x
84. Narvush JA, Gadaga TN (2003) The role of interactions between yeasts and lactic acid bacteria in African fermented milks a review. Int J Food Microbiol 81:51–60
85. Navarette-Bolanos JL (2012) Improving traditional fermented beverages: how to evolve from spontaneous to directed fermentation. Eng Life Sci 12(4):410–418
86. De Vuyst LG, Vrancken F, Ravyts F, Rimaux T, Weckx S (2009) Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. Food Microbiol 26(7):666–675
87. Ravyts F, De Vuyst L, Leroy F (2012) Bacterial diversity and functionalities in food fermentations. Eng in Life Sciences 12:356–367. https://doi.org/10.1002/elsc.201100119
88. Soni SK, Soni R, Janveja C (2013) Production of fermented foods. In: Panesar PS, Marwaha SS (eds) Biotechnology in agriculture and food processing opportunities and challenges. CRC Press, Boca Raton, pp 219–278
89. Muyanja CMBK, Narvhus JA, Treimo J, Langsrud T (2003) Isolation, characterization and identification of lactic acid bacteria from bushera: a Ugandan traditional fermented beverage. Inter J Food Microbiol 80:201–210
90. Smid EJ, Kleerebezem M (2014) Production of aroma compounds in lactic fermentations. Annu Rev Food Sci Technol 5:313–326
91. Annan NT, Poll L, Sefa-Dedeh S, Plahar WA, Akobsen M (2003) Volatile compounds produced by *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei* in single starter culture fermentations of Ghanaian maize dough. J Appl Microbiol 94(3):462–474
92. Nyanga LK, Nout MJR, Tendekayi H, Gadaga H, Theelen B, Boekhout T, Zwietering MH (2007) Yeasts and lactic acid bacteria microbiota from masau (*Ziziphus mauritiana)* fruits and their fermented fruit pulp in Zimbabwe. Inter J Food Microbiol 120:159–166
93. Vieira-Dalodé G, Jespersen L, Hounhouigan J, Moller PL, Nago CM, Jakobsen M (2007) Lactic acid bacteria and yeasts associated with gowé production from sorghum in Bénin. J Appl Microbiol 103:342–349
94. Halm MA, Lillie A, Sorenson KJ, Jakobsen M (1993) Microbiological and aromatic characteristics of fermented maize doughs for kenkey production in Ghana. Int J Food Microbiol 12:531–536
95. Sawadogo-Lingani H, Diawara B, Glover RK, Tano-Debrah K, Traoré AS, Jakobsen M (2010) Predominant lactic acid bacteria associated with the traditional malting of sorghum grains. Afr J Microbiol Res 4(3):169–179
96. Songré-Ouattara LT, Mouquet-Rivier C, Humblot C, Rochette I, Diawara B, Guyot JP (2010) Ability of selected lactic acid bacteria to ferment a pearl millet-soybean slurry to produce gruels for complementary foods for young children. J Fd Sci 75(5):261–269
97. Sawadogo-Lingani H, Diawara B, Glover RK, Tano-Debrah K, Traoré AS, Jakobsen M (2010) Predominant lactic acid bacteria associated with the traditional malting of sorghum grains. Afri J Microbiol Res 4(3):169–179
98. Adimpong B, Nielsen DS, Sørensen KI, Derkx PMF, Jespersen L (2012) Genotypic characterization and safety assessment of lactic acid bacteria from indigenous African fermented products. BMC Microbiol 12:75–89
99. Oguntoyinbo FA, Tourlomousis P, Gasson MJ, Narbad A (2011) Analysis of bacterial communities of traditional fermented west African cereal foods using culture independent methods. Inter J Food Microbiol 145:205–210
100. Owusu-Kwarteng J, Akabanda F, Nielsen DS, Tano-Debrah K, Glover RLK, Jespersen L (2012) Identification of lactic acid bacteria isolated during traditional fura processing in Ghana. Food Microbiol 32(1):72–78
101. Oguntoyinbo FA, Narbad A (2012) Molecular characterization of lactic acid bacteria and in situ amylase expression during traditional fermentation of cereal foods. Food Microbiol 31:254–262

102. Obinna-Echem PC, Kuri V, Beal J (2014) Evaluation of the microbial community, acidity and proximate composition of akamu, a fermented maize food. J Sci Food Agric 94(2):331–340
103. Ekwem OH (2014) Isolation of antimicrobial producing lactobacilli from akamu (a Nigerian fermented cereal gruel). Afr J Microbiol Res 8(7):718–720
104. Ben Omar NH, Abriouel R, Lucas M, Martinez Canamero J, Guyot JP, Galvez A (2006) Isolation of bacteriocinogenic *Lactobacillus plantarum* strains from ben saalga, a traditional fermented gruel from Burkina Faso. Int J Food Microbiol 112:44–50
105. Osungbaro TO (2009) Physical and nutritive properties of fermented cereal foods. Afr J Food Sci 3(2):023–027
106. Odunfa SA, Adeyele S (1985) Microbial changes during traditional production of ogi-baba, a west African fermented sorghum gruel. J Cereal Sci 3:173–180
107. Teniola OD, Odunfa SA (2001) The effects of processing methods on the levels of lysine, methionine and the general acceptability of ogi processed using starter cultures. Int J Food Microbiol 63:1–9
108. Enujiugha VN (2006) Supplementation of ogi, a maize-based infant weaning food, with African oil bean seed (*Pentaclethra macrophylla* Benth). Int J Postharv Technol Innov 1:202–212
109. Michodjèhoun-Mestres L, Joseph H, Joseph D, Christian M (2005) Physical, chemical and microbiological changes during natural fermentation of "gowé", a sprouted or non-sprouted sorghum beverage from West-Africa. Afr J Biotechnol 4:487–496
110. Enujiugha VN, Badejo AA (2017) Probiotic potentials of cereal-based beverages. Critl Rev Food Sc Nutri 57(4):790–804
111. Ukeyima MT, Enujiugha VN, Sanni TA (2010) Current applications of probiotic foods in Africa. African J Biotechnol 9:394–401
112. Adebolu TT, Olodun AO, Ihunweze BC (2007) Evaluation of ogi liquor from different grains for antibacterial activities against some common diarrhoeal bacteria in south-west Nigeria. African J Biotechnol 6:1140–1143
113. Ajiboye TO, Iliasu GA, Adeleye AO, Abdussalam FA, Akinpelu SA, Ogunbode SM, Jimoh SO, Oloyede OB (2014) Nutritional and antioxidant dispositions of sorghum/millet-based beverages indigenous to Nigeria. Food Sci Nutr 2(5):597–604
114. Ajiboye TO, Iliasu GA, Adeleye AO, Ojewuyi OB, Kolawole FL, Bello SA, Mohammed AO (2015) A fermented sorghum/millet-based beverage, Obiolor, extenuates high-fat diet-induced dyslipidaemia and redox imbalance in the livers of rats. J Sci Food Agric 96(3):791–797
115. Oyetayo VO, Osho B (2004) Assessment of probiotic properties of a strain of *Lactobacillus plantarum* isolated from fermenting corn slurry. J Food Agric Environ 2(1):132–134
116. Muyanja CMBK, Narvhus JA, Treimo J, Langsrud T (2003) Isolation, characterisation and identification of lactic acid bacteria from bushera: a Ugandan traditional fermented beverage. International J Food Microbiol 80:201–210
117. Mukisa IMD, Porcellato YB, Byaruhanga K, Muyanja CMBK, Rudi T, Langsrud T, Narvhus JA (2012) The dominant microbial community associated with fermentation of Obushera (sorghum and millet beverages) determined by culture-dependent and culture-independent methods. International J Food Microbiol 160:1–10
118. Abegaz K, Beyene F, Langsrud T, Narvhus JA (2002) Indigenous processing methods and raw materials of borde, an Ethiopian traditional fermented beverage. J Food Technol Africa 7:59–64
119. Gadaga TH, Mutukumira AN, Narvhus JA, Feresu SB (1999) A review of traditional fermented foods and beverages of Zimbabwe. Int J Food Microbiol 53:1–11
120. Mugochi T, Mutukumira T, Zvauya R (2001) Comparison of sensory characteristics of traditional Zimbabwean non-alcoholic cereal beverages, masvusvu and mangisi with mahewu, a commercial cereal product. Ecol Food Nutr 40:299–309
121. Mcmaster LD, Kokott SA, Reid SJ, Abratt VR (2005) Use of traditional African fermented beverages as delivery vehicles for *Bifidobacterium lactis* DSM 10140. Int J Food Microbiol 102:231

122. Namugumya BS, Muyanja CMBK (2009) Traditional processing, microbiological, physiochemical and sensory characteristics of kwete, a Ugandan fermented maize based beverage. African J Food Agric Nutr Dev 9:1046–1059
123. Agarry OO, Nkama I, Akoma O (2010) Production of kunun-zaki (a Nigerian fermented cereal beverage) using starter culture. Int Res Jf Microbiol 1(2):18–25
124. Efiuvwevwere BJ, Akona O (1995) The microbiology of "kunun-zaki", a cereal beverage from northern Nigeria, during the fermentation (production) process. World J Microbiol Biotech 11(5):491–493
125. Mugula JK, Nnko SA, Narvhus JA, Sørhaug T (2003) Microbiological and fermentation characteristics of togwa, a Tanzanian fermented food. Int J of Food Microbiol 80:187–199
126. Prado FC, Parada JL, Pandey A, Soccol CR (2008) Trends in non-dairy probiotic beverages. Food Res Int 41:111–123
127. Kitabatake N, Gimbi DM, Oi Y (2003) Traditional non-alcoholic beverage, Togwa, in East Africa, produced from maize flour and germinated finger millet. Int J Food Sci Nutr 54:447–455
128. Molin G (2001) Probiotics in foods not containing milk or milk constituents, with special reference to *Lactobacillus plantarum* 299v. Am J Clinic Nutr 73:380S–385S
129. Sharma G, Ghosh BC (2006) Probiotic dairy foods and probiotics for health benefits. Indian Food Ind 25:68–73
130. Hassani A, Procopio S, Becker T (2016) Influence of malting and lactic acid fermentation on functional bioactive components in cereal-based raw materials: a review paper. Food Sci Technol 51:14–22
131. Capozzi V, Russo P, Dueñas MT, López P, Spano G (2012) Lactic acid bacteria producing B-group vitamins: a great potential for functional cereals products. Appl Microbiol Biotechnol 96:1383–1394
132. Kedia G, Wang R, Patel H, Pandiella SS (2007) Use of mixed cultures for the fermentation of cereal-based substrates with potential probiotic properties. Process Biochem 42:65–70
133. Holzapfel WH (2002) Appropriate starter culture technologies for small-scale fermentation in developing countries. Int J Food Microbio 75(3):197–212
134. Onyango C, Noetzold H, Ziems A, Hoffmann Bliey T, Henle T (2005) Digestibility and antinutrient properties and extruded maize-fingermilet blendin the production of *uji*. LWT 38:697–707
135. Reddy NR, Pierson MD (1994) Reduction in antinutrient and toxic components in plant foods. Food Res Int 27(3):281–290
136. Giraud E, Brauman A, Kekele S, Lelong B, Raimbault M (1991) Isolation and physiological study of an amylolytic strain of *Lactobacillus plantarum*. Appl Microbiol Biotechnol 36:379–383
137. Humblot C, Turpin W, Chevalier F, Picq C, Rochette I, Guyot JP (2014) Determination of expression and activity of genes involved in starch metabolism in *Lactobacillus plantarum* A6 during fermentation of a cereal-based gruel. Int J Food Microbiol 185:103–111
138. Olukoya DK, Ebigwei SI, Olasupo NA, Ogunjimi AA (1994) Production of DogiK, an improved ogi (Nigerian fermented weaning food) with potentials for use in diarrhoea control. J Trop Pediatr 40(2):108–113
139. Cebrián R, Baños A, Valdivia E, Pérez-Pulido R, Martínez-Bueno M, Maqueda M (2012) Characterization of functional, safety, and probiotic properties of *Enterococcus faecalis* UGRA10, a new AS-48-producer strain. Food Microbiol 30:59–67
140. Sanni AI, Onilude AA, TIbidapo O (1999) Biochemical composition of infant weaning food fabricated from fermented blends of cereal and soybean. Food Chem 65(1):35–39
141. Ogunbanwo ST, Sanni AI, Onilude AA (2003) Characterization of bacteriocin produced by *Lactobacillus plantarum*F1 and *Lactobacillus brevis* OG1. Afr J Biotechnol 28:219–227
142. Lei V, Friis H, Michaelsen KF (2006) Spontarneously fermented millet product as treatment for diarrhea in young children: an intervention study in northern Ghana. Int J Food Microbiol 110(3):246–253

143. Mensah P, Tomkins AM, Draser BS, Harrison TJ (1991) Antimicrobial effect of fermented Ghananian maize dough. J Appl Bacteriol 70:203–210
144. Afolayan AO, Ayeni FA, Ruppitsch W (2017) Antagonistic and qualitative assessment of indigenous lactic acid bacteria in different varieties of ogi against gastrointestinal pathogens. Pan Amer Med J 27:22–30
145. Okereke HC, Achi OK, Ekwenye UN, Orji FA (2012) Antimicrobial properties of probiotic bacteria from various sources. African J Biotechnol 11(39):9416–9421
146. Banwo K, Sanni A, Tan H, Tian Y (2012) Phenotypic and genotypic characterization of lactic acid bacteria isolated from some Nigerian traditional fermented foods. Food Biotechnol 26(2):124–142
147. Collado MC, Isolauri E, Salminen S (2008) Specific probiotic strains and their combinations counteract adhesion of *Enterobacter sakazakii* to intestinal mucus. FEMS Microbiol Lett 285:58–64
148. Saarela M, Mogensen G, Fonden R, Matto J, Mattila-Sandholm T (2000) Probiotic bacteria: safety, functional and technological properties. J Biotechnol 84:197–215
149. Arslan S, Erbas M (2016) Probiotic cereal-based fermented functional foods. In: Montet D, Ray RC (eds) Fermented foods, part 1 biochemistry and biotechnology. CRC Press, Boca Raton, pp 196–212
150. Fischer MM, Egli IM, Aeberli I, Hurrell RF, Meile L (2014) Phytic acid degrading lactic acid bacteria in tef-injera fermentation. Int J Food Microbiol 190:54–60

A Novel Delivering Agent for Bioactive Compounds: Chewing Gum

52

Ibrahim Palabiyik, Haniyeh Rasouli Pirouzian, Nevzat Konar, and Omer Said Toker

Contents

I. Palabiyik (✉)
Faculty of Agriculture, Department of Food Engineering, Namik Kemal University, Tekirdağ, Turkey
e-mail: ipalabiyik@nku.edu.tr

H. R. Pirouzian
Department of Food Science and Technology, Tabriz University of Medical Sciences, Tabriz, Iran
e-mail: rasuly_h@yahoo.com

N. Konar
Faculty of Architecture and Engineering, Department of Food Engineering, Siirt University, Siirt, Turkey
e-mail: nevzatkonar@hotmail.com

O. S. Toker
Faculty of Chemistry and Metallurgical, Department of Food Engineering, Yildiz Technical University, İstanbul, Turkey
e-mail: stoker@yildiz.edu.tr

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_32

Abstract

Functional food concept is one of the hot topics in the food industry. In recent years, people want to consume food products having health beneficial effect as well as nutritive characteristics. Regarding functional food development, foods have also advantages and disadvantages in terms of delivering bioactive compounds due to formulation (interaction of the bioactive compound with other ingredients, calorie value provided) and production process (mechanical and thermal processes applied during production). When considering the factors restricting usage of the food products as a delivery system, chewing gum is one of the most up-and-coming products in many aspects: (i) simplicity of the formulation prevents the activity of bioactive compound by interaction, (ii) level of mechanical and thermal stresses applied during production, (iii) enabling the release of targeted molecule in a controlled and sustained manner, (iv) different consumption behavior abolishing calorie intake concern since it is only chewed without swallowing, and (v) holding time in mouth. Usage of encapsulated bioactive compounds can improve the release behavior of the functional ingredient. Mastication process and the formed matrix/structure of the chewing gum also influence the release of the bioactive compounds. The researches about improving functionality of chewing gum have indicated that chewing gum can be used as a delivery system for transportation of the desired bioactive compound to body/targeted site. However, during functional chewing gum development, formulation, production process, mastication process, and type/form of bioactive compounds should be considered to achieve the product with required functional properties.

Keywords

Chewing gum · Functionality · Bioavailability · Confectionery · Delivery system

Abbreviations

CMG	Chios mastic gum
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
FDA	Food and Drug Administration
FM	Fusion method
HPMC	Hydroxypropyl methylcellulose
MCG	Medicated chewing gum
MCGs	Membrane coating granules
MS	Mutans streptococci
NRT	Nicotine replacement therapy
ODF	Oral disintegrating film
PVAc	Polyvinyl acetates
Qt	Quercetin
TP	Tea polyphenols
UGTs	UDP-glucuronosyltransferases enzymes

1 Introduction

Bioactive compounds have begun to attract attention after people understood the relation between diet and health. Approximately 35 million people die every year because of noncommunicable chronic diseases such as obesity, cardiovascular diseases, cancer, and diabetes which are responsible for 60% and 70% of death ratio in the world and Europe, respectively [1]. Most of such noncommunicable diseases are related with diet/eating behavior of people. Therefore, especially in recent years, functional food term is a part of people's everyday experience, which has motived the producers in industry and researchers to manufacture functional foods and improve functionality of the food products. According to basic definition of functional foods, they must have a positive effect on health as well as nutritiousness, in other words, they have function in terms of sustaining the optimal health and decreasing the noncommunicable disease risk [2]. Functional food term was used firstly in Japan in 1984. The essential features of functional foods [3, 4] are summarized in Fig. 1. As functional foods have one of those features, they have also two or all of them. Widely used bioactive compounds in the food industry can be classified as the following [5]:

Antioxidants: Phytochemicals including polyphenols (curcumin), flavonoids (anthocyanins), isoflavones, resveratrol, carotenoids (lutein).
Antimicrobials: Enzymes (lactoperoxidase, lysozyme), polysaccharides (chitosan), bacteriocins (nisin), herbs, spices, essential oils (terpenes), phenols, acids, aldehydes, ketones, and esters.
Vitamins: Water-soluble (B_1, B_2, B_3, B_5, B_6, B_7, B_9, and B_{12} vitamins and Vitamin C) and fat-soluble (Vitamin A, D, E, and K) vitamins.
Probiotics: Lactobacillus (*Lactobacillus acidophilus*, *L. casei*, *L. plantarum*) and Bifidobacterium genera.

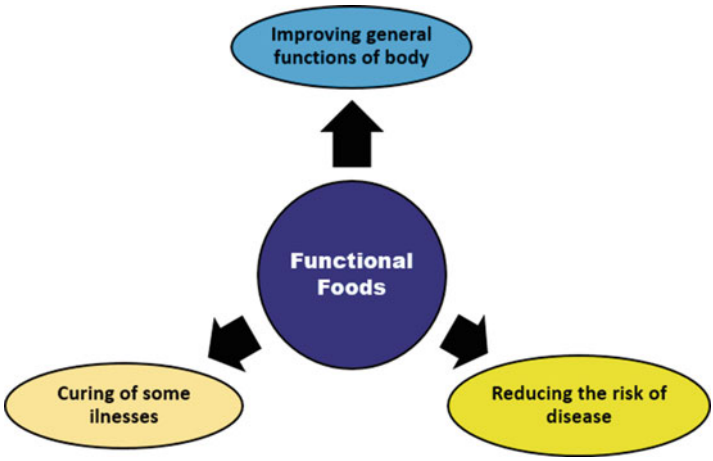


Fig. 1 General characteristics of functional foods

Prebiotics: Fructo-oligosaccharides, inulin, galacto-oligosaccharides, lactulose, polydextrose.
Other compounds: Natural flavorings (oregano, rosemary, β-pinene, sweet orange, lavender essential oils) and minerals.

Bioactive compounds (antioxidants, peptides, carbohydrates, lipids, and glucosinolates) are extra-nutritional agents that generally occur in low concentrations in foods. These constituents vary widely in chemical structure and function. Bioactive compounds actions can reduce the levels of circulating LDL, platelet aggregation, and tumor development. They also exhibit anti-inflammatory effect and estrogen-like activity, helping to maintain healthy bones, breast and increasing menopausal symptoms [6]. Phenolic compounds are present in plants especially in cereals, legumes, nuts, olive oil, vegetables, fruits, tea, and red wine. Most of the phenolic compounds have antioxidant properties, and they have desired effects on thrombosis and tumor-genesis and promotion. Antioxidants are applied in low concentrations to delay free radicals accumulation and hence inhibit oxidation of the food [7].

During functional product development, just adding the bioactive compounds mentioned above is not enough. Delivery system designed for bioactive compounds must provide: (i) maintenance of the activity and stability of bioactive compounds before their releasing, (ii) controlled and sustained release, and (iii) reaching and activating bioactive compounds in the target location [5]. Food matrix used for delivering and bioactive compounds should be taken into consideration with respect to these three factors. Otherwise, the targeted result cannot be achieved from functional food. In addition, as known, most of the bioactive compounds are sensitive to temperature, light, oxygen, and mechanical forces, which should be also considered during developing functional products. Therefore, in order to maintain them, encapsulated forms of the bioactive compounds are widely used in the food industry for preserving them against stresses applied during production and in the conditions of mouth, digestive, and gastric media.

Food matrix (structure of food influences release of the bioactive compound), production process of food products (heat level and duration, mechanical forces applied during production), and compositions of them (some bioactive compounds can interact with food components and lose their activity before reaching target side) directly affect effectiveness of delivery system. Considering all of the factors mentioned, chewing gum is one of the most suitable products for delivering of bioactive compounds in many aspects when compared to other food products:

(i) Production process of chewing gum: Level of applied heat and mechanical forces are not extreme and production process is quite easy in terms of both labor and equipment.
(ii) Formulation of chewing gum: Food products are compact products and they have many compounds such as fat/oil, protein, starch, simple or complex sugars, etc., at high amount. Some of these compounds can prevent activity or release of bioactive compounds.

(iii) Consumption style of chewing gum: Chewing gum differs from most of the foods in terms of consumption. Most of the foods are swallowed and stay at very short time in the mouth. However, regarding chewing gum, most parts of chewing gum (gum base) are discarded without swallowing. This distinctive feature of chewing gum can provide the following advantages: (i) providing less calories when compared with the other products, (ii) chewiness of the chewing gum in the mouth for a long time without being swallowed enable the controlled and sustained release of the bioactive compounds, (iii) only target compound can be released during chewing, and (iv) people of all age savorily consume chewing gums.

Chewing gum can be an attractive bioactive delivery system due to convenience for administration and controlled release of the corresponding bioactive compounds. Because of these favorable characteristics of chewing gums, they are widely used for medical purposes, medicated chewing gum. It was reported that chewing gum is the most convenient tool for delivering of encapsulated and un-encapsulated bioactive compounds due to its production processes where extreme heat and moisture conditions are not performed [8]. Fabrication and consumption characteristics of the chewing gum can provide an opportunity for manufacturing functional, nutritional, and dietetic chewing gums [9]. Concentration difference of the corresponding compound between saliva and chewing gum is a driving force for the transportation of the bioactive compounds from chewing gum to saliva. The nature of saliva in mouth gives rise to release of water-soluble compounds in short times, lower than 5 min, which can be accepted as negative aspects of usage of chewing gum for delivery of bioactive compounds. In order to eliminate the deficiency, chewing gum or bioactive compound/medicinal component can be designed to achieve controlled/sustained release [10]. Chewing gum formulation, encapsulation techniques used for encapsulation of bioactive compounds and encapsulating wall material should be organized in this respect. The interaction between gum base and bioactive compound specifies the release behavior of the functional ingredient. Therefore, selection of optimum gum base of the chewing gum is substantial for achieving targeted delivery system.

As all mentioned above indicates chewing gum is a suitable product for delivering of bioactive compounds and it has many advantages when compared with the other products; however, some attempts are required for enabling/improving achievement of delivery system of chewing gum. In this chapter, we describe formulation and production process of chewing gums and probable usages of chewing gums in delivering of bioactive compounds.

2 Chewing Gum

Chewing gums are defined as an elastic and extendable mass, which has two phases, containing sugar, polyols, gum base, aroma, acidulants, colors, and other ingredients and additives, according to the type of product [11–13]. Although chewing gum generally is classified as a subcategory of confectionery products, it is a food product

that can be expressed in terms of some of the consumption qualities it carries, even though there are different opinions about the definition as a food. The composition providing the winning character consumption or use by the gum mastication characteristics and properties of gum base portion is located mainly in the composition of elements and determines the applied processes [14]. The main feature that makes chewing gum as a special product in the food category is that it is long lasting in the mouth and undergoes the highest level of mechanical effect. Chewing gum is typically manufactured using an insoluble gum base blended with soluble sweeteners and flavorings.

In this part of the chapter, ingredients and types of the chewing gums will be introduced.

2.1 Chewing Gum Ingredients

Chewing gum consists of two phases: (i) water-insoluble continuous phase (gum base) and (ii) water-soluble discontinuous phase (sweeteners, color, flavor, etc.). These phases are generally formulated at a ratio of 1:3, respectively, depending on the chewing gum type [15]. The chewing gum contains a gum center (including a gum base, a bulk portion, and one or more flavoring agents) and an outer coating of layers which includes at least two sugar alcohols (preferably lactitol, erythritol, hydrogenated isomaltulose, or maltitol). Coating layers consist of 50–100% polyol. At this phase, more flavors, colors, and actives can be added. Sugar syrup, Arabic gum, starches, and other binders can be applied to the surface of gum. Optional components include vitamins, cooling and warming agents, menthol, plant extracts, etc. Gum base is the main composition of the chewing gum (20–30%) [11]. It is an inert, nonnutritive, and nondigestive product which is not dissolved while chewing; also it is used as a delivery system to carry sweeteners, flavors, and other substances in chewing gum. Gum base is made of a combination of food-grade hydrophilic and hydrophobic polymers, waxes, and softeners including elastomers, resins, plasticizers, fillers, and antioxidants. A generic chewing gum formulation is shown in Table 1.

2.1.1 Water Insoluble Chewable Gum Base Portion

Elastomer is a polymer and the key ingredient with high elongation properties that provides elasticity and cohesiveness and controls rubbery texture. When present in high quantities, the gums may have a hard texture, while low concentrations render the lack of elasticity of the product [16]. Latex or natural gums such as Jelutong, Lechi Caspi, Perillo, and Chicle are natural elastomers applied in chewing gum [17]. Butadiene-styrene copolymers, polyisobutylene, isobutylene isoprene copolymers are synthetic elastomers used in chewing gum formulations [18]. Also synthetic elastomers such as polyethylene mixtures and nontoxic vinyl polymer, such as polyvinyl alcohol, are widely used bases [19]. Types and quantity of elastomers determines the ability of formulation process.

Table 1 Typical chewing gum formulation

Ingredients	Examples	Functions
Gum base: Elastomer	Natural: Smoked or liquid latex, guayule, Jelutong, Lechi-caspi, Perillo, sorva, rosadinha, chicle, massaranduba balata, massaranduba chocolate, nispero Synthetic: Polyisobutylene, isobutylene, isoprene copolymer, styrene-butadiene copolymers, polyvinyl acetate	*Provides elasticity and cohesiveness*
Anticaking agent	Precipitated silicon dioxide, solid carbon dioxide	*Preventing agglomeration*
Plasticizer/ softener	Glycerin, lecithin, aqueous sweetener solutions, sorbitol, hydrogenated starch hydrolysate, corn syrup, tallow, cocoa butter, glycerol monostearate, glycerol triacetate, fatty acid (palmitic, stearic, oleic)	*Regulating the cohesiveness and modifying the texture*
Antioxidant	Ascorbic acid, tocopherol, butylhydroxytoluene	*Prevent oxidation of gum base*
Antitack agent	Slip-agent can be used as this purpose which may be comprised of α-cellulose and vegetable proteins Alkaline metal phosphate, maltodextrin	*Helps chewing gum not adhere to denture fillings and natural teeth*
Emulsifier	Mono-, di-, tri-stearyl acetate; lactylic esters	*Dispersing immiscible compounds*
Filler/ texturizer	Magnesium and calcium carbonate; ground limestone; silicate types; clay; alumina; talc; titanium oxide; mono, di-, tri-calcium phosphate; cellulose polymers	*Modifying the texture of gum base*
Elastomer solvents 15%	Ester gums: Glycerol esters, pentaerythritol esters of rosins (hydrogenated dimerized and polymerized rosins) Synthetic: Terpene resins	*Softening elastomeric material*
Colorants	Fruit and vegetable extracts, titanium oxide	*Improving color*
Sugar	Sucrose, dextrose, maltose, dextrin, dried invert sugar, fructose, levulose, galactose, corn syrup	*Bulk material, texture, and improving taste*
Polyols	Sorbitol, mannitol, xylitol, hydrogenated starch hydrolysate, maltitol	*Bulk material and texture*
Flavor	Essential oils, synthetic flavors, mixture (citrus oils, fruit essences, peppermint oil, spearmint oil, clove oil, oil of wintergreen and anise)	*Improving/providing flavor*
High-intensity sweeteners	Sucralose, aspartame, salts of acesulfame, alitame, saccharin	*Production of sugar-free product*

In addition, flavor release properties of the gum base are affected by the type of elastomer used. For instance, gum bases made with poly isobutylene showed higher affinity for flavor agents compared to polyvinyl acetate [20]. Resins are the conventional elastomer solvents aiding the softening of the elastomer base component.

They act as binders and softeners between elastomers and texturizers. Occurrence of solvents in low concentrations leads to unacceptable chewing properties [16]. Moreover, high quantities of solvent results in stickiness to dental surfaces. Terpinene resins such as polymers of alpha-pinene or beta-pinene, methyl, glycerol, or pentaerythritol esters of resins or modified resins and gums, such as hydrogenated, dimerized, or polymerized resins or mixtures, are applied.

Emulsifier is added in order to optimize the chewability and mouth-feel of the gum and also it allows two indissoluble phases to disperse in one another and can improve softness. Also it reduces gums adhesive nature. Emulsifier helps for ingredients to be mixed and hydrated while chewing. They include mono-, di-, tri-stearyl acetate, lactylic esters. Plasticizers or softeners such as lanolin, palmitic acid, oleic acid, stearic acid, sodium stearate, potassium stearate, glyceryl triacetate, glyceryl lecithin, glyceryl monostearate, propylene glycol monostearate, acetylated monoglyceride, glycerin, tallow, cocoa butter, natural and synthetic waxes, hydrogenated vegetable oils, polyurethane waxes, paraffin waxes, microcrystalline waxes, fatty waxes, sorbitan monostearate, propylene glycol are used to obtain desirable texture and adjust cohesiveness of chewing gum [19]. It reduces the brittleness and gives the elastomers softness.

Antioxidants such as propyl gallate, butylated hydroxy anisole, and butylated hydroxy toluene are applied in the product to prevent oxidation of gum base and also flavors during shelf-life. Fillers or textural agents provide overall texture and improve chewing ability. Texturizer's amount determines the cud size and stretching of the product. Commonly used fillers are magnesium and calcium carbonate, ground limestone, silicate types, clay, alumina, talc, titanium oxide, mono-, di-, tricalcium phosphate, and cellulose polymers [21]. Antitack agents such as slip-agent (comprised of α-cellulose and vegetable proteins), alkaline metal phosphate, and maltodextrin eliminate self-adhesiveness of resins because they have a tendency to stick together. It reduces fragmentation of the gum during mastication and terminates attaching to the teeth [22].

Natural elastomers such as latex or vegetable sources such as chicle, which is composed of cis- and trans-polyisoprene [23], were predominantly used in the past. However, synthetic elastomers are more commonly used today in their place [24]. When environmental safety and waste control are taken into consideration, it can be seen that natural and biodegradable elastomers in the gum composition have been re-emphasized instead of synthetic elastomers [25, 26]. There is a scarce study on the use of natural and biodegradable elastomers. When these studies are examined, it is seen that especially zein is used as an alternative natural elastomer [26–28]. The release amount of bioactive substance was determined to be 95%, as a result of the study conducted with medicated chewing gum produced using corn-originated zein. It is also a positive result that the textural properties are compatible with the control samples obtained using conventional synthetic gum base [27]. Plant-based polymers also show functional properties and have a potential in chewing gum production as a gum base. In a study, Kenger plant (*Gundelia tournefortii*) was used in the production of antimicrobial and antioxidant chewing gum. It was found that methanolic extract of gum exhibited 195.6 gallic acid equivalents (GAE) mg/100 g gum

antioxidant activity and 17.9 mm inhibition zone for *Escherichia coli* O157:H7 as an antimicrobial activity [29]. Production of functional chewing gum from such natural sources is very important for functional industry and such functional and natural products will attract attention and they will be the most preferred food products in the future.

2.1.2 Water Soluble Bulk Portion

Sweeteners provide the sweetness of chewing gum and also it improves the taste of the product. In addition, it masks the harshness of some flavor agents such as menthol and menthone. There are two types of sweeteners: aqueous and bulk. Aqueous sweeteners such as sorbitol, hydrogenated starch hydrolysates, and corn syrups are applied to blend components and keep the product fresh and flexible. Moreover, they act as a plasticizer or softening and binding agents [30]. Examples of bulking agents include sucrose, dextrose, maltose, dextrin, dried invert sugar, fructose, levulose, and galactose. Polyols such as sorbitol, mannitol, malitol, and xylitol are widely used as bulking agents; also a lower calorie gum is produced. High-intensity sweeteners (alitame, aspartame, acesulfame K, sucralose, saccharin, thaumatin, and neotame) are used when a lower calorie product is needed.

Coloring agents are added to the chewing gum in order to improve the color of the formulation. Colorants produce gentle and soft color in the product [31]. The coloring agents include fruit and vegetables extracts and also titanium oxide.

A variety of flavoring agents such as essential oils, synthetic flavors, and mixtures (citrus oils, fruit essences, peppermint oil, spearmint oil, clove oil, oil of wintergreen and anise) are suitable for providing an acceptable flavor and also they mask the bitter taste of some drugs incorporated in the product as an active ingredient [32]. Silicon dioxide and solid carbon dioxide are anticaking materials that are used to prevent agglomeration. These agents improve flowability and rehydration. They are added to the formulation to extend shelf life.

2.1.3 Sugar-Free Chewing Gums

There is a need to use alternative sweeteners to control sugar, carbohydrate, and calorie intakes. Low-calorie sweeteners aid in weight maintenance and also assist in the management of diabetes [33]. There are some reliable reviews and statements indicating that sucrose-free chewing gum can help reducing the risk of dental cavities, teeth demineralization, and other periodontal problems [34]. Bulking agents with a sweet taste are commonly used for the manufacture of sugar-free chewing gums. Polyols are soluble in water, thus they can quickly deplete from chewing gum during mastication.

The low sweetness of sugar alcohols can be enhanced by high intensity sweeteners. Acesulfame K-containing chewing gum has a pleasant sweet taste due to its fast onset of sweetness. Acesulfame K has good solubility; therefore it can be dissolved quickly by the saliva. Prolonged sweetness may be achieved by encapsulation of some of the sweeteners or usage of their combinations. Application of intense sweeteners like neotame and aspartame positively extends both sweetness

and flavor. Therefore, these sweeteners have flavor-enhancing property and may extend the flavor of chewing gum up to four times longer [35].

However, there is a challenge for formulation of sucrose-free chewing gums due to incongruity of some sweeteners with ingredients of the chewing gum matrix. High-intensity sweeteners are affected by factors such as pH, moisture, temperature, microbial growth, and chemical reactions. For example, heat, moisture, alkaline pH can destabilize aspartame. Also, the sweetening power of aspartame can be notably reduced in the presence of aldehyde-based flavors such as cinnamon. Likewise in the chewing gum formulations containing sodium pyrophosphate and aspartame can be degraded rapidly in the formulation. Aspartame can be stabilized by acid treatment prior to its incorporation into a cinnamon-flavored chewing gum [36] or it can be entrapped within a hydrophobic matrix consisting of lecithin, fatty acids, and synthetic waxes [37].

Haahr et al. [38] showed that the release of mint flavors from xylitol-containing chewing gums was higher than the gums made with sorbitol. This finding may be explained by the high water solubility of xylitol (2350 g/L) in comparison to sorbitol (182 g/L). According to the results obtained from Potineni and Peterson [15], release of flavors from a sugar-free phase did not follow the log P model. In a sugared chewing gum, the release of cinnamaldehyde was stable throughout the 8-min chewing time. In contrast, the cinnamaldehyde release from the sugar-free chewing gum was more rapid. Using tandem mass spectrometry, the researchers stated that hemiacetal products formed due to reaction of cinnamaldehyde with sorbitol. These components were more polar and unstable under slight alkaline conditions; therefore the more polarity of these products would result in a more rapid release rate of cinnamaldehyde.

Streptococcus mutans and *Streptococcus sobrinus* (mutans streptococci) combine water-insoluble glucans from sucrose and produce large amounts of acid, which is involved in tooth demineralization. The acid tolerance of these bacteria is extremely high, therefore allowing colonization and persistence under cariogenic conditions [39]. The chewing gum containing xylitol and sorbitol has been suggested to reduce caries rates. Xylitol is not fermented or used by *Streptococcus mutans* as a growth substrate; hence it can inhibit the microorganism growth in saliva [40]. In addition, the consumption of xylitol can decrease the metabolism of acidogenic flora and hence decrease the level of caries occurrence. Thaweboon et al. [41] investigated the effect of xylitol chewing gum on mutans streptococci (MS) in saliva and dental plaque. They observed that chewing 100% xylitol gum caused a considerable reduction in the salivary MS count. Chewing gum sweetened with 100% and 55% xylitol was well accepted by all the participants in the study. Adverse reactions like gastrointestinal discomfort or transient loose stools were not reported. The usage of sorbitol in chewing gum production was shown to be effective in reducing teeth remineralization due to maintaining interproximal plaque pH (>5.5). A clinical study conducted by Kleber et al. [42] showed the beneficial effect of baking soda on removing plaque and reducing gingivitis in a polyol-containing chewing.

Çaglar et al. [43] evaluated the effect of xylitol and probiotic (*Lactobacilli reuteri*) chewing gums on salivary mutans streptococci (MS). They examined the

oral effects of probiotic and xylitol together. The results demonstrated that a 3-week consumption of probiotic bacteria or xylitol in chewing gum significantly reduced ($P < 0.05$) the levels of salivary mutans streptococci; however, the combination of the two agents did not enhance the beneficial effect.

2.1.4 Medicated Chewing Gum, a Novel Drug Delivery System

Chewing gum can be applied as an oral drug delivery system because it can deliver pharmaceuticals and/or nutrients which are known as medicated chewing gum (MCG) and non-MCG. MCG adjusts a continuous release of medicine entrapped in the product [44]. Due to MCG's convenient administration, proven health, nutrition and cognitive benefits, it became acceptable all around the world [45]. The stability of the active substance incorporated in the chewing gum is good because it protects the drug from oxygen, light, and humidity. MCG has widespread application in medicinal and food industry because it has the ability to release active and functional components into oral cavity and also the release action is rapid and steady. Caffeine, nicotine, sodium fluoride, dimenhydrinate, chlorhexidine, acetyl salicylic acid, and vitamin C and are drug samples that were formulated in the form of chewing gum [46, 47]. The advantages and disadvantages of medicated chewing gums are briefly listed in Table 2.

Chewing gum as a drug delivery system can be used to cure and prevent the dental caries, pain, smoking cessation, obesity, motion sickness, acidity, and diabetes. To prevent tooth decay, several factors such as the amount of fluoride intake, good health, and decreasing the carbohydrates consumed by the bacteria is important.

Table 2 The advantages and disadvantages of medicated chewing gums [44]

Advantages	1. Increasing rate of effectiveness 2. Termination of drug delivery due to removal of gum any time 3. Reduced risk of overdosing 4. Requiring no water to drink 5. Protects the susceptible drugs from chemical or enzymatic attack in gastrointestinal tract 6. Suitable for both systemic and local drug delivery 7. Appropriate for rapid delivery 8. Fewer side effects 9. Reduced risk of intolerance to gastric mucosa 10. Great stability against light, oxygen, and moisture 11. Extirpation of xerostomia and help tasting and swallowing in people with dry mouth 12. Reducing hypoglycemic shocks in patients taking antidiabetic drugs 13. Help reduce food cravings
Disadvantages	1. disappearing of drug in oral cavity following salivary dilution 2. different release profiles because the chewing styles are different 3. short time of administration due to eating, speaking, and drinking 4. allergic reaction to artificial sweeteners 6. teeth decay through being coated by sugar 7. stomach irritations, aches, gastric ulcer through continuous swallowing of saliva and even flatulence because of presence of sorbitol in some formulations

Antimicrobial substances are used for treatment of gingivitis, periodontitis, plaque growth, and other oral and pharyngeal infections. For example, studies have proved that the use of chlorhexidine in chewing gum formulations was effective in reducing plaque and gingivitis and in masking its undesirable taste [48]. The roots, stems, and branches of evergreen shrub called *S. persica L.* have been used for oral hygiene for centuries. Extracts from *S. persica L.* roots and stems consists of powerful antimicrobial substances such as sulfur. Moreover, fluoride is also found in remarkable amounts, which is easily dissolved and released in water.

Large amounts of fluoride and antimicrobial agents in the plant lead to strong anticaries effects. The extract seems to have a slight bitter taste due to its volatile oils. The use of chewing gum leads to low and uniform contribution of fluoride in salvia. This is the most helpful way to prevent tooth decay [49]. In addition, there is no need to increase the drug release of fluoride salts because they are soluble in water.

Aslani et al. [50] formulated the medicinal gum by *Salvadora persica L.* in order to prevent tooth decay. Their results pointed out that 1 g of *S. persica L.* contained 0.04 μg of fluoride. The best sweetener and flavoring agent for persica gum were xylitol and peppermint, respectively. Also, the desired organoleptic characteristics were achieved by combination of xylitol and peppermint. The combination with the least elasticity and ductility increased the softness of chewing gum. It was concluded that the hard gum leads to lower drug release.

Nicotine is the basic active compound in cigarette that augments individual smoking behavior. Nicotine replacement therapy (NRT) can help smokers to quit smoking by replacing some of the nicotine attained from cigarettes [51]. One of the widely accessible NRT products is chewing gum. It can be used as smoking cessation aid and was confirmed by Food and Drug Administration (FDA) in 1984. Aslani and Rafiei [52] showed that the addition of 2 and 4 mg nicotine and aspartame as a sweetener and cherry and eucalyptus as flavoring agents had optimal chewing hardness and highest acceptability among smokers. The release of nicotine from 2 to 4 mg chewing gums at 45 min was reported 92% and 93%, respectively.

Aloe vera is a plant which has anti-inflammatory, antiseptic, anticancer, antiviral, antibacterial, antitumor, antioxidant, and antidiabetic effects. It contains several compounds such as vitamins, sugars, enzymes, minerals, lignin, saponins, salicylic acids, amino acids, and anthraquinone. One gram of *Aloe vera* powder contains 5.16 ± 0.25 mg/g of phenolic compounds and 104.63 ± 4.72 mg/g of carbohydrates [21]. Aslani et al. [21] designed the *Aloe vera* chewing gum. Results showed that the chewing gum containing maltitol, aspartame, and sugar sweeteners was selected as the optimal formulation according to its physicochemical and organoleptic properties. Also the peppermint flavor had the most acceptances between consumers.

3 Factors Affecting Release Rate and Amount

The composition, physicochemical properties of the chewing gum and active substances (solubility in saliva, crystallinity, polarity, and physical form like solid, liquid, emulsion, microcapsule), active ingredients amount, and duration of chewing

will determine the in vivo and in vitro release of active agents and flavors from a chewing gum matrix. The release and absorption of a drug through mucosa is dependent on several factors such as stability of gum base and its compounds to enzymes, molecular mass, and ionization. Lipid-soluble drugs are slowly released into oral cavity because they are bounded to lipophilic phase (gum base). Ingredients more soluble in water will be instantly released within few minutes of chewing; however, substances with hydrophobic characteristics are first released into gum base and then into salvia. Moreover, type and speed of chewing characteristics of different consumers will affect the release rate of active components. The difference mentioned all lead to variable results of drug release [44].

Although it was proved that chewing gum can be used as a delivery system for bioactive compounds, there are limited studies in the literature; therefore, aroma release and bioactive compounds release can be associated. Gum bases have tendency to absorb flavors with hydrophobic character, therefore complicates the flavor release from the chewing gum matrix [34]. The slow release of active ingredients can be achieved by coating them with appropriate materials (modifying their water solubility), or hydrophilic/hydrophobic balance of the chewing gum formulation could be manipulated through reducing the concentration of the gum base. Also, undesired interactions between different ingredients can be prevented by encapsulation of some ingredients by appropriate substances.

Encapsulation technique is used for coating food ingredients such as preservatives, oxidation-reduction agents, colors, sweeteners, enzymes, antioxidants, acids, buffers, flavors, nutrients, and cross-linking agents [53]. Microencapsulation can minimize the probability of interaction between ingredients in order to protect color, flavor, and texture of food [54]. It is evident that by inserting the microencapsulated bioactive compounds in chewing gum, the microcapsules contents will be released in a controlled/sustained manner by chewing (physical rubs) or mucus (chemical activity) [55].

Microencapsulation technique is carried out for acids or other components to produce long-lasting taste or to improve the releasing time of special tastes or flavors. Abbasi et al. [9] investigated the capability of microwave energy as a low-cost, rapid, and simple technique to microencapsulate citric acid powders coated using casein and inulin. Microcapsules were incorporated in chewing gum formulation. The results illustrated that citric acid powder without coating caused weakening and hollowing of the products texture. Probably citric acid can affect gum base negatively and therefore degenerates the gums structure and lattice. Chewing gum treated with inulin-coated citric acid gained the highest overall acceptance. Due to the prebiotic effect of inulin the findings are admirable. This functional nutritional and dietetic chewing gum contained 10%w/w of inulin.

Physical and chemical properties of a flavor ingredient determine their release from a food matrix. Two main factors including volatility and hydrophobicity of the flavor compounds affect their release in a chewing gum matrix [56].

The release profile of volatile compounds from chewing gum is determined by the hydrophilicity. Hydrophilic ingredients (mostly taste components) ($\log P < 1.8$) are released faster; however, compounds with $\log P > 1.8$ have an increasing release

rate over the whole chewing duration [57]. The release of flavors is a two-phase process; in the first 5 min of mastication period, the primary flavor release is dependent on the gum base to water partition coefficient of the compounds (water-soluble ingredients dissolve in the saliva). In this phase, combination of the water-soluble portion of the chewing gum compounds occurs and new surfaces are formed. During the second phase, the release of hydrophobic compounds and the extraction of non-polar ingredients occur [58].

The distribution of the flavor ingredients between the two phases is dependent on the compound affinity for each phase and mainly is related to the compound hydrophobicity. For example, components which are more hydrophobic would interact more with the gum base, therefore it results in a relatively low release rate during mastication. For various hydrophobic ingredients, a nonequilibrium partition model has been used for investigating flavor release mechanism [58]. According to this model, during the first 5 min of chewing, the flavor release is linearly dependent on gum base to water partition coefficient (Log cP). This phase is thermodynamic control. However, after the 5-min period, the air to water (saliva) partitioning coefficient controls the flavor release and is called diffusion control.

Harrison et al. [59] used the stagnant layer theory with the interfacial mass transfer from gum to saliva as a rate limiting step. Also the interaction of flavor compounds with the olfactory epithelium as a controlling factor was evaluated. Generally, the release rate was faster for flavor compounds with low chewing gum-to-saliva portioning coefficients, while flavor ingredients with a high chewing gum-to-salvia portioning coefficient were found to release slower. Both models indicate that the gum base acts as a determinative factor in release rate of diverse flavor ingredients.

Ferrazzano et al. [60] produced new chewing gums enriched with the polyphenol quercetin (Qt). The in vivo experiments were performed for Qt release in the saliva. The antibacterial effect of this bioactive compound against *S. mutans* was assessed after 14 days of consumption. The release analysis illustrated that due to the high solubility of Qt in the artificial saliva Qt released mainly in the first 10 min of chewing. Also adding Qt to the chewing gums did not change the saliva pH values, therefore the anticaries effect of Qt was not related to its buffering capacity. In addition, after 7 days of consumption, a significant reduction of the concentration of *S. mutans* strains in saliva was observed. The authors concluded that the biochemical mechanism of inhibition of quercetin against *S. mutans* growth is likely based on two different mechanisms of interaction with bacterial DNA or with the ATP binding site of bacterial gyrase.

Potineni and Peterson [12] reported that cinnamaldehyde release rate was correlated to the dissolution of the sugar alcohol. It did not follow the previous pattern predicted by the log *P* value. Because cinnamaldehyde forms transient hemiacetals within the sugar alcohol phase during chewing gum production. Upon mastication due to slight alkaline conditions in the mouth these hemiacetal products would degrade and again cinnamaldehyde and sugar alcohol forms. Also any alcoholic compound such as glycerine (4.0% of the chewing gum composition) can be involved in this proposed hemiacetal reaction mechanism. Glycerine release was

correlated to the polyol release phase. However, hemiacetals formed with polyols such as sorbitol seem to be more hydrophilic compared to hemiacetals formed with glycerin [15]. Over all cinnamaldehyde release in chewing gum is a two-phase process: (1) the release of hemiacetal bonded cinnamaldehyde compounds during the dissolution of sugar alcohol phase (dominate mechanism during the initial stage of mastication; 0–4 min) and (2) the release from the gum base as predicted by the log cP value (dominate after 6 min).

It is evident that the type of sweetener and its content will influence the release of flavor compounds from a chewing gum matrix; however, mechanism for this impact is still to some extent unclear. Hansson et al. [61] stated that mono- and di-saccharides affect the flavor release by salting out effect mechanism. Polysaccharides can interact with flavor in many ways due to their different structures. For examples starches and hydrocolloids such as carboxymethylcellulose and pectin may change viscosity of food systems by gel formation at a critical concentration, causing a reduced flavor perception [62]. Moreover, softer gels have been shown to release volatiles faster than harder gels, therefore resulting in increased intensity for flavor perception [63]. The formation of inclusion complexes, especially with starch and cyclodextrins, is the other way of interactions of carbohydrates with flavors. Hydrophobic interactions lead the flavor components to be trapped inside these complexes, and consequently retardation of flavor release or sometimes unavailable release occurs. Some authors have stated that lower affinity between the gum base and flavor agents leads to faster release or short lastingness during mastication and vice versa [20]. This theory is based on thermodynamic data. However, the release profile of flavor compounds from chewing gum during mastication would be correlated to the polyol release rate. These factors affecting flavor release can be taken into consideration to improve efficiency of the delivery of bioactive compounds with flavor of chewing gum.

4 The Effects of Chewing Gum Production Process and Mastication on Bioactive Compounds

Two different processes should be considered when targeting the quality characteristics and the use of the bioactive components in the chewing gum as a delivery system:

(a) Production process
(b) Mastication process

As in most food products, mechanical and thermal processes are applied in the production of chewing gum. Mechanical, chemical, and low-intensity heat treatment is also applied during different periods of consumption. Some consumers replace the chewing gum with new one after chewing for short times (<10 min) [64], although some continue to consume chewing gum for half a day or even longer [65]. The production process and consumption behavior of the chewing gum should be taken

into account as well as release characteristics mentioned in the previous part during use of the chewing gum as a delivery system.

The main characteristics to be considered when examining the stability of bioactive compounds present in the gum matrix can be listed as the following:

(a) Mechanical stability
(b) Thermal stability
(c) Oxidative stability
(d) Chemical stability
(e) Interactions with the other constituents

When considering the chewing gum system, there are two potential components for carrying bioactive agents. The first is the coating material used for the coating of chewing gum, while the other is the water-soluble base of chewing gum. However, considering the product range, it can be stated that the chewing gum is a more accurate carrier since coating part is more susceptible to environmental stresses such as light, oxygen, and temperature which can negatively influence the activity of the bioactive compounds. The presence of bioactive components in the gum base composition may be indicated as an up-and-coming approach with considerable strengths and disadvantages. In general, the gum base, main ingredient of chewing gum, is a raw material supplied directly by a limited number of producers/suppliers and its nonbiodegradable and artificial characteristics disturb the consumers. Furthermore, the processes applied in the production of chewing gum and the conditions of these processes may be mentioned as a disadvantage in terms of the stability of the bioactive components.

4.1 Production Process

The production processes of the chewing gum are presented in Fig. 2 [14]. The production of chewing gum can be carried out mainly by three different methods: (i) conventional/traditional method (fusion); (ii) freezing, grinding, and tabletting; and (iii) direct compression method [14]. However, the most common method is the conventional method named Fusion Method (FM). The method of freezing, grinding, and tabletting is stated to be disadvantageous in terms of oxidative stability, especially for bioactive compounds compared to other methods because of moisture content. The direct compression technique involves less severity heat treatment. Because, in conventional methods, the chewing gum is softened between 70–120 °C and mixed with the liquid plasticizer for 2–8 min with or without emulsifier(s). However, if the bioactive components are not involved in the process at this stage, the effects of the potential disadvantages of the FM on thermal stability will be reduced. In the FM, after gum base softening and mixing with the plasticizer, it is mixed for a further 1–4 min by adding 2/3 of the total sweetener and colorant. While the mass is slowly stirred, the remaining sweetener and flavor ingredients are added and then stirred for another 1–4 min. As a final step, fillers, moisturizers, and antioxidants are added and mixed for another 1–4 min [16].

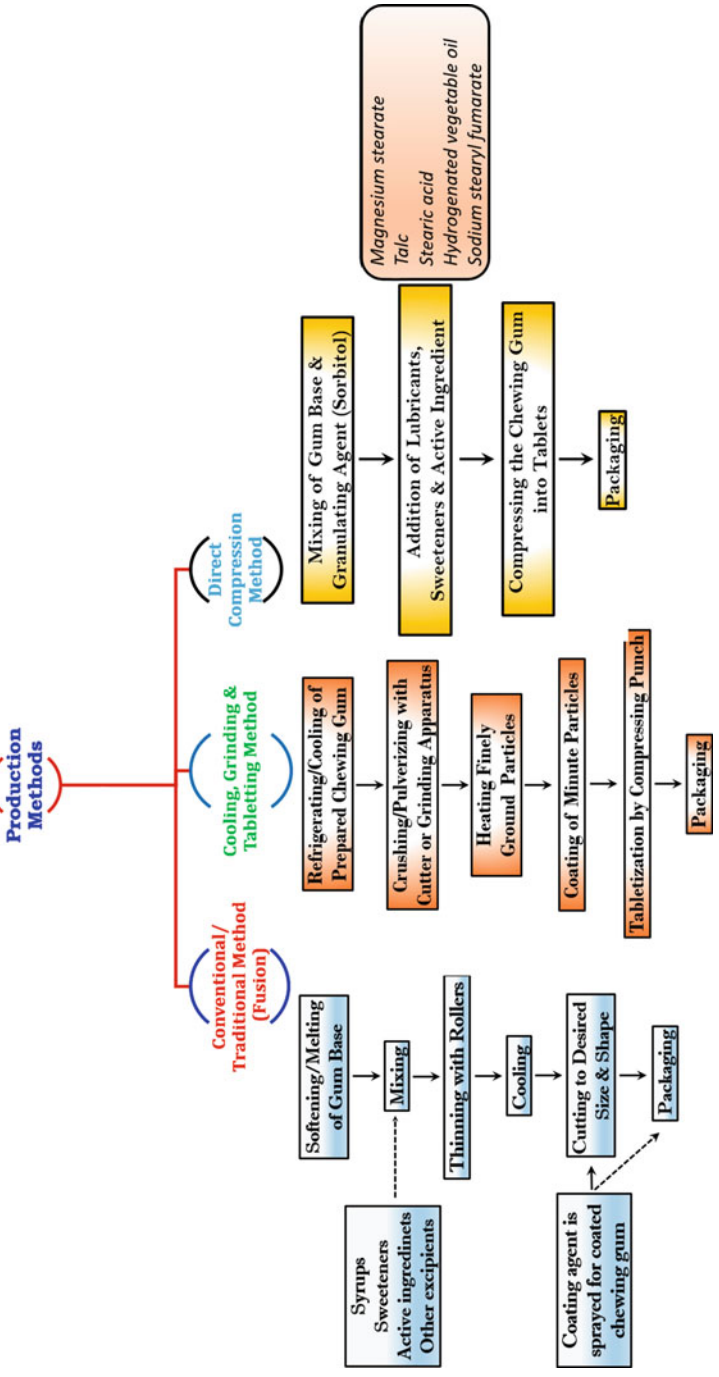


Fig. 2 Production processes of chewing gums

When considering the chewing gum process and the solubility and chemical properties of all the components, the water-soluble base part can be specified as a suitable carrier for bioactive components. Also considering the homogeneous distribution and dissolution properties of the bioactive components, they may also be advantageous, especially for thermal, oxidative, and mechanical process stability, to be incorporated at the same stage with the process colorants or aroma substances. The largest volumes of encapsulating matrices used in the industry are water-soluble and dissolve when in the presence of water [66]. The widespread use of encapsulation for the development of stability of bioactive components supports the fact that inclusion of the process as part of the water-soluble base in chewing gum is a more appropriate option.

The formed structure of the chewing gum presents good mechanical and thermal stability, and the encapsulation technologies used determine the extent of protection obtained and the stability of the functional compound during storage [67]. The number of studies for applying microencapsulated bioactive compounds in chewing gum matrixes is also very limited [8–10, 68]. However, flavor companies are nowadays interested in microscopically encapsulated aromas, which do not release directly but under precisely defined circumstances, for example, under mechanical stress such as chewing the chewing gum [69].

Among the existing stabilization methods, microencapsulation is a promising approach since it protects antioxidants from heat, light, and oxygen, allowing stability, bioavailability, flavor masking, and controlled release while maintaining their functional properties and increasing ease of handling [70]. Stability (process and storage), solubility characteristics, sensory and textural properties are the main factors that should be kept in mind in the encapsulation method. Also, the required features of the encapsulated products are dependent on the type of product. For example, sustained release of flavor compounds is desirable for chewing gum, while for powdered foods, flavors should be released by reconstitution (rehydration) [71]. Fast-release of the water-soluble bioactive compounds can also be arranged by encapsulation techniques/materials.

Oil-soluble bioactive components can also be present in the chewing gum as a water-soluble base component. For this purpose, for example, double or water-in-oil-water ($W_1/O/W_2$) emulsions can be used. However, the actual use of $W_1/O/W_2$ emulsions in food industry is still scarcely developed probably because they are highly susceptible to a variety of factors, generating destabilization mechanisms that are responsible for the system breakdown [72]. Also, during emulsification and encapsulation processes, solubility and interaction with saliva should be considered. The inconvenience of particle size after encapsulation may be caused by sandiness during consumption; therefore, it should be optimized during using chewing gum as a delivery vehicle.

One of the most characteristic features of the gum process is the nature and mechanism of the applied mixing/kneading process. Blade mixers are used with various deformable or plastic solids and high-consistency pastes to achieve a kneading and mixing action accompanied by heating or cooling. They are used for mixing the components of soft sugar confectioneries containing chewing gum [73].

The process involves compressing the fluid mass flat, folding it over on itself, and then compressing it again. The material is usually torn apart, and high shear is produced between the moving and stationary fluid elements. The mixing is usually performed by two Z-shaped heavy blades rotating in opposite directions at different speeds on parallel horizontal shafts [73]. When considering the fact that mechanical forces can damage the activity of bioactive compounds, the forces generated during mixing/kneading process should be considered during enrichment of chewing gums with bioactive compounds.

In manufacturing process, after kneading, extrusion process is applied; for this aim Z-blade type equipment are widely used. Extrusion cooking is a process of forcing a material to flow under a variety of conditions through a shaped hole (die) at a predetermined rate to achieve various products [73]. The principle of extrusion, developed for many nonfood products, has been applied successfully in the confectionery industry [74]. Three major types of extruders are used in the food industry: piston, roller-type, and screw extruders [73]. For some products, such as chewable candies and chewing gum, the roller extruder has modified to develop the multiple screw extruder [74]. Screw extruders utilize single, twin, or multiple screws rotating within a metal cabinet called the barrel. The screws convey the material forward and through a small orifice called a die, which can take many shapes and sizes. Several external parameters such as screw speed and configuration, the temperature of the barrel, the size and shape of the die, and the length of the barrel affect the properties of the final product [73]. The parameters of extrusion processes, temperature and shear stresses, can also affect the bioactive compound activity; therefore, the amount or form of bioactive compounds (encapsulated or not, heat stable or not) used in the chewing gum formulation should be selected attentively.

Shaping is carried out in the final stage of the chewing gum production. Products in the form of transaction, strips, pellets, or balls are gained. The most common is the strip form with different thicknesses and sizes. For this purpose, rolling and cutting operations are carried out after the extruder. In order to obtain chewing gum in the form of a ball, shaping is carried out after the extruder. Although strong mixing has disadvantagous for stability of bioactive compounds during chewing gum production, level and duration of thermal treatment in production cause advantages in terms of heat and oxidative stability of them. In functional product development studies, the selection of chewing gum for the delivery of a large number of active ingredients [65] and the positive results are indicative of the potential use of chewing gum as a bioactive component carrier and functional food [14].

During production of chewing gum or consumption, the potential influence of bioactive components on gum base should be considered and suitable gum base must be selected, accordingly. It is possible to obtain functional chewing gum with better bioactive component stability and bioavailability by studies carried out using different gum bases.

As can be seen, there are different parts of chewing gum process which include thermal and mechanical processes at different levels. Regarding high sensitivity of the most bioactive compounds to environmental and process conditions, there are

two main possible actions that can be done to achieve production of the functional product with desired bioactive compound content:

1. Optimizing the process conditions depending on the bioactive compounds used in the chewing gum.
2. Designing/selecting the suitable form/type of bioactive compounds.

In addition, in all circumstances, as optimization of processes or selecting the most bioactive compound type can be performed, an amount of bioactive compound is lost during production process; therefore, this deprivation should be tolerated by adding higher amount than targeted value.

4.2 Mastication Process

The mastication process is the first transformation to which food is subjected during eating/chewing [75]. Chewing gum can be defined as the product which remains in the mouth for a longer time and undergoes various speed, trajectory, and severe mastication process during this time, as mentioned above. During the mastication process, the substances contained in the composition are subjected to the mechanical effects of chewing. In addition, the temperature of the oral cavity ($<30°C$) and the presence and flow of saliva cause chemical and physical effects. These interactions may affect the bioavailability and stability of bioactive components in functional chewing gums, as well as on the product quality. Saliva plays an important early role in digestion where with the initial chewing of a portion of food, it contributes to the formation of a cohesive food bolus, covered by a mucin film, which facilitates the swallowing process [76, 77]. Mixing of saliva with food can have a diluting effect and play a role by initial breakdown of food, by affecting flavor release, transport of taste compounds to the taste buds, precipitation of proteins by tannins, e.g., resulting in a sensation of astringency, and acting as a buffering system, affecting the degree to which we perceive sourness [78]. Emulsifying agents such as lecithin or glyceryl monostearate, which are present in the chewing gum composition, play an important role in moisturizing the chewing gum with saliva.

Also, one factor that should be kept in mind is the need for saliva transfer for the transportation of bioactive compounds from gum matrix to the mouth. Otherwise, the active material will remain in the gum bolus and will be discarded at the end of consumption without any effect or bioavailability. This is not only in terms of bioavailability perspective, but also in terms of production costs. Minimizing the level of bioactive compounds to be thrown by the bolus will lead to optimum utilization in production. This will also optimize product costs. The bioavailability is a critical factor to determine the efficiency of bioactive compounds orally ingested, therefore it is necessary to develop different delivery systems to enhance the in vivo bioavailability of nutraceuticals orally administered [79].

Food characteristics are changing while the food is masticated, and they consequently influence the oral processes. It is therefore a dynamic process, where food

structure and perception change over time [75]. Chewing gum is not swallowed, it is consumed by chewing in the mouth [80], and various components are released during chewing. During chewing, the bioactive compound present in the chewing gum product is released from the matrix into the saliva and can be absorbed from the oral mucosa or metabolized by reaching the mast for gastrointestinal absorption [65]. For instance, parts of the encapsulated ingredients are released from the food structure due to the deformation observed in the product during chewing and digestion [71]. In vitro and in vivo studies need to be conducted in the field of functional gum development that determine the amount of bioactive ingredient passing to saliva. Factors that influence the amount and the stability of the bioactive components along the masticatory process are summarized as the following:

(a) Saliva temperature, amount, and flow rate
(b) Saliva composition, therefore, saliva viscosity
(c) Mastication time
(d) Mastication mechanism

In recent years, researchers reported that it is possible to improve the level of release of bioactive components in pretreatment and mastication processes. For example, oral disintegrating film (ODF) is a dosage form produced with water-soluble polymers and when placed in oral cavity, it is quickly hydrated by saliva, adhered to mucosa, and disintegrated in seconds releasing the active ingredient for mucosal absorption, which results in a rapid absorption and instant bioavailability due to high blood flow regions such as the sublingual [81]. Rapid and effective release of bioactive components can also be considered independent of the optimum size of chewing gum consumption. Taking bioactive compound from chewing gum matrix to mouth is related with the diffusibility of the saliva into chewing gum. Diffusibility of the saliva in the chewing gum affects amount and release rate of the bioactive compound; therefore, size of the product should be optimized. The important level of bioavailability is the release of the targeted level before the mastication process is complete. Since the duration of this process varies widely according to the consumption habits and product qualities, it is more advantageous to realize the release as soon as possible depending on the aim of the usage and behavior of active substance.

The total volume of saliva secreted per day has been estimated to be about 0.6 L [82]. Several studies have reported that the mean flow rate of unstimulated/resting whole saliva in healthy persons during the day is in the range of 0.3–0.4 mL/min but with a large standard deviation [73, 83]. The saliva flow rate in response to chewing gum consumption was 3.4 mL/min [84], and this ratio was used to stimulate the saliva flow [85]. The longer chewing gum period of each food bolus may result with the longer time for odor release and the greater incorporation of saliva, since the chewing gum process and gustatory stimulation both increase salivary flow rate while chewing flavored gum, as opposed to chewing gum base [76, 86]. Therefore, the fact that the use of bioactive components is not in the negative direction of the effect on flavor release in chewing gum may be asserted as a factor that can improve bioavailability.

The highest constituent in the salivary composition is water with 99% [76]. It is of great importance that the water-soluble bioactive component is preferred in the chewing gum production. Also, salivary fluid is composed of a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins, represented by enzymes, immunoglobulins and other antimicrobial factors, mucosal glycoproteins, traces of albumin, and some polypeptides and oligopeptides of importance to oral health. There are also glucose and nitrogenous products, such as urea and ammonia [87]. The main protein and also digestive enzyme in saliva is alfa-amylase (1,4-glucan 4-glucanohydrolase) [88]. The presence and level of this enzyme should also be taken into account for the stability and bioavailability of the bioactive components. Saliva has a strong potential to interact with all food macronutrients because of the diversity of its enzymatic composition. Its amylolytic activity is the most well-known, but proteolytic and lipolytic activities have also been reported in human saliva. The active role of these enzymatic activities at the early stages of the digestion may be limited in terms of food degradation because of the short duration of contact with food in the mouth [89].

In the process of mastication, the mechanism of the physical effects of chewing gum should be understood and taken into account in product development studies. The human chewing system is a complex system of upper and lower jaws with teeth on it [90]. It also includes the movement of tongue and cheek as well as saliva production. While chewing, the lower jaw (mandible) is moved with the muscles attached between the upper jaws [91]. The chewing movement starts with the opening of the mandible, so there is a gap between the teeth in the skull and the mandible. The tongue places the food particles that need chewing into the gaps in one side of the mouth. The mandibular then closes these food particles and separates the pieces and then falls into the tongue to reposite the particles during the next cycle. The opening of the mouth within chewing cycle is approximately vertical [92]. The opening phase of the mandible slows at the beginning and increases when the mouth is opened [93]. When the mouth begins to close, the mandibula moves laterally outward, and initially returns to the teeth, rapidly closing and slowing down for occlusion [90].

Texture is perhaps the most important sensory attribute linked to food structure [75]. The consistency of oral processing (chewing, swallowing) may be affected by the texture of the chewing gum thereby affecting the profile of flavor delivery in both the temporal and intensity dimensions [94]. The texture of the chewing gum affects the product quality and the mechanical effects that will be experienced during the mastication. Different food particles depending on their shape and texture are used to chew up. These differences create a different chewing mechanism for different food products. If a vertical chewing gesture is used, the teeth use their heads to break food particles [90]. If more lateral chewing is used, the teeth use sharp edges to function like blades and cut food particles [95]. Another important factor is the size of the bolus. According to the composition of chewing gum, the size of the bolus may vary during the mastication process. In this case, it is also possible that there may be differences in mechanical effects during mastication.

Simulations of chewing gum's mastication can be performed using different devices by in vitro studies [96]. One factor to be considered while designing the devices is the quality of the human mastication system, a perfect endowed art that cannot be completely imitated. Several studies have been conducted for this purpose [97–99]. It will also be useful to consider that panelists do not have the same severe mechanical effects during mastication and consumers perform slow and fast mastication over time [12]. For this purpose, the principles of sensory analysis studies carried out with the Time-Intensity technique can be taken into account. Also, chewing gum is an example of a product where the perceived intensity of flavor characteristics changes over time. Many sensory properties of the chewing gum can change, such as the flavor and texture. Flavor compounds differ in their rate of release as gum is chewed [26].

The material to be used as a natural gum base elastomer in functional and/or conventional chewing gums should be able to demonstrate the functions imparted to the product by synthetic elastomers. The main functions of these ingredients are elasticity, pliability, and stickiness. For example, if the chewing gum exhibits a high degree of adhesion to the teeth and/or palate due to the gum base to be obtained by the use of an alternative elastomer, the functional properties of this product will be acceptable to the consumer even if the functional properties are improved. It is also possible that there is a similar property, at the same time, that there are also adhesion problems in the packaging material. It is also important that, during consumption, the chewing gum cannot be cured, bent, and bended by elasticity and exhibit elastic behavior. Otherwise consumption will be difficult and consumption time will be shortened. Shortening of the consumption period will bring disadvantages in terms of completing the release of the bioactive components in the structure.

5 Buccal Delivery of Bioactive Compounds by Chewing Gum

Consumers are increasingly looking to nonnutrient therapeutic compounds for added benefits from their food which may pay the way for health promotion, disease prevention, and performance improvement. However, one barrier to obtaining high systemic levels of a bioactive in a delivery agent is that the body especially the digestive system or gut has a number of enzymes which rapidly changes molecules prior to their entry into the circulatory system. This presystemic metabolism is also known as the "first-pass" effect. Glucuronidation process occurred in the first-pass in the gut or liver and catalyzed by UDP-glucuronosyltransferases enzymes (UGTs) causes poor bioavailability of orally administered bioactive compounds and decreases their therapeutic effects [100]. Efficacy of bioactive compounds depends on the bioavailability; therefore, novel delivery systems should be developed to improve in vivo bioavailability of beneficial compounds orally administered [79]. Due to ease of usage and special physiological properties, buccal delivery of drugs has attracted particular attention. Moreover, it has been discovered that bioavailability of anthocyanins is enhanced in mouth by human saliva and oral microflora. It was found that deglycosylation of the parent anthocyanin to its respective aglycone

increases chemopreventive impact by removing the sugar groups which could cause steric hindrance and salivary β-glucosidase mostly produced by oral microflora hydrolyzed black raspberry anthocyanins which increased its chemopreventive effect against oral cancer [101].

Delivery of bioactive compounds can be conducted in two ways within the oral mucosal cavity: (i) topical delivery, which is delivery into local parts of oral cavity and (ii) intravenous delivery either via the buccal mucosa or sublingual mucosa. Since sublingual mucosa is thinner than the buccal mucosa and has a rich blood supply and surface area, it is more permeable. Although washing effect of saliva and tongue movement can decrease the durability of the bioactive compound, this route is preferred when a rapid onset is desired; in particular, for the treatment of acute disorders. On the other hand, buccal mucosa has an even surface therefore it is easier to place and remove controlled-release systems.

Buccal delivery of bioactive compounds is very advantageous for bioavailability due to avoidance of first pass effect. However, buccal delivery of bioactives exhibits the following disadvantages:

(a) Enzymes in saliva can modify chemical structure.
(b) Salivary secretion and swallowing can dilute and remove bioactive compounds from the absorption side.
(c) Limited surface area and the barrier feature of the buccal mucosa may cause ineffectiveness of this delivery route.

Total effective area for bioactive compound delivery on oral membrane is 31.5 cm^2 and thickness of oral mucosa varies between 100 and 800 μm. The amount of saliva in mouth is approximately 1.1 ml, 1% of which is organic and inorganic materials and depending on the secretion rate, salivary pH changes between 5.5 and 7.0. However, salivary secretion rate is between 0.5 and 2.0 L/day. Saliva can help transportation of bioactive compounds from bulk to absorption sites. However, dilution also occurs due to the presence of saliva which may reduce the amount of absorbed material.

5.1 Absorption Mechanism of Bioactive Compounds

Two different routes exist for the passage of bioactive compounds namely extracellular pathway through the intercellular space and intracellular pathway through passage into and across the cell. The former favors the absorption of hydrophilic substances due to its hydrophilic nature. However, the latter route includes the lipophilic cell membrane which can be favorable for the absorption of lipophilic compounds.

5.2 Obstacles for Oral Delivery

Oral route is convenient and maybe the most preferred to both clinicians and patient among wide range of routes for bioactive compounds delivery. Absorption of

bioactive compound generally depends on thickness and keratinization of oral mucosa and absorption increases by the decrease of these parameters. Therefore, it was found that permeability is the highest in sublingual mucosa and the lowest in the palatal mucosa [102]. However, membrane coating granules (MCGs) which are intercellular lipid substances cause the most important obstacle for bioactive compound permeability. By covering the apical surface of the cell, MCGs merge with the cell membrane and form stable barrier for absorption. Moreover, bioactive compound should be stable in first pass metabolism or encapsulated before incorporation into the delivery system to withstand enzymes included in saliva such as esterases, carbohydrases, and phosphatases.

5.3 Important Factors for Buccal Delivery

The crucial parameters for buccal delivery are sensorial and physicochemical properties, physiological conditions, and ingredients of dosage form. As diffusivity depends on molecular size and weight, small molecules lower than 75 Da is generally absorbed rapidly by oral mucosa. Hydrophilic materials also have better absorption characteristics. Another important factor is the dissociation constant (Ka or pKa) of the bioactive molecules that is dissociation of the molecules at the oral cavity pH which is around 6.8 and causes obstacle for the passage of the bioactive molecules.

The sensory properties of delivery agent can affect the consumer compliance or the acceptance of the product. Also it should be considered in the design of delivery agent that effective surface area of absorption site is about 2 cm^2; therefore, deliverable amount of bioactive compound in the delivery agent is limited. For this reason, buccal delivery route can be suitable for bioactive compounds, effective dose of which should be in the order of a few milligrams. Ingredients of delivery agent should not be irritant and toxic.

Concerning the physiological conditions, mucoadhesive polymers and absorption enhancers may be needed to improve bioactive compounds absorption by increasing residence time and absorption of bioactive compounds when dilution with saliva, swallowing, and the presence of MCGs severely limit the absorption of the compounds [103]. The important point for the mucoadhesive performance is the pH stability in the oral cavity. Resveratrol-loaded mucoadhesive tablets were found to prevent and/or cure inflammatory lesions of the oral cavity [104]. Another approach to increase the solubility and thus bioavailability of phenolics is developing a food-grade self-nanoemulsifying system. Wang et al. [105] achieved to improve the dissolution and absorption of ellagic acid by using self-nanoemulsifying delivery technique. This can be used in chewing gums to increase absorption of insoluble bioactive compounds.

5.4 Chewing Gum as a Modern Carrier

General factors affecting carrier capability of chewing gum is summarized in Fig. 3. Chewing gum is a novel and convenient carrier to deliver bioactive compounds via

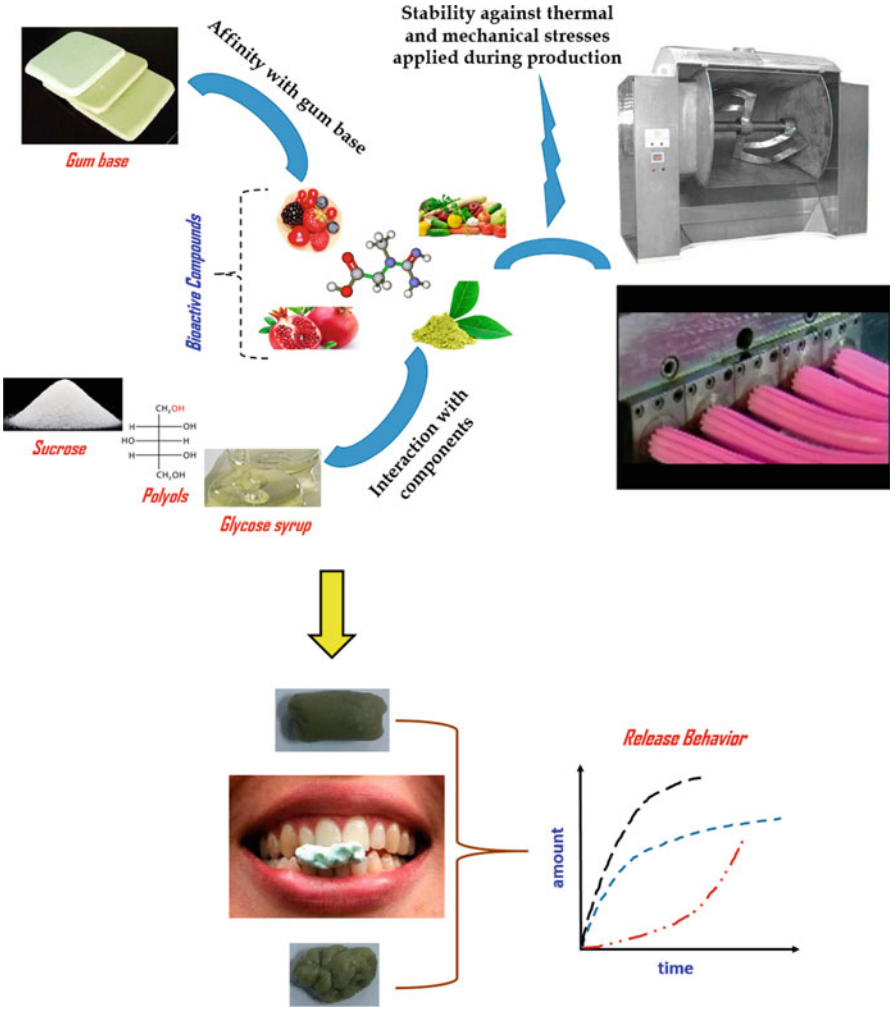


Fig. 3 General scheme for factors considered during functional chewing gum development

oral transmucosal route. Because chewing gum is generally kept in mouth much longer than capsules, rinses, and gels, this dosage form can increase bioavailability by delivering therapeutic agents for an extended period of time. Moreover, bioactive compounds can be released slowly and continuously depending on chewing gum formulation and production process. For this reason, some pharmaceutical companies invented special chewing gums to orally deliver drugs, in addition to the most common tablets and capsules [106]. It is also discovered that saliva increased the solubilization of lipophilic polyphenols present in fruits and plant beverages boosting their potent antioxidant effects and increased the stickiness of polyphenols to oral surfaces contributing to the enhancement of the redox status of the oral cavity

[107]. Therefore, chewing gum containing lipophilic polyphenol would have advantages by prolonged contact with saliva.

As mentioned before, the same factors can affect the absorption of bioactive compounds incorporated in chewing gum, namely, pKa of the compound, oral pH, saliva secretion rate and composition, enzyme content, permeability and thickness of the mucosa, keratinization and composition, cell renewal rate. Other factors related with chewing gum are bioactive compound solubility and interaction with other chewing gum ingredients, release rate of gum base, chewing rate, frequency, intensity, and duration.

5.5 Functional Chewing Gum Developing Studies

High amount of chewing gum products has been formulated and commercialized as vehicles for delivering health active agents. Progression in microencapsulation has allowed the design of carriers and systems which can exactly target a desired release site at a predesignate rate. Active substances ingested via the oral route can be schemed to be delivered using two paths: (i) local and (ii) systemic. Dental caries prevention, halitosis, angina relief, and xerostomia symptoms are examples of local effects delivery. Smoke cessation and pain relief are examples where confectionary products have demonstrated their effectiveness as systemic delivery carriers [34].

For the treatment of gingivitis and plaque build-up, Trivedi et al. [108] found that addition of rosemary extract to chewing gum prevented this kind of oral diseases by supplying antibacterial, antioxidant, and/or anti-inflammatory effect in the oral cavity. Rosemary extract includes ursolic acid and carnosic acid. Most of the cases, the extract is isolated from leaves of *Rosmarinus officinalis*. Rosemary oil, which is another product from *Rosmarinus officinalis*, is derived from steam extraction of flowering parts of the plant as well as the leaves. Although rosemary oil has been suggested for use in dentifrices along with other oils such as eucalyptol and menthol, it is found that the chemical composition of rosemary oil differs a great deal from rosemary extract and rosemary extract gave better results. Enhanced effects can also be possible by including triclosan or other phenolic antibacterial agents.

Quercetin (Qt)-containing chewing gum has been developed to inhibit the growth of oral *Streptococcus mutans* strains which are responsible for tooth decay [60]. In vivo release kinetics and antibacterial effects of Qt were studied. The release analysis in saliva of young volunteers demonstrated that the most of Qt release took place in the first minutes of chewing without changing saliva pH values. Moreover, in vivo antibacterial analysis showed after 14 days daily consumption that concentration of *S. mutans* strains in saliva decreased after 7 days of consumption. Therefore, Qt-included chewing gums can provide an effective anticaries concentration in saliva, without changing salivary pH values.

Bioactive compounds having low water solubility and high lipophilicity are released extremely slowly in chewing gums due to lipophilic nature of chewing gum base. Therefore, cyclodextrin can be used to enhance release rate of this kind of substances. Jacobsen et al. [109] studied chewing gums containing hydroxypropyl-

β-cyclodextrin inclusion complexes of imidazole antimycotics (miconazole, econazole, and clotrimazole) for local treatment of fungal infections in the oral cavity. In conclusion, antimycotic release from the chewing gum was increased by using both the econazole β-cyclodextrin inclusion complex and the miconazole hydroxypropyl-β-cyclodextrin kneaded product in the formulation of chewing gum.

Another approach which can be used in chewing gums to enable systematic release of therapeutic agents and increase their bioavailability is the utilization of oral disintegrating films. It is a dosage form produced with water-soluble polymers and when placed in oral cavity, it is quickly hydrated by saliva, adhered to mucosa, and disintegrated in seconds releasing the active ingredient for mucosal absorption, which results in a rapid absorption and instant bioavailability due to high blood flow regions. Tedesco et al. [81] investigated the release of peanut skin phenolic compounds from disintegrating films including gelatin and hydroxypropyl methylcellulose (HPMC). Higher gelatin containing films caused lower release due to cross-linking between gelatin and polyphenols. However, HPMC films showed better maintaining and release behavior. In vitro analysis demonstrated that 80% of phenolics were released in 5 min and 60% of phenolics remained stable according to accelerated stability tests.

Anthocyanins are suitable candidates to be used in chewing gum as a vehicle because they show chemoprotective activities in the oral cavity. Bioavailability of red grape and chokeberry juice anthocyanins were investigated in vivo [110]. Twelve volunteers kept red grape or chokeberry juice in the mouth for 5 min and anthocyanin stability, mucus binding, and uptake into epithelial cells were measured. Among the other anthocyanin-glucosides in red grape juice, major loss was found in the amount of delphinidin-3-glucoside in red grape juice exceeded that of other, and lesser mucus binding observed for delphinidin- and petunidin-glucosides, suggesting the degradation of this anthocyanin. Whereas in chokeberry juice, the most reduction occurred in the amount of cyanidin-3-xyloside and it was found that cyanidin-3-glucoside preferentially accumulated in epithelium cells. These results implied that chemical structure of anthocyanin influenced stability and buccal cell uptake and therefore the bioavailability of anthocyanin-rich products for the promotion of oral health.

For the treatment of supragingival plaque, chlorhexidine is usually used; however, it causes tooth staining. Smith et al. [111] investigated the effect of chewing gum incorporated with chlorhexidine on the plaque, gingivitis, and stain formation. Bleeding scores and plaque were less for volunteers who used chlorhexidine gum for 4 and 8 weeks. Moreover, stain intensity was significantly lower for the chlorhexidine gum than rinse at week 8. This result showed that controlled treatment of supragingival plaque can be done without causing tooth staining by using chewing gum formulation.

Apart from bioavailability of bioactive compounds, the typical human diet is no longer rich in sources of phytochemicals. Moreover, normal dietary sources of phytochemicals are likely not ideally suited for delivery of these substances to a subject in order to obtain maximum bioavailability. Moreover, normal dietary sources of phytochemicals do not contain ideal combinations of phytochemicals to

produce the maximum therapeutic or nutritional benefit. Therefore, chewing gum formulation with desirable combinations can be developed to deliver phytochemicals in a controlled manner for the treatment or prevention of diseases. For instance, a functional chewing gum was patented containing green tea polyphenol, black tea polyphenol, white tea polyphenols consisting of epigallocatechin-3-gallate, gallocatechin gallate, gallocatechin, catechin, catechin gallate, epicatechin, epicatechin gallate and epigallocatechin, ginger phenolics, chlorogenic acid, retinoids, carotenoids, narcotics, theaflavins, and garlic extract [112].

Tea polyphenols (TP) have been extensively used as an antioxidant in foods and cosmetics. EC (epicatechin), EGC (epigallocatechin), EGCG (epigallocatechin gallate), and ECG (epicatechin gallate) are the basic active components in TP. According to recent progress in clinical study, TP could be used as an oral drug for prevention and treatment of caries, plaque, and laryngopharyngitis and prevent the development of cancer [113]. Catechins are water-soluble active agents. It had been stated that water-soluble substances usually release easily and rapidly from chewing gum. Polyvinyl acetate (PVAc), an elastic component, could be used as a retarding release agent for active ingredients such as catechins [114]. Phenolic isomersmagnolol and honokiol from Magnolia bark extract was shown to permeate the oral mucosal barriers and reduce salivary bacterial count, biofilm growth, salivary mutans streptococci, and plaque [115]. Chewing gum prepared with tea extract, *Camellia sinesis* (rich in epigallocatechin gallate), was able to reduce gingivitis and oral microbial growth [116].

Lee et al. [117] explored the beneficial effects of green tea catechins. It was shown that chewing or holding tea leaves in the oral cavity is an effective method in distributing tea polyphenols to the oral cavity. Long chewing time and direct contact with buccal mucosa yielded great salivary levels of polyphenols. Therefore, chewing gum can be used as a delivery vehicle for bioactive compounds. Moreover, catechins found in green tea were aimed to the beneficial effects of reducing the risks of oral cancer, cardiovascular disease, and tooth decay.

Yang et al. [10] produced chewing gums containing catechins that were prepared by applying a novel solid dispersion and hot-melt fluid bed coating method. The active material was granulated with PVAc and the pellets were coated with acrylic insoluble polymer. Researchers observed that the releases of catechins from the product were well extended with the growing coating level of Eudragit to the granules. The results illustrated that the release rate of catchins could be controlled by altering the coating thickness of Eudragit. The kinetic parameters indicated that the release rate of the formulation tended to change inversely with the level of Eudragit coating. The mechanism of controlled release was found to be close to the Higuchi model. The chewing gum prepared with Eudragit and by particular coating methods retained the effective therapeutic concentrations of catechins over a possible chewing period (approximately 30–40 min) in vivo.

Starch inclusion complexes could be applied for controlled release of bioactive compounds, due to enhancing the stability, dispersibility, and quite possibly bioavailability of bioactive compounds encapsulated within a starch helix [118]. Blair [118] used the chewing gum as a delivery vehicle for bioactive polyphenols found in

green tea. They evaluated the release of the polyphenols from the chewing gum matrix to the saliva in free form and as a starch inclusion complex. The overall stability of catechins found in green tea can be increased when bounded to the complex because it is susceptible to oxidative reactions.

The consumption of plant foods like ginger, a well-known used plant, decreases the risk of obesity, diabetes, heart disease, and overall mortality. It consists of active phenolics agents such as gingerols and shogaols that improve gastrointestinal problems and motion sickness. Ginger has antioxidant and cancer-protective characteristics due to its free radical scavenging activity. Flavonoids and phenolic components of ginger contain gallic acid, tannic acid, catechin, epicatechin, rutin. Some other volatile oils constituents exist in ginger, e.g., cineole, linalool, citral, phellandrene [119]. It has phenolic content of 61.50 ± 5.27 mg/g and 76.75 ± 5.45 mg/g of concentrated extract as gallic acid and tannic acid equivalents, respectively. Chewing gum can be used as a pleasant food to deliver beneficial ginger active agents to the body due to the lack of hepatic first pass metabolism. Aslani et al. [119] found that the chewing gum formulations consisting ginger released almost 100% of their active agents after 60 min.

Chios Mastic Gum (CMG) is the dried exudate of the shrub-like tree *Pistacia lentiscus* L. var. *chia.* CMG contains triterpenes/triterpenoids, essential oil, phenolic compounds such as tyrosol and p-hydroxy-benzoic, p-hydroxy-phenylacetic, vanillic, gallic, and trans-cinnamic acids. The unique physicochemical properties of CMG and its composition combined with its health-promoting potential have encouraged the eastern Mediterranean and the Middle East residents to exploit the CMG for preparation of several traditional foods and beverages. The major ratio of the CMG could be used as a natural gum base in the manufacture of Chios chewing gum. Kehayoglou et al. [120] stated that the Mastiha particles act as a plasticizing agent when applied in chewing gum. The environment conditions, method of collection, and also the duration of product storage are effective factors on the polymeric fractions. For example, a steam process is applied for the recovery of essential oils. This procedure results in an increase in hardness due to elimination of the oil constituents because this polymeric fraction has plasticizing effect. However, incorporation of food additives such as wax and lecithin in mastic gum would reduce final hardness of the product.

There are some people who are keen to chew the natural Mastiha, due to its full advantage of flavor and health-promoting characteristics. But the natural Mastiha has poor textural characteristics and during chewing it sticks to the teeth. A wide variety of food additives such as sweeteners, stabilizers, gums, and plasticizers can be used for improving flavor and textural properties of the natural Mastiha [121].

One of the most important features of bioactive compounds delivery vehicles is organoleptic properties or consumer compliance. For instance, for the treatment of diabetes, prediabetes, polycystic ovary disease, and obesity, metformin and its pharmaceutically acceptable salts are utilized to reduce plasma glucose levels (particularly, postprandial glucose levels), hepatic glucose production, lipid levels, and intestinal absorption. Moreover, metformin influences without resulting in hypoglycemia. However, oral formulations of metformin may lead to a bitter

aftertaste, causing loss of appetite. Moreover, many people do not prefer it because of its side effects, including gastrointestinal disturbance and large pill size. The reason for the side effects such as vomiting, diarrhea, abdomen discomfort is due to abrupt release of active drug in gastrointestinal tract. These often give rise to the failure of consumers to comply with taking the therapeutic agents, i.e., "compliance issues." Compliance issues are prevalent in individuals of all ages, especially children, who typically do not want to take medicines that taste bad. Well-formulated chewing gum can mask bad tastes and prevent gastrointestinal disturbance. Therefore, metformin hydrochloride including chewing gum was developed as a drug carrier to increase organoleptic properties and solve compliance problem successfully [122]. Also, Mostafavi et al. [123] formulated metformin chewing gum having dosage content of 86.2%. After 5 min of chewing 70% of the metformin was released. Freeze-dried acesulfame-isomalt sweeteners were used to mask the bitter taste of drug. Therefore, metformin chewing gum prevented bitter taste with a suitable release rate.

Another medicinal material with having bitter taste is chokeberry. It includes large quantities of antioxidants, and shows a prophylactic effect against cancer, heart diseases, and Alzheimer's disease. However, chokeberry could not be used in food products and medicinal products for oral administration due to its sensorial properties. Therefore, chewing gum formulation can be developed to deliver chokeberry's therapeutic compounds with good acceptable taste. Porsgaard [124] developed a chewing gum containing chokeberry which masked its inherent bitterness. It is claimed that this effect was resulted from the slow release rate of chokeberry from gum base. Moreover it was believed that developed chewing gum can be used for removal of plaque and/or biofilm from the teeth by preventing colonization of streptococci in the initial phase of dental plaque formation.

Proanthocyanidins are natural antioxidants, free radical scavenging ability of which is 50 times of vitamin E and 20 times of vitamin C. By reaching maximum plasma concentration in 20 min, proanthocyanidins have a rapid oral absorption and half-life of up to 7 h. Moreover, proanthocyanidins have several positive health effects including skin protection, decreasing degenerative eye spots and the incidence of cataract, preventing cancer, and decreasing risk of diabetes. Grape seeds contain large amounts of oligomeric proanthocyanidins and seed extract is rich in anthocyanins. Therefore, chewing gum including grape seed extract was formulated [125].

Tree of heaven, *Ailanthus altissima* Mill. Swingle (Simaroubaceae), is rich in therapeutic compounds and used for the treatment of colds and gastric diseases in the Chinese traditional medicine as a bitter aromatic drug. Oil of its ripened form is rich in vitamin C, vitamin E, and other vitamins and minerals, sterols, saponins, seven kinds of essential amino acids, and unsaturated fatty acids including oleic acid, linoleic acid, alpha-linolenic acid. It helps to regulate blood pressure and gastrointestinal function, relieve diabetic complications, and sleep well. Samara oil including chewing gum was formulated to provide slow absorption through oral mucosa for maximum therapeutic effects [126].

6 Conclusions

Chewing gum, unlike other foods, is a product which is chewed without swallowing. Chewing gum is composed of water-soluble (flavor, polyols, sugar, glucose syrup, glycerin, sweeteners) and water-insoluble phases (gum base). There are different types of chewing gums: (i) sugar gum, (ii) sugar-free gum, and (iii) medicated chewing gum. Regarding medicated chewing gums, they can be used as a carrier of caffeine, nicotine, sodium fluoride, dimenhydrinate, chlorhexidine, acetyl salicylic acid, and vitamin C. Medicated chewing gums have been drawing attention in a pharmaceutical industry in terms of preventing and curing dental caries, pain, smoking cessation, obesity, motion sickness, acidity, and diabetes. Rosemary extracts, quercetin, anthocyanins, proanthocyanidins, tea polyphenols, cathechins, and ginger were added to chewing gum formulations for enrichment aim and the produced functional products have substantial health beneficial effects. All of the studies showed that production process of the chewing gum, consumption behavior of the chewing gum (chewed without swallowing in the mouth for a long time), and formulation of the product make it an important tool for delivering bioactive substances. However, organoleptic properties of the chewing gum should be considered during improving functionality since they directly specify the consumer acceptability of the products.

After production of the chewing gum with functional characteristics, it is important to observe release manner in the mouth. It is desired from the functional product that required amount of bioactive compounds release from the matrix and reach the targeted site of the body. During chewing, mass transfer occurs between chewing gum and saliva in the mouth. Regarding saliva composition, great part, higher than 99%, is made up of water; therefore, depending on the structure/strength of the matrix of the chewing gum, water-soluble bioactive compounds can be easily solved by saliva and transported to mouth. The research findings indicated that water-soluble compounds could release from chewing gum within lower than 10 min, which can enable advantages/disadvantages based on the bioactive compound characteristics and aim of chewing gum use. Controlled/sustained release might be desired in some cases where encapsulated bioactive compounds can be used or the structure of the chewing gum can be arranged by using suitable gum base. As known, all of the bioactive compounds are not water-soluble, and some of them are lipophilic nature, where types of compounds can be also encapsulated or emulsions can be formed to carry such compounds by chewing gum into mouth. Depending on the target site, encapsulation also benefits for transporting the compound without damaging further parts of the digestive system.

Mastication process performed during consumption is also important for releasing bioactive compounds. If the matrix is not degraded at desired level, the aimed release cannot be achieved. Therefore, chewing gum matrix should be optimized considering release behavior of the product or bioactive compound should be designed with respect to textural characteristics of the chewing gum. More studies should be conducted in detail to observe releasing different functional ingredients from chewing gum. Saliva viscosity is also another important factor affecting

release; therefore, ingredients affecting viscosity should be carefully used in the formulation of the product. In addition, chewing gum size and particle size of the compounds be used in the formulation should be considered since they directly affect the solubility of the compound in saliva. As can be seen, from formulation to production/quality parameters of the chewing gum (especially texture)/type or form of the bioactive compounds, there are many factors affecting release behavior; therefore, all of them should be optimized with respect to targeted outputs.

Consumer behavior against preference has tended to products prepared with natural ingredients instead of artificial ones. From this reason, usage possibilities of natural ingredients such as colorants, flavoring agents should be investigated. In addition, one of the most important concerns about chewing gums is associated with gum base, nonbiodegradable and artificial, used in the formulation. Therefore, researchers should expend energy to discover natural and bio-degradable gum base used in the chewing gum production. By eliminating disadvantages of using artificial ingredients and optimization of critical factors mentioned above relating both with chewing gum and bioactive compound delivered, chewing gum will be the most important delivery system among all food products.

References

1. EPHAC (2010) Towards a healthier, more sustainable CAP (The European Agriculture and Public Health Consortiums position paper). http://eurohealthnet.eu/sites/eurohealthnet.eu/files/publications/EPHAC-Position
2. Granato D, Nunes DS, Barba FJ (2017) An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statics in the development of functional foods: a proposal. Trends Food Sci Technol 62:13–22
3. Mark-Herbert C (2004) Innovation of a new product category-functional foods. Technovation 24:713–719
4. Menrad K (2003) Market and marketing of functional food in Europe. J Food Eng 56:181–188
5. Simões LDS, Madalena DA, Pinheiro AC, Teixeira JA, Vicente AA, Ramos LÓ (2017) Micro- and nano bio-based delivery systems for food applications: *In vitro* behavior. Adv Colloid Interf Sci 243:23–45
6. Hooper L, Cassidy A (2006) A review of the health care potential of bioactive compounds. J Sci Food Agric 86:1805–1813
7. Halliwell B (1995) How to characterize an antioxidant: an update. Biochem Soc Symp 61:73–101
8. Santos MG, Carpinteiro DA, Thomazini M, Rocha-Selmi GA, da Cruz AG, Rodrigues CEC, Favaro-Trindade CS (2014) Coencapsulation of xylitol and menthol by double emulsion followed by complex coacervation and microcapsule application in chewin gum. Food Res Int 66:454–462
9. Abbasi S, Rahimi S, Azizi MH (2009) Influence of microwave-microencapsulated citric acid on some sensory properties of chewing gum. J Microencapsul 26:90–96
10. Yang X, Wang G, Zhangi X (2004) Release kinetics of catechins from chewing gum. J Pharm Sci 93:293–299
11. Valduga E, Lazzari MR, Xardanega R, Di Luccio M (2012) Evaluation of sugar inversion in chewing gum added of sodium lactate. J Food Process Eng 35:37–53
12. Potineni RV, Peterson DG (2008) Influence of flavor solvent on flavor release and perception in sugar-free chewing gum. J Agric Food Chem 56:3254–3259

13. Fritz D (2008) Formulation and production of chewing gum and bubble gum. Kennedy's Books Ltd, Essex
14. Konar N, Palabiyik I, Toker OS, Sagdic O (2016) Chewing gum: production, quality parameters and opportunities for delivering bioactive compounds. Trends Food Sci Technol 55:29–38
15. Potineni RV, Peterson DG (2008) Mechanisms of flavor release in chewing gum: Cinnamaldehyde. J Agricultural Food Chem 56:3260–3267
16. Cherukuri RS, Marschall-helman E, Hriscisce FT (1985) Non-adhesive chewing gum base composition. New York, Warner-Lambert Company
17. Pratik S, Asif K, Ramana MV, Mitul P, Mahesh K (2011) Chewing gum: a modern era of drug delivery. Int Res J Pharm 2:7–12
18. Gadhavi AG, Patel BN, Patel DM, Patel CN (2011) Medicated chewing gum a 21st century drug delivery system. Int J Pharm Sci Res 2:1961–1974
19. Ingole B, Daga AS, Joshi UM, Biyani KR (2012) Chewing gum: a mobile drug delivery system. Int J Pharm Sci Rev Res 14:106–114
20. Niederer B, Le A, Cantergiani E (2003) Thermodynamic study of two different chewing-gum bases by inverse gas chromatography. J Chromatogr A 996:189–194
21. Aslani A, Ghannadi A, Raddanipour R (2015) Design, formulation and evaluation of aloe vera chewing gum. Adv Biomed Res 4:175
22. Sameja K, Raval V, Asodiya H, Patadiya D (2011) Chewing gum: a modern approach to oral mucosal drug delivery. Int J Pharm Res Dev 4:001–016
23. Rose K, Steinbüchel A (2005) Biodegradation of natural rubber and related compounds: recent insights into a hardly understood catabolic capability of microorganisms. App Environ Microbiol 71:2803–2812
24. Farber TM, Clewell AE, Endres JR, Hauswirth J (2010) Safety assesment of a novel ingredient for removable chewing gum. Food Chem Toxico l48:831–838
25. Cook RB (1996) Confections comprising a proteinaceous chewable base. US patent 5,482,722
26. Mcgowan BA, Padua GW, Lee S-Y (2005) Formulation of corn zein chewing gum and evaluation of sensory properties by the time-intensity method. J Food Sci 70:475–481
27. Mehta FF, Triverdi P (2015) Formulation and characterization of Biodegredable medicated chewing gum delivery system for motion sickness using corn Zein as gum former. Trop J Pharm Res 14(5):753–760
28. Mehta F, Rajagopalan R, Trivedi P (2013) Formulation and texture characterization of environment friendly chewing gum. Int J of Pharm Tech Res 5(1):222–232
29. Palabiyik I, Toker OS, Konar N, Öner B, Demirci AS (2017) Development of a natural chewing gum from plant based polymer. J Polym Environ. https://doi.org/10.1007/s10924-017-1094-2
30. Nagasamy VD, Toprani PS, Mukherejee S, Tulasi K (2014) Medicated chewing gums – a review. Int. J Pharm Sci 4:581–586
31. Asija R, Patel S, Asija S (2012) Oral dosages forms: medicine containing chewing gum: a review. J Drug Deliv Ther 2:90–95
32. Smith AP, Woods M (2012) Effects of chewing gum on the stress and work of university students. Appetite 58:1037–1040
33. Nabors LO (2001) Alternatives sweeteners. Marcel Dekker, New York
34. Lakkis JM (2016) Encapsulation and controlled release technologies in food systems. Wiley Blackwell, UK
35. Bahoshy BJ, Klose RE, Nordstrom HA (1976) Chewing gums of longer lasting sweetness and flavor. General Foods Corp US 3:943,258
36. Bunczek MT, Urensis P (1993) Aspartame stability in chewing gum using an acid gelatin system. US Patent 5,192,561
37. Sharma SC, Yang KY (1986) Chewing gum compositions containing novel sweetener delivery systems and method of preparation. US Patent 4,597,970

38. Haahr AM, Pilsgaard CF, Stahnke LH, Bredie WLP, Refsgaard HHF (2003) Effect of sweetener on release of flavor compounds from chewing gum. In: Le Quere JL, Etievant PX (eds) Flavor research at the Dawn of the twenty-first century, proceedings of the 10th Weurman flavor research symposium. Intercept LLC, Paris
39. Tanzer JM, Freedman ML, Fitzgerald RJ (1984) Virulence of mutants defective in glucosyltransferase, dextranmediated aggregation, or dextran activity. In: Magenhagen S, Rosan B (eds) Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington
40. Edwardson S, Birkhed D, Majare B (1977) Acid production from lycasin, maltitol, sorbitol and xylitol by oral streptococci and lactobacilli. Acta Odontol Scand 35:257–263
41. Thaweboon S, Thaweboon B, Soo-Ampon S (2004) The effect of xylitol chewing gum on mutans streptococci in saliva and dental plaque. Southeast Asian J Trop Med Public Health 35:1024–1027
42. Kleber CJ, Milleman JL, Putt MS, Nelson BJ, Proskin HM (1998) Clinical effects of baking soda chewing gum on plaque and gingivitis. J Dent Res 77:A290
43. Çaglar E, Kavaloglu SC, Kuscu OO, Sandalli N, Holgerson PL, Twetman S (2007) Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli. Clin Oral Invest 11:425–429
44. Aslani A, Rostami F (2015) Medicated chewing gum, a novel drug delivery system. J Res Med Sci 20:403–411
45. Surana AS (2010) Chewing gum: a friendly oral mucosal drug delivery system. Int J Pharm Sci Rev Res 4:68–71
46. Semwal R, Semwal DK, Badoni R (2010) Chewing gum: a novel approach for drug delivery. J Appl Res 10:115–123
47. Rassing MR (1994) Chewing gum as a drug delivery system. Adv Drug Deliv Rev 13:89–121
48. Imfeld T (2006) Chlorhexidine-containing chewing gum. Schweiz Monatsschrz 116:476–483
49. Bijella MFTB, Brighenti FL, Bijella MFB, Buzalaf MAR (2005) Fluoride kinetics in saliva after the use of a fluoride containing chewing gum. Braz Oral Res 19:25–260
50. Aslani L, Ghannadi A, Mortazavi S, Torabi M (2013) Design, formulation and evaluation of medicinal chewing gum by the extract of Salvadora Persica. Life Sci J 10:47–55
51. Kralikova E, Kozak JT, Rasmussen T, Gustavsson G, Houezec JL (2009) Smoking cessation or reduction with nicotine replacement therapy: a placebo-controlled double blind trial with nicotine gum and inhaler. BMC Public Health 9:433
52. Aslani A, Rafiei S (2012) Design, formulation and evaluation of nicotine chewing gum. Adv Biomed Res 1:1–6
53. Reineccius GA (1993) Controlled release techniques in food industry. In: Risch SJ, Reineccius GA (eds) Encapsulation and controlled release of food ingredients, ACS Symposium Series, vol 590. American Chemical Society, Washington DC
54. Greenblatt HC, Dombroski M, Klishevich W, Kirkpatrik J, Bajwa I, Garrison W, Redding BK (1993) Encapsulation and controlled release of flavors and fragrances. In: Karsa DR, Stephenson RA (eds) Encapsulation and controlled release. Royal Society of Chemistry (RSC), London
55. Lew CW (2000) Encapsulation additives. US Patent 6,056,992
56. Taylor AJ (2002) Release and transport of flavors in vivo: physicochemical, physiological, and perceptual considerations. Comp Rev Food Sci Food Safety 1:45–57
57. Sostmann K, Potineni PV, McMillan E, Antenucci RN (2009) In: Hansel A, Dunkl J (eds) 4th international conference on proton transfer reaction mass spectrometry and its applications. Innsbruck, Innsbruck University Press
58. De Roos KB, Wolswinkel K (1994) Non-equilibrium partition model for predicting flavor release in the mouth, in trends in flavor research, proceedings of the 7th Weurman flavor research symposium, Noordwijkerhout, The Netherlands, 15-18 1993. In: Maarse H, van den Heij DJ (eds). Elsevier, Amsterdam

59. Harrison M (2000) Mathematical models of release and transport of flavors from foods in the mouth of the olfactory epithelium. In: Roberts DD, Taylor AJ (eds) Flavor Release. Oxford University Press, Washington, DC
60. Ferrazzano GF, Cantile T, Coda M, Alcidi B, Sangianantoni G, Ingenito A, Stasio MD, Volpe MG (2016) In vivo release kinetics and antibacterial activity of novel polyphenols-enriched chewing gums. Molecules 21:1–11
61. Hansson A, Andersson J, Leufven A (2001) The effect of sugars and pectin on flavor release from a soft drink-related model system. Food Chem 72:363–368
62. Roberts DD, Elmore JS, Langley KR, Bakker J (1996) Effects of sucrose, guar gum, and carboxy-methylcellulose on the release of volatile flavor compounds under dynamic conditions. J Agric Food Chem 44:1321–1326
63. Baek I, Linforth RST, Blake A, Taylor AJ (1999) Sensory perception is related to the rate of change of volatile concentration in-nose during eating of model gels. Chem Senses 24:155–160
64. Delarue J, Loescher E (2004) Dynamic of food preferences. A case study with chewing gums. Food Qual Prefer 15:771–779
65. Chandran S, Ravi S, Vipin KV, Augusthy AR (2014) Formulation and evaluation of medicated chewing gums containing methyl prednisolone IP. Int J ChemTech Res 6:4810–4816
66. Ko S, Gunasekaran S (2014) Controlled release of food ingredients. In: Nano- and microencapsulation for foods. Wiley, Chichester, pp 325–343
67. Peltzer MA, Salvay AG, Delgado JF, Wagner JR (2017) Use of edible films and coatings for functional food developments: a review. In: Functional foods: sources, health effects future perspectives. Nova Science Publishers, New York, pp 1–26
68. Charanioti C, Nikoloudaki A, Tzia C (2015) Saffron and beetroot extracts encapsulated in maltodextrin, gum arabic, modified starch and chitosan: incorporation in chewing gum system. Carbohyd Polym 127:252–263
69. Arvanitoyannis IS, Varzaka TH (2008) Vegetable waste management: treatment methods and potential uses of treated waste. In: Arvanitoyannis IS (ed) Waste management for the food industries. Elsevier. Academic Press, London
70. Aguiar J, Estevinho BN, Santos L (2016) Microencapsulation of natural antioxidants for food application – the specific case of coffee antioxidants – a review. Trends Food Sci Technol 58:21–39
71. Nakagawa K (2014) Nano- and microencapsulation of flavor in food systems. In: Kwak HS (ed) Nano- and microencapsulation for foods. Wiley, Oxford
72. Marquez AL, Perez MP, Wagner JR (2017) Double emulsions: potential applications for the elaboration of functional foods. In: Nelson DL (ed) Functional foods: sources, health effects and future perspectives. Nova Science Publishers, New York
73. Mohos F (2010) Confectionery and chocolate engineering: principles and applications. Willey, Oxford
74. Minifie BW (1989) Chocolate, cocoa and confectionery: science and technology, 3rd edn. AVI Book, New York
75. Rey A, Gonzalez R, Martinez-de-Juan JL, Bendito J, Mulet A (2007) EMG assessment of chewing gum behaviour for food evaluation: influence of personality characteristics. Food Qual Prefer 18:585–595
76. Dawes C, Pedersen AML, Villa A, Ekström J, Proctor GB, Vissink A, Aframian D, McGowan R, Aliko A, Narayana N, Sia YW, Joski RK, Jensen SB, Kerr AR, Wolf A (2015) The functions of human saliva: a review sponsored by the world workshop on oral medicine VI. Arch Oral Bio 60:863–874
77. Katschinski M (2000) Nutritional implications of cephalic phase gastrointestinal responses. Appetite 34:89–96
78. Engelen L (2004) A rough guide to texture. Oral physiology and texture perception of semi solids. Dissertation, University of Utrecht

79. Ting Y, Jiang Y, Ho CT, Huang Q (2014) Common delivery systems for enhancing in vivo bioavailability and biological efficacy of nutraceuticals. J Funct Foods 7:112–128
80. Yang Y, Yin J, Shao B (2011) Simultaneous determination of five aluminum lake dyes in chewing gum by HPLC with photodiode array detection. Food Addit Contam 28:1159–1167
81. Tedesco MP, Monaco-Lourenço CA, Carvalho RA (2017) Characterization of oral disintegrating film of peanut skin extract-potential route for buccal delivery of phenolic compounds. Int J Biol Macromol 97:418–425
82. Watanabe S, Dawes C (1988) The effects of different foods and concentrations of citric acid on the flow rate of whole saliva in man. Arch Oral Biol 33:1–5
83. Heintze U, Birkhed D, Björn H (1983) Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. Swed Dent J 7:227–238
84. Richardson CT, Feldman M (1986) Salivary response to food in humans and its effect on gastric acid secretion. Am J Phys 250:G85–G91
85. Edgar M, Dawes C, O'Mullane D (2004) Saliva and oral health, 3rd edn. BDJ Books, London
86. Dawes C, Macpherson LMD (1992) Effects of nine different chewing gums and lozenges on salivary flow rate and pH. Caries Res 26:176–182
87. De Almeida PDV, Gregio AMT, Machado MAN, De Lima ADS, Azevedo AAS, Azevedo LR (2008) Salvia composition and functions: a comprehensive review. J Comtemp. Dent Pract 9:72–80
88. Woolnough JW, Bird AR, Monro JA, Brennan CS (2010) The effect of a brief salivary a-amylase exposure during chewing on subsequent in vitro starch digestion curve profiles. Int J Mol Sci 11:2780–2790
89. Neyraud E, Palicki O, Schwartz C, Nicklaus S, Feron G (2012) Variability of human saliva composition: possible relationships with fat perception and liking. Arch Oral Biol 57:556–566
90. WL X, Lewis D, Broundloud JE, Morgenstern MP (2008) Mechanism, design and motion control of a linkage chewing device for food evaluation. Mech Mach Theory 43:376–389
91. Lucas PW (2004) The structure of the mammalian mouth. In: Dental functional morphology: How teeth work. Cambridge University Press, Cambridge
92. Lucas PW (2004) How the mouth operates. In: Dental functional morphology: How teeth work. Cambridge University Press, Cambridge
93. Mongini F, Tempia-Valenti G, Benvegnu G (1986) Computer-based assessment of habitual mastication. J Prosthet Dent 55:638–649
94. Blee N, Linforth R, Yang N, Brown K, Taylor A (2011) Variation in aroma release between panelists consuming different types of confectionary. Flavour Fragr J 26:186–191
95. Lucas PW (2004) Tooth shape. In: Dental functional morphology: How teeth Work. Cambridge University Press, Cambridge
96. Krause AJ (2010) Real-time release of volatile and non-volatile components from chewing gum using a mechanical chewing device. Dissertation, University of Minnesota
97. Anderson K, Throckmorton GS, Buschang BH, Hayasaki H (2002) The effects of bolus hardness on the masticatory kinematics. J Oral Rehabil 29:689–696
98. Peyron MA, Lassauzay C, Woda A (2002) Effects of increased hardness on jaw movement and muscle activity during chewing of visco-elastic model foods. Exp Brain Res 142:41–51
99. Foster K, Woda A, Peyron M-A (2006) Effect of texture of plastic and elastic model foods on the parameters of mastication. J Neurophysiol 95:3469–3479
100. Wu B, Kulkarni K, Basu S, Zhang S, Hu M (2011) First-pass metabolism via UDP-glucuronosyltransferase: a barrier to oral bioavailability of phenolics. J Pharm Sci 100:3655–3681
101. Mallery SR, Budendorf DE, Larsen MP, Pei P, Tong M, Holpuch AS, Larsen PE, Stoner GD, Fields HW, Chan KK, Ling Y, Liu Z (2011) Effects of human oral mucosal tissue, saliva, and oral microflora on intraoral metabolism and bioactivation of black raspberry anthocyanins. Cancer Prev Res 4:1209–1221
102. Satheesh Madhav NV, Shakya AK, Shakya P, Singh K (2009) Orotransmucosal drug delivery systems: a review. J Control Release 140:2–11

103. Mizrahi B, Domb AJ (2008) Mucoadhesive polymers for delivery of drugs to the oral cavity. Rec Pat Drug Deliv Formul 2:108–119
104. Martins ICF, Raposo NRB, Mockdeci HR, Polonini HC, de Oliveira FA, Fabri GMC, das Graças AMCM (2017) Delivering resveratrol on the buccal mucosa using mucoadhesive tablets: a potential treatment strategy for inflammatory oral lesions. Curr Drug Deliv. https://doi.org/10.2174/1567201814666170726102558
105. Wang ST, Chou CT, Su NW (2017) A food-grade self-nanoemulsifying delivery system for enhancing oral bioavailability of ellagic acid. J Funct Foods 34:207–215
106. Pagare PK, Satpute CS, Jadhav VM, Kadam V (2012) Medicated chewing gum: a novel drug delivery system. J Appl Pharm Sci 2:40–54
107. Ginsburg I, Koren E, Shalish M, Kanner J, Kohen R (2012) Saliva increases the availability of lipophilic polyphenols as antioxidants and enhances their retention in the oral cavity. Archives Oral Biol 57:1327–1334
108. Trivedi H, Xu T, Worrell C, Panaligan K (2005) US Patent 11/256,861
109. Jacobsen J, Bjerregaard S, Pedersen M (1999) Cyclodextrin inclusion complexes of anti-mycotics intended to act in the oral cavity–drug supersaturation, toxicity on TR146 cells and release from a delivery system. Eur J Pharm Biopharm 48:217–224
110. Kamonpatana K, Failla ML, Kumar PS, Giusti MM (2014) Anthocyanin structure determines susceptibility to microbial degradation and bioavailability to the buccal mucosa. J Agri Food Chem 62:6903–6910
111. Smith AJ, Moran J, Dangler LV, Leight RS, Addy M (1996) The efficacy of an antigingivitis chewing gum. J Clin Periodontol 23:19–21
112. Blumenthal G (2005) US Patent 11/166,543
113. Ooshima T, Minami T, Aono W, Tamura Y, Hamada S (1994) Reduction of dental plaque deposition in humans by oolong tea extract. Caries Res 28:146–149
114. Edgar KJ, Buchanan CM, Debenham JS (2001) Advances in cellulose ester performance and application. Prog Polym Sci 26:1605–1688
115. Greenberg M, Urnezis P, Tian M (2007) Compressed mints and chewing gum containing magnolia bark extract are effective against bacteria responsible for oral malodour. J Agric Food Chem 55:9465–9469
116. Gelski J (2006) Tea's weight loss potential cited as additional benefits. Food business News 28:38–40
117. Lee MJ, Lambert JD, Prabhu S, Meng X, Lu H, Maliakal P, Ho CT, Yang CS (2004) Delivery of tea polyphenols to the oral cavity by green tea leaves and black tea extract. Cancer Epidemiol Biomark Prev 13:132–137
118. Blair DW (2010) Use of starch inclusion complexes for improved delivery of dietary polyphenols to the oral cavity by chewing gum. Dissertation, the Pennsylvania State University
119. Aslani A, Ghannadi A, Rostami F (2016) Design, formulation and evaluation of ginger medicated chewing gum. Adv Biomed Res 5:130
120. Kehayoglou A, Doxastakis G, Kiosseoglou V (1994) Compressional properties of Chios mastic. In: Charalambous G (ed) Food flavors, ingredients and composition, proceedings of the 7th international flavor conference Samos, Greece, 1993. Elsevier, Amsterdam
121. Paraskevopoulou A, Kiosseoglou V (2016) Chios mastic gum and its food applications. In: Kristbergsson K, Otles S (eds) Functional properties of traditional foods. Springer, New York
122. Gluskin AE, Qazi MW (2006) US Patent 11/887,284
123. Mostafavi SA, Varshosaz J, Arabian S (2014) Formulation development and evaluation of metformin chewing gum with bitter taste masking. Adv Biomed Res 3:92
124. Porsgaard TK (2005) US Patent 12/159,524. https://www.google.com/patents/US20080299250
125. Liping L, Xihui Z (2009) Chinese Patent CN 200810114357. http://www.google.com/patents/CN101595935A?cl=en
126. Si H (2016) Chinese Patent CN 201610642022. https://www.google.com/patents/CN106260468A?cl=en&hl=tr

Bioactive Molecules in Edible and Medicinal Mushrooms for Human Wellness

53

Chia-Wei Phan, Elson Yi-Yong Tan, and Vikineswary Sabaratnam

Contents

Abstract

Mushrooms are now gaining popularity not only as an ordinary culinary ingredient, but as a healthy and whole functional food. This chapter describes three major categories of bioactive molecules found in edible and medicinal mushrooms. First is the mushroom's polysaccharide which is widely accepted as a superior immune-modulatory agent. The mushroom β-glucans differ from the bacterial and plant glucans. Mushroom β-glucans consist of linear β-(1→3)-linked backbones with β-(1→6)-linked side chains of varying length and distribution. Several important β-glucans like lentinan, schizophyllan, grifolan, as well as polysaccharide krestin (PSK) and polysaccharopeptide, will be discussed. Next, the triterpenes family, which are highly conserved in *Ganoderma* species,

C.-W. Phan (✉) · E. Y.-Y. Tan
Mushroom Research Centre, University of Malaya, Kuala Lumpur, Malaysia

Department of Pharmacy, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
e-mail: phancw@um.edu.my; elsontan@outlook.com

V. Sabaratnam
Mushroom Research Centre, University of Malaya, Kuala Lumpur, Malaysia

Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia
e-mail: viki@um.edu.my

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_83

will be elaborated further in this chapter. Finally, the indole alkaloids, which are important in mushroom as pigmentation inducer and hallucinogens, will be briefly discussed with emphasis on the psilocin and its derivatives. Other pharmacologically important mushroom-derived alkaloids will also be included. Overall, the potential to develop mushrooms as nutraceutical foods for human wellness, and their bioactive molecules for drugs, is huge.

Keywords
Mushroom · Polysaccharides · Glucans · Triterpenes · Alkaloids · Indole

1 Introduction

Mushrooms are not only valued as a food source but also their long history of beliefs in curative abilities both from the western and oriental traditional medicine systems. What are mushrooms? Mushrooms are the macrofungi with distinctive fruiting bodies commonly occurring in fungi of the class Basidiomycetes and occasionally in the class Ascomycetes [1, 2]. Fruiting bodies are also used interchangeably with basidiocarps (the sexual fruiting body of Basidiomycetes) or ascocarps (the sexual fruiting body of Ascomycetes) [3]. Interestingly, even though the Basidiomycetes demonstrate a wide variety of fruiting body shape, the Ascomycete species still outnumbered the Basidiomycetes [4]. An overview of the mushroom species and the basic terminology used for a typical Basidiomycete mushroom is presented in Fig. 1.

In terms of food, mushroom consumption is considered popular in six countries known as the G-6 (USA, Germany, UK, France, Italy, and Canada) [5]. The six countries make up to 85% of the world consumption of mushroom. According to the Food and Agriculture Organization (FAO), the main exporters of fresh mushrooms in 2012 are Poland, Netherlands, China, Ireland, and Canada. The main importers of

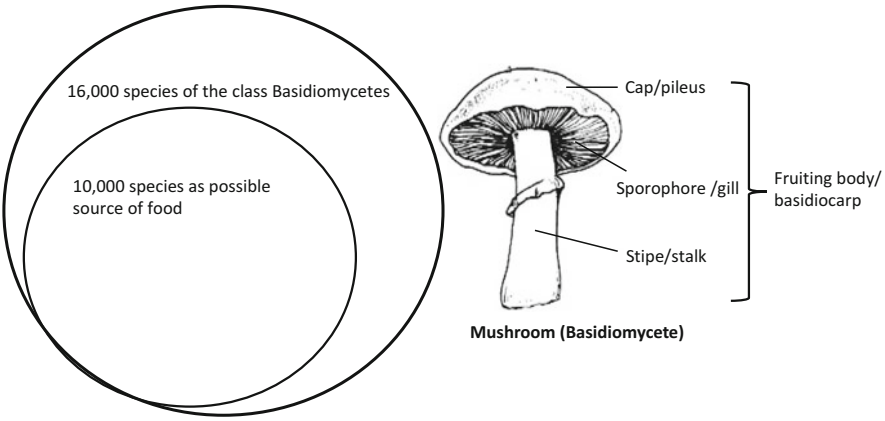


Fig. 1 A pie chart illustrates the number of species of the class Basiciomycetes (left) and the common term to describe the parts of a typical mushroom of Basidiomycete class (right)

fresh mushrooms in the world are the United Kingdom, Germany, Russia, France, and USA. To date, China is still the main producer of mushrooms in the world, producing 5.15 million tons of fresh and processed products yearly.

Indeed, there are many other important roles that mushrooms play in the world. Their expediency to man as food, as tonics and medicines, and also in the bioconversion of waste organic materials are all of great benefit to both man and nature [6]. Phan and Sabaratnam [7] have recently reviewed that the spent mushroom substrate can serve as a reservoir to recover important lignocellulosic enzymes like laccase, xylanase, lignin peroxidise, cellulose, and hemicellulase. In recent years, mushrooms are also known as "mycoremediation tools" because of their use in remediation of different types of pollutants [8, 9].

Mushrooms are now popular for their medicinal properties. There is also a fast mounting volume of in vitro and in vivo animal trials describing a range of medicinal and health promoting properties of mushrooms, including antitumor, anticancer, brain and cognitive function, immunomodulatory, and antiobesity [10]. However, as emphasized by Roupas et al. [11], there are still inadequate direct human intervention trials of the edible and medicinal mushrooms. Figure 2 shows the six most widely investigated edible and medicinal mushrooms [*Ganoderma lucidum* (Fr)

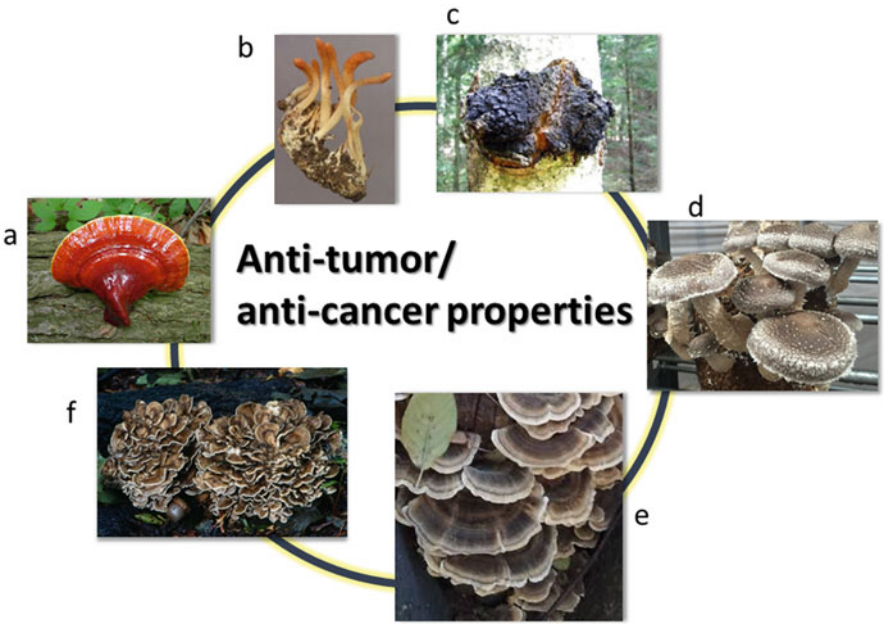


Fig. 2 The six most researched edible and medicinal mushrooms in the field of cancer therapy and oncology according to Scopus. a: *Ganoderma lucidum* (Fr) P. Karst, b: *Cordyceps militaris* (L.:Fr.) Link, c: *Inonotus obliquus* (Ach. ex Pers.) Pilát, d: *Lentinula edodes* (Berk.) Pegler, e: *Trametes versicolor* (L.) Lloyd, and f: *Grifola frondosa* (Dicks.: Fr.). All photos and names are retrieved from mycobank.com. All the mushrooms are of Basidiomycetes class, except for *C. militaris* (Ascomycete). Note the variety of shapes and colors of the basidiocarps

P. Karst, *Cordyceps militaris* (L.:Fr.) Link, *Inonotus obliquus* (Ach. ex Pers.) Pilát, *Lentinula edodes* (Berk.) Pegler, *Trametes versicolor* (L.) Lloyd, and f: *Grifola frondosa* (Dicks.: Fr.)] to fight cancer. The most recent research findings of the six mushrooms on antitumor and anticancer are stipulated in Table 1 [12–27].

In this chapter, we describe and emphasize on the highly sought after bioactive compounds (polysaccharides, triterpenes, and alkaloids) isolated from mushrooms. We also report on the recent advances in our understanding of these mushroom-derived compounds as well as their mode of actions.

2 Abundance of Nutraceuticals in Mushrooms

2.1 Polysaccharides

Bioactive polysaccharides are abundantly found in plants, yeast, bacteria, and fungi. In mushrooms, they exist in the form of α- and β-glucans [28]. Notably, each type of β-glucan comprises a different molecular backbone. They differ among bacterial, cereal, oat, yeast, and fungal glucans. The structural diversity of mushroom β-glucans has been reviewed recently [29]. Essentially, mushroom β-glucans consist of linear β-(1→3)-linked backbones with β-(1→6)-linked side chains of varying length and distribution [30, 31]. They can form tertiary structures stabilized by interchain hydrogen bonds. Some variations include 1→4 linkages, α-glucan moieties, protein complex, and alternate sugars [32]. Figure 3 shows the structure of a fungal (mushroom) β-glucan.

The involvement and importance of polysaccharides in tumor and cancer treatment were first recognized more than 100 years ago when it was found that certain polysaccharides could induce complete remission in patients with cancer. Ever since antitumor activity of macrofungal polysaccharides was first published by Chihara in the 1960s [33], researchers have isolated structural diversified polysaccharides with strong antitumor activity [34]. Unlike traditional antitumor drugs, these substances produce an antitumor effect by activating various immune responses in the host and cause no harm to the body [35].

Carrying out an extensive study in 1966, Gregory isolated the active substances from fruiting bodies of more than 200 Basidiomycetes mushroom species [36]. The polysaccharides isolated from 22 mushroom species and 50 culture media displayed an inhibitory effect on tumor cells, including Sarcoma S-180, adenocarcinoma 755, and leukemia L-1210 [36, 37]. Bioactive polysaccharides can be isolated from mycelium, the fruiting body, and sclerotium, which represent three different forms of a macrofungi in the life cycle [38, 39].

The most famous and most-talk-about polysaccharides isolated from mushrooms are the lentinan derived from *L. edodes*, tremellan from *Tremella fuciformis* Berk., polysaccharide krestin (PSK) and polysaccharopeptide from *T. versicolor*, ganoderan from *G. lucidum*, schizophyllan from *Schizophyllum commune* Fr.,

Table 1 The six mushroom species which are highly researched on their antitumor/anticancer activities. The most recent literature on the antitumor/anticancer properties of these mushrooms is included along with this table.

Mushrooms	Common name	Compound	Description	References
Ganoderma lucidum	Lingzhi, reishi	Ergosterol	Effectively inhibited tumor growth in Hepa1-6-bearing C57 BL/6 mice	[12]
		Genistein		
		Kaempferol		
		Ganoderic acid A		
		Ganoderic acid B		
		Ganoderic acid C2		
		Ganoderic acid D		
		Ganoderic acid H		
		Ganoderic acid Y		
		Ganoderenic acid A		
		Ganoderenic acid D		
		Ganoderenic acid G		
		Polysaccharides	The mushroom polysaccharides enhanced the radiosensitivity of hepatocellular carcinoma cell line HepG2 through Akt signaling pathway	[13]
		Triterpenes	Inhibited the proliferation of human prostate cancer cells and induced apoptosis	[14]
		Polysaccharides	Inhibited prostate cancer cell migration	[15]
Cordyceps militaris	Dongchongxiacao, caterpillar mushroom, winter caterpillar summer grass	Cordycepin	Inhibited malignant transformation, increased cell apoptosis, and decreased cell mitosis in a murine oral cancer model	[16]
		Cordycepin	Induced apoptotic cell death of human brain cancer	[17]
Inonotus obliquus	Chaga mushroom, black tree fungus	Polysaccharides	Inhibited NF-κB nuclear translocation in human nonsmall cell lung carcinoma (NSCLC)	[18]
		Lanostane-type triterpene (inonotusanes D)	Exhibited strong cytotoxicity against the 4T1 (mouse breast cancer) cell line	[19]

(*continued*)

Table 1 (continued)

Mushrooms	Common name	Compound	Description	References
Lentinula edodes	Shiitake	Polysaccharides	Exhibited inhibition of cell proliferation on HCT-116 and HeLa cells	[20]
		Acid heteropolysaccharides	Showed inhibition against A549 human lung cancer cells, SGC7901 gastric cancer cells, MCF-7 breast cancer cells, U937 histiocytic lymphoma cells, and MG-63 human osteosarcoma cells	[21]
		Selenium-containing polysaccharides	Cytotoxic against PC3 and HeLa cancer cells	[22]
Grifola frondosa	Yunzhi, maitake mushroom, hen of the woods	D-fraction polysaccharide	Showed synergistic effects with vitamin C against SMMC-7721 hepatocarcinoma cells	[23]
		D-fraction polysaccharide	Modulated mammary tumor progression	[24]
		Polysaccharide	Enhanced immunostimulatory activity	[25]
Trametes versicolor	Turkey tail	Extracts combined with metronomic zoledronic acid	Attenuated breast tumor propagation	[26]
		Glucan	Exhibited antitumor activity on Sarcoma-180 cells	[27]

grifolan from *G. frondosa*, and pleuran from *Pleurotus ostreatus* (Jacq.) P. Kumm [40] (Fig. 4).

Most recently, Ahn et al. [41] studied the effects of lentinan from *L. edodes* on mouse bone marrow-derived macrophages with and without inflammasome triggers. Lentinan was found to upregulate pro-inflammatory cytokines like interleukin (IL)-1β, IL-18, or caspase-1. However, lentinan was found to attenuate IL-1β secretion when the macrophages were treated with bacteria *Listeria monocytogenes* or lipopolysaccharide (LPS). This clearly shows that lentinan, a bioactive polysaccharide, can act as a "double edge sword." It increases the pro-inflammatory cytokines to initiate immune response when infection commences, and on the other hand, exerts anti-inflammation when an infection persists.

Interestingly, Zhang et al. [42] reported on the combinational effects of oral polysaccharides like lentinan and tremellan on mice which were immunized with inactivated H1N1 influenza vaccine. The results showed that mice in the

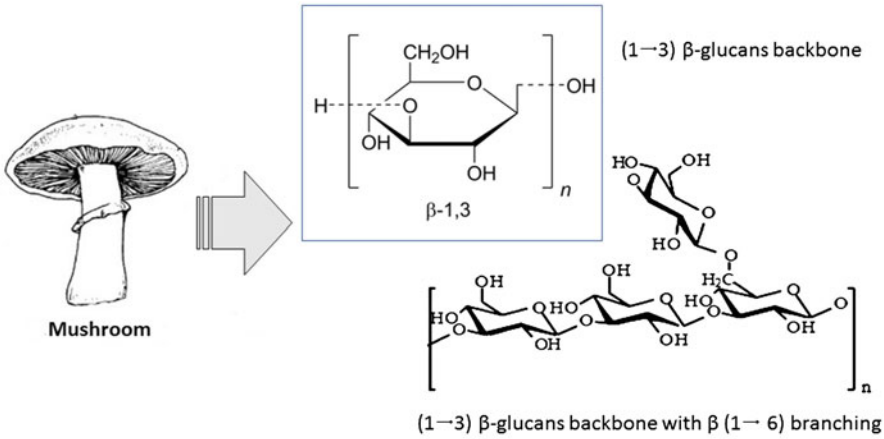


Fig. 3 Mushroom β-glucans consisting of linear β-(1→3)-linked backbones with β-(1→6)-linked side chains

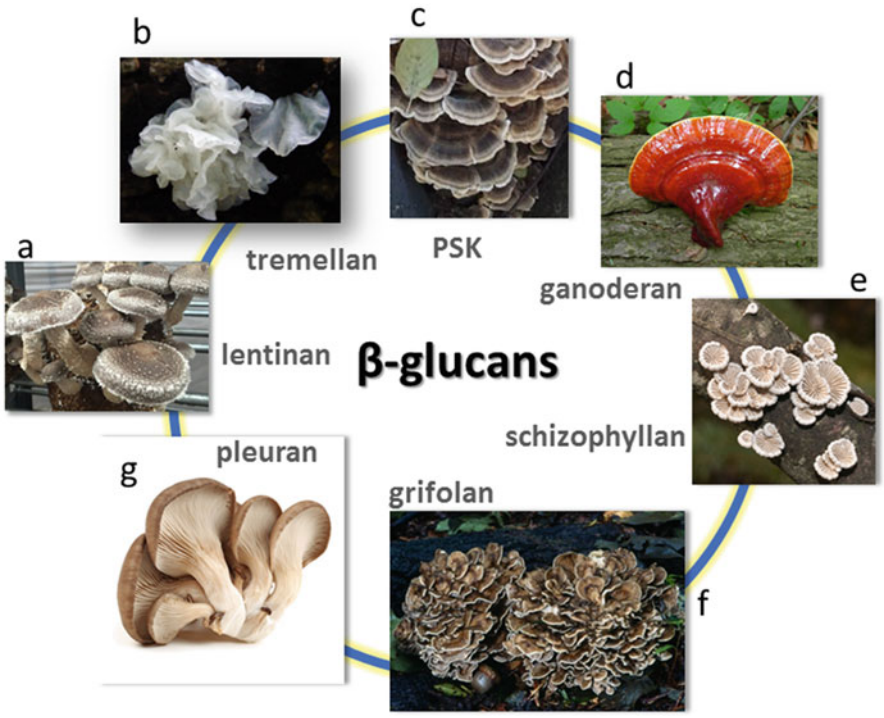


Fig. 4 The different names of bioactive β-glucan found in their respective mushroom origins. (**a**) *L. edodes*, (**b**) *T. fuciformis*, (**c**) *T. versicolor*, (**d**) *G. lucidum*, (**e**) *S. commune,* (**f**) *G. frondosa*, (**g**) *P. ostreatus. All photos and names are retrieved from mycobank.com.*

polysaccharides with vaccine groups had improved viral clearance. The immunized rat which were fed with lentinan and tremella recovered faster than the mice receiving only the vaccine after infection.

Some polysaccharides isolated from mushroom might possess an acidic or a neutral characteristic due to different types of glycosidic linkages. Some are bound to protein or peptide residues such as polysaccharide-protein/-peptide complexes. One example is the protein-bound polysaccharide K (PSK) from *T. versicolor*. PSK is very popular among the patients with gastrointestinal cancer (GIC) and they consume it with or without chemotherapy [43, 44]. Most recently, a network meta-analysis revealed that PSK combined with chemotherapy can increase the patient overall survival by 3–5 years [45]. Another meta-analysis and systematic review study also showed that PSK can extend survival in lung cancer patients [46]. As exemplified by PSK, in addition to the primary structure, a higher structure of polysaccharides, such as chain conformation, plays an important role in their antitumor activities.

Ganoderan represents an immunomodulatory polysaccharide from *G. lucidium*. It is an antioxidant polysaccharide that was shown to prevent and control cerebral arteriosclerosis by regulating the NADPH oxidizing enzyme expression [47]. It was also shown to exert protective effects in rats with chronic glomerulonephritis [48]. On the other hand, pleuran, which was extracted from *P. ostreatus*, was first reported to be formulated as a β-glucan-based cream. The pleuran-based cream was found to be effective in mild to severe atopic dermatitis [49]. In fact, β-glucan-rich *P. ostreatus* was found to be a functional food as it demonstrated hypoglycemic effect in diabetic mice, and it is capable of improving hyperlipidemia in obese mice [50, 51].

According to Zhang et al. [52], schizophyllan is a nonionic, water-soluble homoglucan which possesses a β-(1→3)-linked backbone with single β-(1→6)-linked glucose side chains at approximately every third residue. Schizophyllan is probably one of the oldest β-glucan discovered from mushroom. Since the mushroom *Schizophyllum commune* is an efficient wood-degrading fungus, it can directly utilize woody substances like corn fibers for the production of schizophyllan [53, 54]. Besides serving as a potential prebiotic with immunomodulating properties [55], schizophyllan is now being developed for bulk biomaterial applications, such as in enhanced oil recovery and as a component of bio-lubricants [56–58].

Grifolan is a branched β-(1→3) glucan extracted from *G. frondosa* [59]. The proposed mechanism by which grifolan exerts antitumor effect includes first the enhancement of immunity against the bearing tumors and secondly, a direct anti-tumor activity to induce the apoptosis of the tumor cells [60]. Grifolan also can be used for the prevention of the oncogenesis by oral administration (cancer-preventing activity). D-fraction, on the other hand, is a protein-bound β-1,6 and β-1,3 glucan (proteoglucan) extracted from *G. frondosa*. D-fraction was reported for the first time in 2017 with the ability to act directly on mammary tumor cells [24].

Overall, mushroom polysaccharides exert their bioactivity mainly via immunomodulation [61]. They help the host to adapt to various biological stresses

and exert a nonspecific action on the host, supporting some or all of the major systems. Most importantly, mushroom polysaccharides are nontoxic and place no additional stress on the body. Therefore, they are regarded as "biological response modifiers" with the potential as prebiotic to safeguard our gut microbiome [62].

2.2 Triterpenoids

Triterpenes are highly oxidized lanostanes. Triterpenes are widely reported in *Ganoderma* species. Zhou et al. [63] had reviewed that triterpene is one of the main components responsible for the claimed therapeutic efficacy of *Ganoderma*. In fact, the potential of *Ganoderma* triterpenoids against various cancer targets had been well documented [64].

Triterpenes reported in *Ganoderma lucidium* include but not limited to ganoderic acid A **(1)**, C **(2)**, F **(3)**, U **(4)**, V **(5)**, W **(6)**, X **(7)**, Y **(8)**, lucidimol A **(9)**, B **(10)**, ganoderiol F **(11)**, ganodermanondiol **(12)**, and ganodermanontriol **(13)** [65–68]. Ganoderiol F and ganodermanontriol were found to be active as anti-HIV-1 agents [69]. Ganoderic acid is a member of highly oxygenated C30 lanostane-type triterpenoids. Ganoderic acids and their derivatives are reported to modulate the signaling network in cancer signaling pathways, and they primarily target nuclear factor-kappa B (NF-κB), 3′,5′-cyclic adenosine monophosphate (cAMP), rapidly accelerated fibrosarcoma mitogen activated protein kinases (RAS-MAPK), phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR), and cell cycle resulting in apoptosis [70]. Figure 5 shows the chemical structures of triterpenoids **(1–13)** from *G. lucidum*.

Although ganoderic acids are popular for their anticancer properties, their beneficial effects on the nervous system are widely pursued by researchers. The triterpenoids isolated from *G. lucidum*, namely, ganoderic acid A **(1)**, 7-oxo-ganoderic acid Z **(14)**, ganolucidic acid A **(15)**, methyl ganoderic acid A **(16)**, methyl ganoderic acid B **(17)**, ganoderic acid S1 **(18)**, ganodermic acid TQ **(19)**, and ganodermatriol **(20)**, have shown NGF- and BDNF-like neuronal survival-promoting effects [71], as well as neuroprotection activity [72]. Figure 6 shows the chemical structures of triterpenoids **(14–20)** from *G. lucidum*.

Besides *G. lucidium*, *G. tsugae* Murrill [73], *G. concinna* [74], and *G. pfeifferi* Bres. [75] were also reported to produce new lanostane-type triterpenoids. Tsugaric acid C **(21)**, tsugarioside B **(22)**, and tsugarioside C **(23)** from *G. tsugae* were found to be effective against human hepatoma cells [73]. Besides that, three new lanostanoids, i.e., 5α-lanosta-7,9(11),24-triene-3β-hydroxy-26-al **(24)**, 5α-lanosta-7,9(11),24-triene-15α-26-dihydroxy-3-one **(25)**, and 8α,9α-epoxy-4,4,14α-trimethyl-3,7,11,15,20-pentaoxo-5α-pregnane **(26)**, were isolated from *G. concinna* [76]. All the three compounds were found to induce apoptosis in human promyelocytic leukemia HL-60 cells. In 2003, Mothana et al. [75] discovered three new antiviral lanostanoid triterpenes from *G. pfeifferi*, namely ganodermadiol **(27)**, lucidadiol **(28)**, and applanoxidic acid G **(29)**, all of which showed antiviral activity against influenza virus type A and HSV type 1. Figure 7 shows the chemical

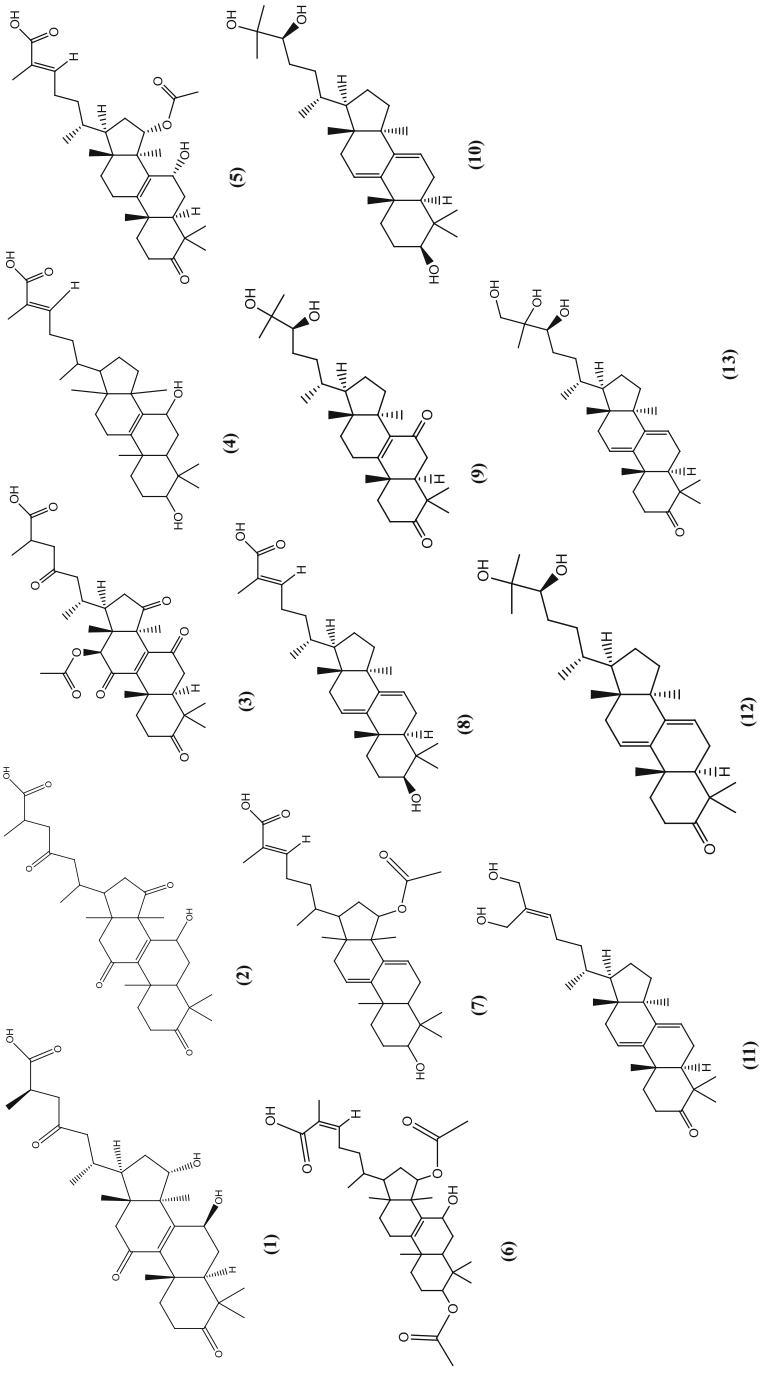
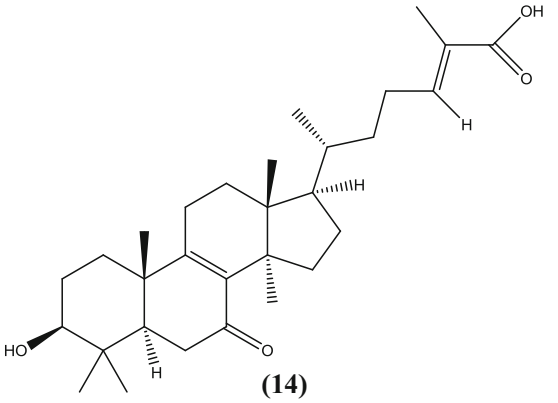
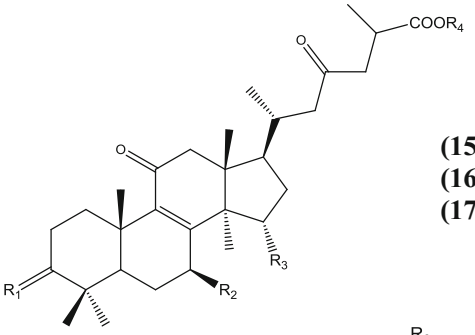


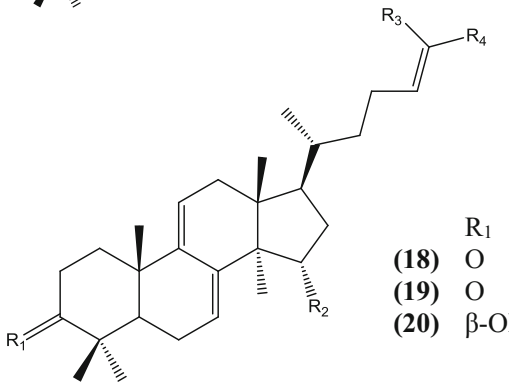
Fig. 5 Different types of ganoderic acids and derivatives isolated from *G. lucidum*



(14)



	R_1	R_2	R_3	R_4
(15)	O	H	α-OH,H	H
(16)	O	OH	α-OH,H	CH_3
(17)	β-OH,H	OH	O	CH_3



	R_1	R_2	R_3	R_4
(18)	O	H	COOH	CH_3
(19)	O	OAc	COOH	CH_3
(20)	β-OH,H	H	CH_2OH	CH_2OH

Fig. 6 Neuroactive triterpenoids (**14**–**20**) isolated from *G. lucidum*

structures of different *Ganoderm*a-derived triterpenoids **(21**–**29)** from *G. tsugae*, *G. concinna*, and *G. pfeifferi*.

Ganoderma colossum (=*Tomophagus colossus* (Fr.) Murrill), found in Vietnam, was reported to possess anti-HIV-1 protease activity [76–78]. El Dine et al. [76] have reported the isolation of four new lanostane triterpenes, namely, colossolactone V

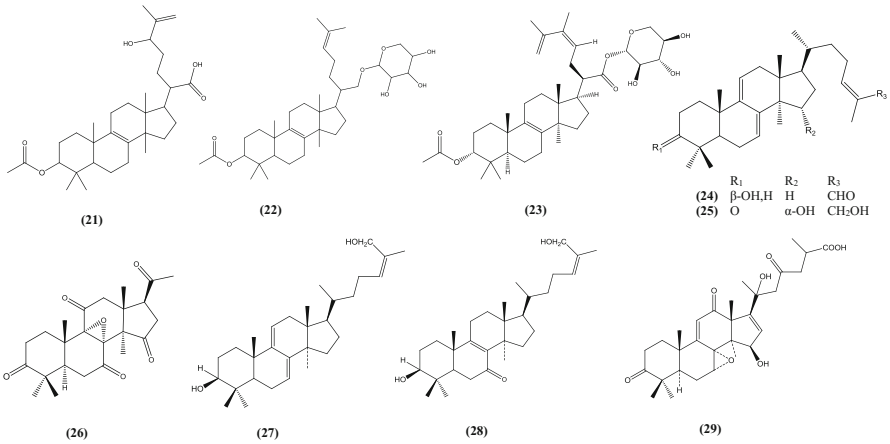
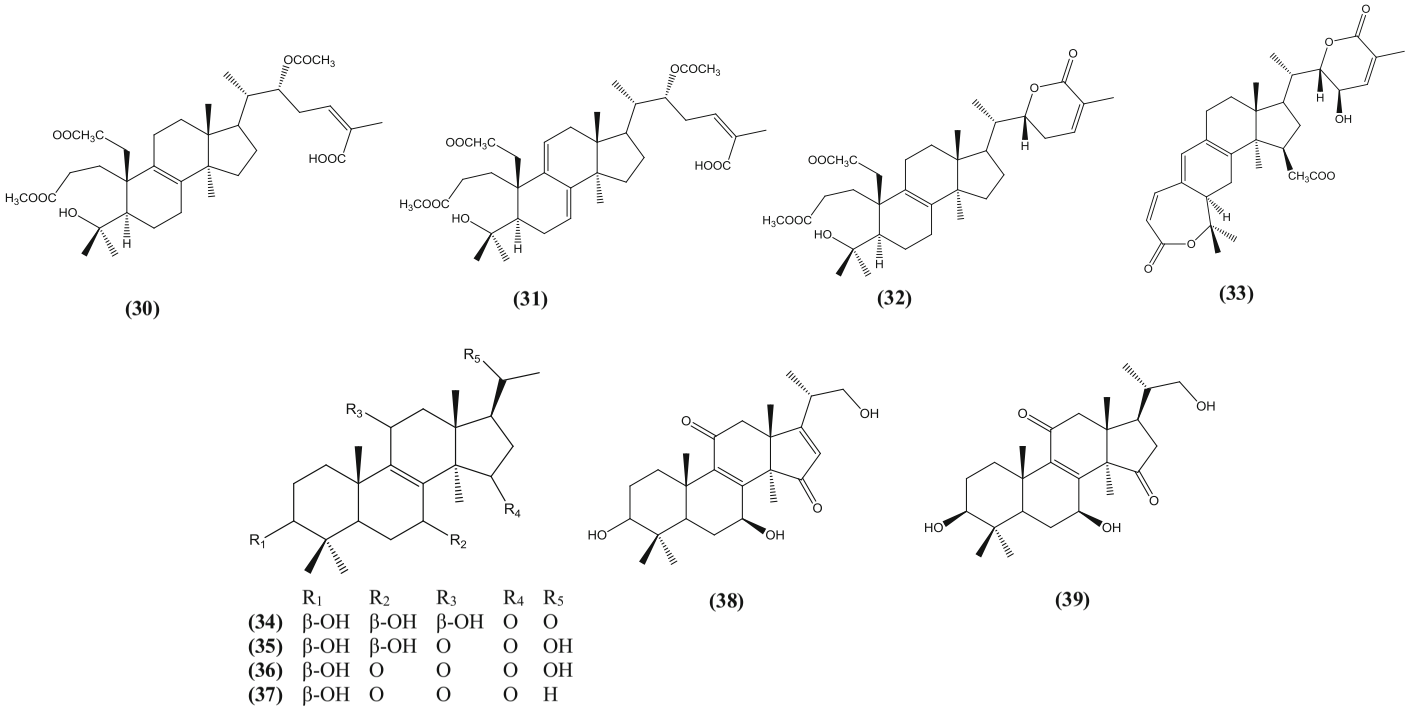


Fig. 7 Triterpenoids **(21–29)** from *G. tsugae*, *G. concinna*, and *G. pfeifferi*

(30), colossolactone VI **(31)**, colossolactone VII **(32)**, and colossolactone VIII **(33)** (Fig. 8), from the Vietnamese mushroom. Furthermore, nortriterpenoids, a derivation of lanostane-type triterpenoids due to degradation of side chains, have also been found in *G. resinaceum* Boud [79]. In the study of Chen et al. [79], six new nortriterpenoids **(34–39)** (Fig. 8) were separated and purified from the basidiocarps of *G. resinaceum*. The new compounds were identified as lucidone I **(34)**, lucidone J **(35)**, lucidone K **(36)**, lucidone I **(37)**, ganosineniol B **(38)**, and ganosineniol C **(39)**, based on high resolution mass spectrometry (HRMS), nuclear magnetic resonance (NMR), infrared (IR), and ultraviolet (UV). However, only compounds **34**, **35**, **38**, and **39** showed a significant α-glucosidase inhibitory activity.

There are several research groups that reported the presence of rare triterpenes in mushrooms such as *Piptoporus betulinus* (=*Fomitopsis betulina*) [80]. Annual white to brownish basidiocarps of the "Iceman" mushroom *F. betulina* can be found on trees in the northern hemisphere [81]. Several experiments had revealed that the extracts of *F. betulina* showed potential cytotoxic activities against several human cancer cell lines [82–84]. Lately, Tohtahon et al. [80] described the isolation and identification of five new lanostane triterpenoids, namely, piptolinic acids A-E **(40–44)** (Fig. 9). The authors also described their cytotoxicity effects against HL-60 and THP-1 human leukemia cell lines.

Astraeus odoratus Phosri, the earth-star mushroom, is popular as food in Thailand despite its strange looking star-shaped figure [85]. The mushroom is expensive due to the limitation of natural occurrence and the difficulty of artificial cultivation. Isaka et al. [86] reported the isolation of twelve new lanostane triterpenoids, astraeusins A–L (**45–56)** (Fig. 10) from the methanol extracts of *A. odoratus.* All the compounds, except for astraeusin F due to sample shortage, were subjected to antibacterial activities against *Bacillus cereus* and *Enterococcus faecium.* Astraodoric acid A **(57)** (Fig. 10) inhibited the proliferation of both *B. cereus* and *E. faecium* with minimum inhibitory concentration (MIC) of 6.25 μg/mL.



	R_1	R_2	R_3	R_4	R_5
(34)	β-OH	β-OH	β-OH	O	O
(35)	β-OH	β-OH	O	O	OH
(36)	β-OH	O	O	O	OH
(37)	β-OH	O	O	O	H

Fig. 8 Colossolactones **(30–33)** from *G. colossum* and nortriterpenoids **(34–39)** from *G. resinaceum*

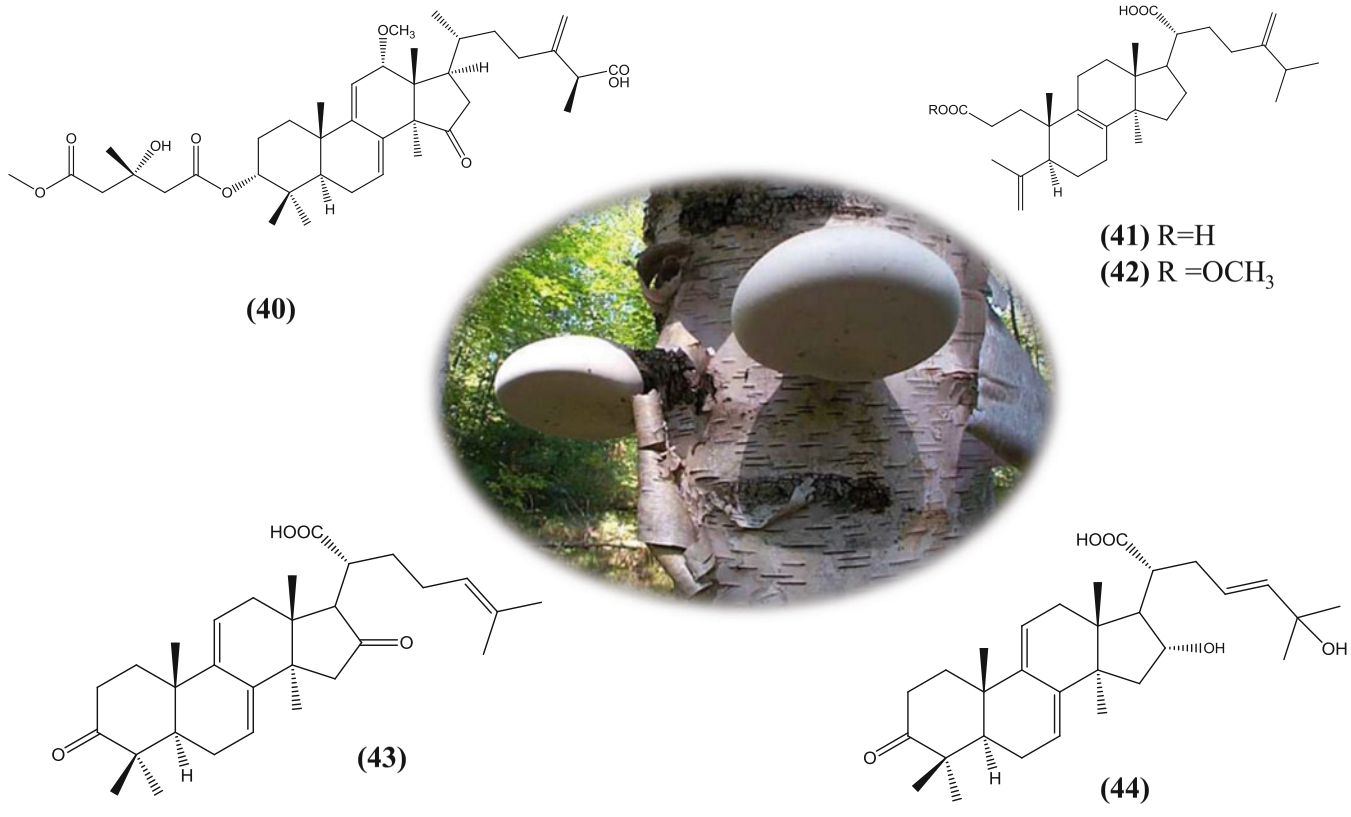


Fig. 9 Piptolinic acids isolated from the "iceman" mushroom, *F. betulina.* Mushroom picture retrieved from mushroomexpert.com

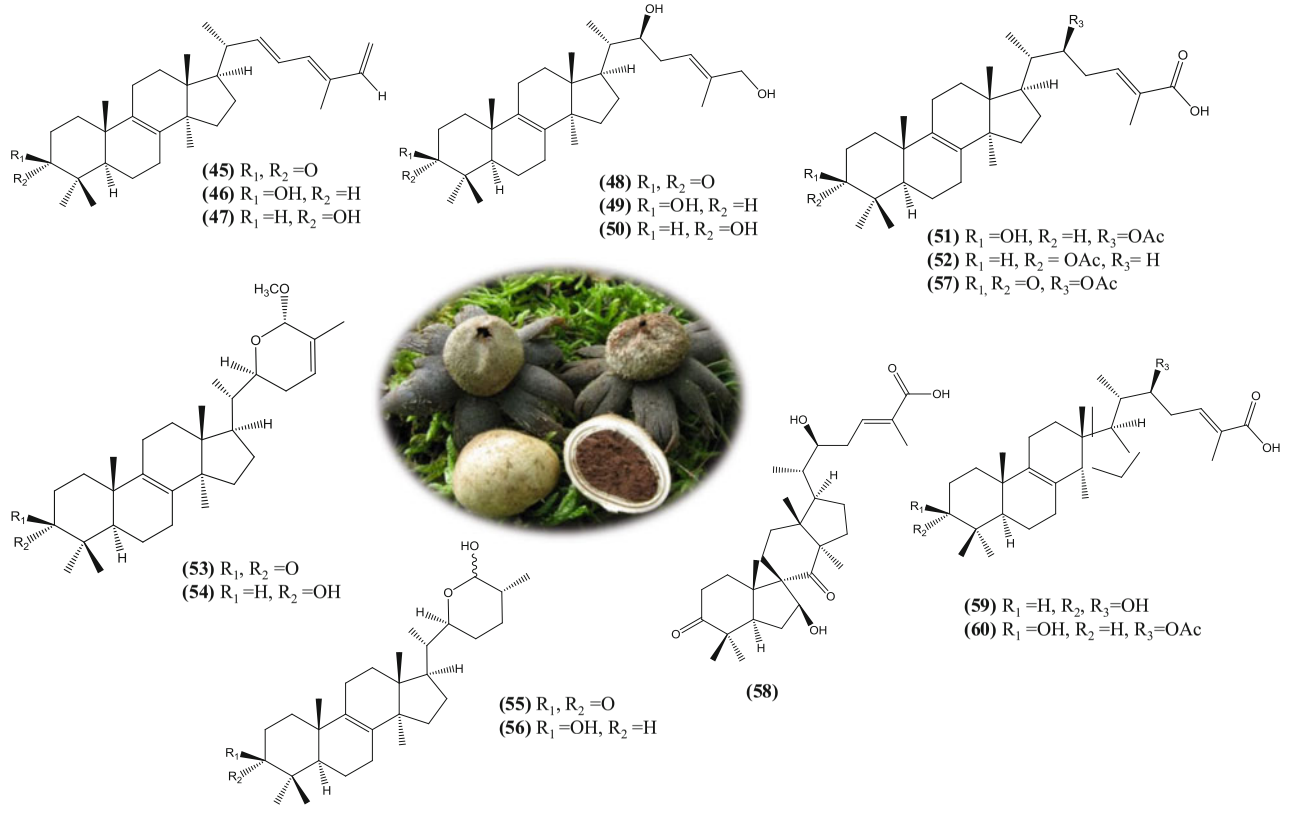


Fig. 10 Astraeusins A–L (**45**–**56**), **a**straodoric acids A, E, and F (**57**, **59**, **60**), and spiro-astraodoric acid (**58**) isolated from *Astraeus odoratus*. Mushroom picture retrieved from mushroomexpert.com

Srisurichan et al. [87] also reported three new lanostane-type triterpenoids which exhibited a varying degree of cytotoxicity against human cancer cells depending on the different side chains they contain. The triterpenoids were named as spiro-astraodoric acid **(58)** and astraodoric acids E **(59)** and F **(60)** (Fig. 10). Interestingly, compound **58** possesses a spirocyclic lanostane triterpenoid structure (Fig. 9). The authors suggested that the presence of an acetoxy group on a lanostane side chain increased the cytotoxicity of the lanostane triterpenoids.

2.3 Alkaloids

Alkaloids are nitrogen-containing heterocyclic compounds. Till now, fungal alkaloids are mostly known because of their toxicological relevance [88]. Perhaps the most widely known mushroom alkaloids are the hallucinogenic indole derivatives which encompass psilocin **(61)** and psilocybin **(62)**, found in "magic mushrooms." In fact, determination of psilocin and psilocybin (Fig. 11) is an important task of forensic analysis and researchers often used HPLC (high-performance liquid chromatography) for quantification.

Homer and Sperry [89] have recently reviewed on the isolation of mushroom-derived indole alkaloids, along with their associated biological activities. The alkaloid compounds can be found in different quantities based on mushroom species, their developmental stages, climatic conditions, and the availability of soluble nitrogen and phosphorous in the soil [90].

Psilocin **(61)**, an indole alkaloid found in the genus *Psilocybe*, is considered a natural monophenol which exhibit various properties including toxicity, and even antioxidant and therapeutic action [91]. It demonstrates bioactivity similar to other psychoactive tryptamines by inducing psychoactive effects like alternation of mood, ease of anxiety, and relief of depression [92]. Its mode of action is believed to occur through serotonin, a monoamine that regulates numerous physiological responses including those in the central nervous system [93, 94]. Most recently, Lenz et al. [95] reported the identification of ω-N-methyl-4-hydroxytryptamine (norpsilocin, **63**) (Fig. 11) from the carpophores of *Psilocybe cubensis* (Earle) Singer. Interestingly,

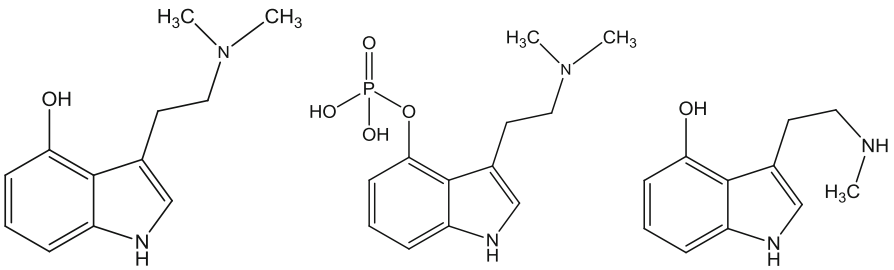


Fig. 11 The chemical structures of psilocin **(61)**, psilocybin **(62)**, and norpsilocin **(63)**

norpsilocin has not been previously reported as a natural product. According to the authors, it is probably liberated from its 4-phosphate ester derivative, which is a known natural product baeocystin. However, no bioactivity was reported for norpsilocin yet.

A recent clinical trial has shown that psilocybin exerts a pharmacological action against depression with no serious or unexpected adverse events in the patients on trial [96]. Subsequently, the authors reported on the possible mechanisms of the post-treatment brain effects of psilocybin and found out that decreased amygdala cerebral blood flow is likened with reduced depressive symptoms [97].

Overall, mushroom-derived indole alkaloids are generally screened from a variety of extracts, which are then found to exert some sort of beneficial bioactivity in vitro. Some of the examples include L-tryptophan **(64)**, which serves as a source of essential amino acid in human diet (Fig. 12). Other examples include corralocin B **(65)** and C **(66)** (Fig. 12), two indole alkaloids identified from the coral-alike lion's mane mushroom, *Hericium coralloides* (Scop.) Pers.[98]. The compounds were found to stimulate neurotrophin expression in human 1321N1 astrocytes. Corralocin B was reported to show antiproliferative activity against human umbilical vein endothelial cells (HUVEC) and human cancer cell lines MCF-7 and KB-3-1.

The basidiocarps of an nonedible but medicinal "bitter cap" mushroom, *Cortinarius infractus* (Pers.) Fr., also contained infractopicrin **(67)** and 10-hydroxyinfractopicrin **(68)** (Fig. 12) [99]. The acetylcholinesterase-inhibiting activity of these compounds was comparable to that of galantamine, a drug used for the treatment of mild to moderate Alzheimer's disease. Notably, the alkaloids present in *C. infractus* are responsible for the distinctive "bitterness" of this mushroom species [100, 101].

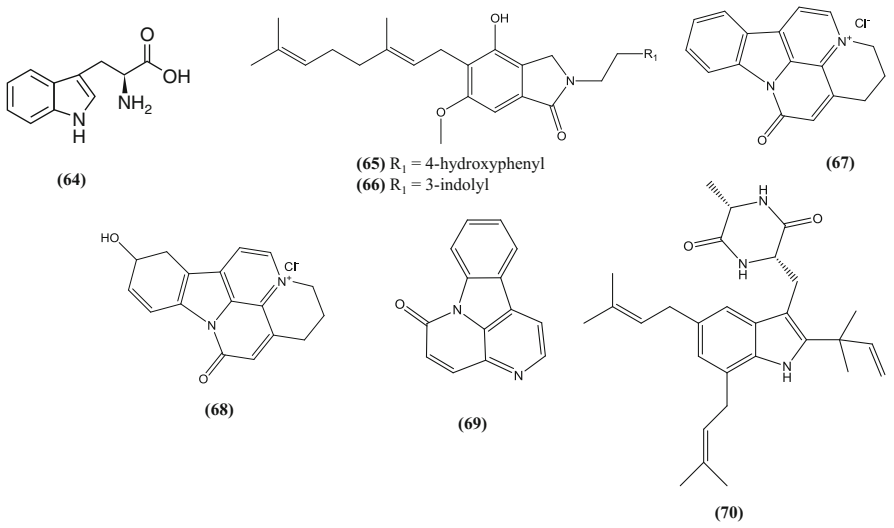


Fig. 12 The chemical structures of alkaloids **(64–70)** from mushrooms

Boletus curtisii Berk., an edible mushroom which is yellow when young and turns brown when old, produces an interesting collection of sulfur-containing β-carboline derivatives, one of which is canthin-6-one **(69)** (Fig. 12) [102]. This indole alkaloid which is responsible for the bright yellow pigmentation is also found in a variety of higher plants and possesses cytotoxic properties against leukemic cells [103]. Other alkaloids from edible mushroom also include echinulin (**70**), a triprenylated tryptophan-based diketopiperazine from an aromatic mushroom, *Lentinus strigellus* Berk [104, 105].

3 Conclusion

The study of mushrooms is growing in popularity because of their attributed health benefits. The aforementioned bioactive molecules found in mushrooms, i.e., polysaccharides, glucans, triterpenes, and alkaloids, contribute greatly to their curative properties like anticancer, anti-inflammatory, antivirus, and even anti-Alzheimer's disease. Indeed, edible and medicinal mushroom has huge demand as "whole functional food," as well as developed as a healthcare product. Overall, the mechanisms of action of the bioactive compounds, which were discussed in this chapter, still elude scientific inquiry and scientists are still working towards unraveling the biochemical pathways leading to the curative effects.

Acknowledgments We acknowledge the support of this work by the University of Malaya BKP grant (BK011-2017). This work was also supported by the University of Malaya High Impact Research MoE Grants, namely UM.C/625/1/HIR/MoE/SC/02 and UM.C/625/1/HIR/MOHE/ASH/01(H-23001-G000008).

References

1. Chang S, Miles P (1992) Mushroom biology – a new discipline. Mycologist 6:64–65
2. Miles PG, Chang ST (1997) Mushroom biology: concise basics and current developents. World Scientific, Singapore
3. Chang S-T, Buswell JA (2008) Development of the world mushroom industry: applied mushroom biology and international mushroom organizations. Int J Med Mush 10:195–208. https://doi.org/10.1615/IntJMedMushr.v10.i3.10
4. Lindequist U, Niedermeyer THJ, Jülich W-D (2005) The pharmacological potential of mushrooms. Evid Based Complement Alternat Med 2:285–299. https://doi.org/10.1093/ecam/neh107
5. Mat-Amin MZ, Harun A, Abdul-Wahab MAM (2014) Status and potential of mushroom industry in Malaysia. Econ Technol Manag Rev 9b:103–111
6. Chang ST (1996) Mushroom research and development - equality and mutual benefit. In: Royse DJ (ed) Mushroom biology and mushroom products. Pennsylvania State University, University Park, pp 1–10
7. Phan C-W, Sabaratnam V (2012) Potential uses of spent mushroom substrate and its associated lignocellulosic enzymes. Appl Microbiol Biotechnol 96:863–873. https://doi.org/10.1007/s00253-012-4446-9
8. Adenipekun C, Lawal R (2012) Uses of mushrooms in bioremediation: a review. Biotechnol Mol Biol Rev 7:62–68. https://doi.org/10.5897/BMBR12.006

9. Kulshreshtha S, Mathur N, Bhatnagar P (2014) Mushroom as a product and their role in mycoremediation. AMB Express 4:1–7. https://doi.org/10.1186/s13568-014-0029-8
10. Rathore H, Prasad S, Sharma S (2017) Mushroom nutraceuticals for improved nutrition and better human health: a review. Pharm Nutr 5:35–46. https://doi.org/10.1016/j.phanu.2017.02.001
11. Roupas P, Keogh J, Noakes M et al (2012) The role of edible mushrooms in health: evaluation of the evidence. J Funct Foods 4:687–709. https://doi.org/10.1016/j.jff.2012.05.003
12. Zhao R, He Y (2018) Network pharmacology analysis of the anti-cancer pharmacological mechanisms of *Ganoderma lucidum* extract with experimental support using Hepa1-6-bearing C57 BL/6 mice. J Ethnopharmacol 210:287–295. https://doi.org/10.1016/j.jep.2017.08.041
13. Yu Y, Qian L, Du NAN et al (2017) *Ganoderma lucidum* polysaccharide enhances radiosensitivity of hepatocellular carcinoma cell line HepG2 through Akt signaling pathway. Exp Ther Med 14:5903–5907. https://doi.org/10.3892/etm.2017.5340
14. Qu L, Li S, Zhuo Y et al (2017) Anticancer effect of triterpenes from *Ganoderma lucidum* in human prostate cancer cells. Oncol Lett 14:7467–7472. https://doi.org/10.3892/ol.2017.7153
15. Zhao X, Zhou D, Liu Y et al (2018) *Ganoderma lucidum* polysaccharide inhibits prostate cancer cell migration via the protein arginine methyltransferase 6 signaling pathway. Mol Med Rep 17:147–157. https://doi.org/10.3892/mmr.2017.7904
16. Hsu P, Lin Y, Yeh E et al (2017) Cordycepin and a preparation from *Cordyceps militaris* inhibit malignant transformation and proliferation by decreasing EGFR and IL-17RA signaling in a murine oral cancer model. Oncotarget 8:93712–93728
17. Chaicharoenaudomrung N, Jaroonwitchawan T, Noisa P (2018) Toxicology in vitro cordycepin induces apoptotic cell death of human brain cancer through the modulation of autophagy. Toxicol in Vitro 46:113–121. https://doi.org/10.1016/j.tiv.2017.10.002
18. Lee JS, Lee KR, Lee S et al (2017) Polysaccharides isolated from liquid culture broth of *Inonotus obliquus* inhibit the invasion of human non-small cell lung carcinoma. Cell 51:45–51. https://doi.org/10.1007/s12257-016-0458-0
19. Zhao F, Xia G, Chen L et al (2016) Chemical constituents from *Inonotus obliquus* and their antitumor activities. J Nat Med 70:721–730. https://doi.org/10.1007/s11418-016-1002-4
20. Zhao Y, Wang J, Wu Z et al (2016) Extraction, purification and anti-proliferative activities of polysaccharides from Lentinus edodes. Int J Biol Macromol 93:136–144. https://doi.org/10.1016/j.ijbiomac.2016.05.100
21. Zhang Y, Liu W, Xu C et al (2017) Characterization and antiproliferative effect of novel acid polysaccharides from the spent substrate of shiitake culinary-medicinal mushroom Lentinus edodes (Agaricomycetes) cultivation. Int J Med Mush 19:395–403. https://doi.org/10.1615/IntJMedMushrooms.v19.i5.20
22. Klimaszewska M, Górska S, Dawidowski M et al (2017) Selective cytotoxic activity of Se-Methyl-Seleno-L-Cysteine– and Se-Polysaccharide–containing extracts from shiitake medicinal mushroom, Lentinus edodes (Agaricomycetes). Int J Med Mush 19:709–716. https://doi.org/10.1615/IntJMedMushrooms.2017021250
23. Zhao F, Wang YF, Song L et al (2017) Synergistic apoptotic effect of D-fraction from *Grifola frondosa* and vitamin C on hepatocellular carcinoma SMMC-7721 cells. Integr Cancer Ther 16:205–214. https://doi.org/10.1177/1534735416644674
24. Alonso EN, Ferronato MJ, Gandini NA et al (2017) Antitumoral effects of D-fraction from *Grifola Frondosa* (Maitake) mushroom in breast cancer. Nutr Cancer 69:29–43. https://doi.org/10.1080/01635581.2017.1247891
25. Meng M, Cheng D, Han L et al (2017) Isolation, purification, structural analysis and immunostimulatory activity of water-soluble polysaccharides from *Grifola frondosa* fruiting body. Carbohydr Polym 157:1134–1143. https://doi.org/10.1016/j.carbpol.2016.10.082
26. Ko C-H, Yue GG-L, Gao S et al (2017) Evaluation of the combined use of metronomic zoledronic acid and *Coriolus versicolor* in intratibial breast cancer mouse model. J Ethnopharmacol 204:77–85. https://doi.org/10.1016/j.jep.2017.04.007
27. Awadasseid A, Hou J, Gamallat Y et al (2017) Purification, characterization, and antitumor activity of a novel glucan from the fruiting bodies of *Coriolus versicolor*. PLoS One 12:1–15. https://doi.org/10.1371/journal.pone.0171270

28. Zhang L, Li CG, Liang H, Reddy N (2017) Bioactive mushroom polysaccharides: immunoceuticals to anticancer agents. J Nutr Food Sci 2:6
29. Zhu F, Du B, Bian Z, Xu B (2015) β-glucans from edible and medicinal mushrooms: characteristics, physicochemical and biological activities. J Food Compos Anal 41:165–173. https://doi.org/10.1016/j.jfca.2015.01.019
30. Brown GD, Gordon S (2005) Immune recognition of fungal β-glucans. Cell Microbiol 7:471–479. https://doi.org/10.1111/j.1462-5822.2005.00505.x
31. Wasser SP (2002) Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol 60:258–274. https://doi.org/10.1007/s00253-002-1076-7
32. Friedman M (2016) Mushroom polysaccharides: chemistry and antiobesity, antidiabetes, anticancer, and antibiotic properties in cells, rodents, and humans. Foods 5:80. https://doi.org/10.3390/foods5040080
33. Chihara G, Maeda Y, Hamuro J et al (1969) Inhibition of mouse sarcoma 180 by polysaccharides from Lentinus edodes (Berk.) sing. Nature 222:687–688. https://doi.org/10.1038/222687a0
34. Finimundy TC, Dillon AJP, Henriques JAP, Ely MR (2014) A review on general nutritional compounds and pharmacological properties of the Lentinula edodes mushroom. Food Nutr Sci 5:1095–1105. https://doi.org/10.4236/fns.2014.512119
35. Zhang M, Cui SW, Cheung PCK, Wang Q (2007) Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. Trends Food Sci Technol 18:4–19. https://doi.org/10.1016/j.tifs.2006.07.013
36. Gregory F (1966) Studies on antitumor substances produced by. Basidiomycetes. Mycologia 58:80–90
37. Xu SL, Choi RCY, Zhu KY et al (2012) Isorhamnetin, a flavonol aglycone from *Ginkgo biloba* L., induces neuronal differentiation of cultured PC12 cells: potentiating the effect of nerve growth factor. Evid Based Complement Alternat Med 2012:278273. https://doi.org/10.1155/2012/278273
38. Zhang M, Zhang L, Cheung PC et al (2003) Fractionation and characterization of a polysaccharide from the sclerotia of *Pleurotus tuber-regium* by preparative size-exclusion chromatography. J Biochem Biophys Methods 30:281-9.
39. Xiao J-H, Xiao D-M, Chen D-X et al (2012) Polysaccharides from the medicinal mushroom Cordyceps taii show antioxidant and immunoenhancing activities in a D-galactose-induced aging mouse model. Evid Based Complement Alternat Med 2012:273435. https://doi.org/10.1155/2012/273435
40. Ren L, Perera C, Hemar Y (2012) Antitumor activity of mushroom polysaccharides: a review. Food Funct 3:1118. https://doi.org/10.1039/c2fo10279j
41. Ahn H, Jeon E, Kim JC et al (2017) Lentinan from shiitake selectively attenuates AIM2 and non-canonical inflammasome activation while inducing pro-inflammatory cytokine production. Sci Rep 7:1–12. https://doi.org/10.1038/s41598-017-01462-4
42. Zhang Q, Hu M, Xu L et al (2017) Effect of edible fungal polysaccharides on improving influenza vaccine protection in mice. Food Agric Immunol 28:981–992. https://doi.org/10.1080/09540105.2017.1323326
43. Shinbo T, Fushida S, Tsukada T et al (2015) Protein-bound polysaccharide K suppresses tumor fibrosis in gastric cancer by inhibiting the TGF-β signaling pathway. Oncol Rep 33:553–558. https://doi.org/10.3892/or.2014.3636
44. Sun C, Rosendahl a H, Wang XD et al (2012) Polysaccharide-K (PSK) in cancer – old story, new possibilities? Curr Med Chem 19:757–762
45. Ma Y, Wu X, Yu J et al (2017) Can polysaccharide K improve therapeutic efficacy and safety in gastrointestinal cancer? A systematic review and network meta- analysis. Oncotarget 8:89108–89118
46. Fritz H, Kennedy DA, Ishii M et al (2015) Polysaccharide K and *Coriolus versicolor* extracts for lung cancer: a systematic review. Integr Cancer Ther 14:201–211. https://doi.org/10.1177/1534735415572883

47. Feng ZL, Fang TJ, Qian YX, Rong WH (2014) The clinical research for Ganoderan's effect on preventing and treating cerebral arteriosclerosis through inhibiting NADPH oxidizing enzyme expression. Pak J Pharm Sci 27:1107–1111
48. Zhong W-D, He H-C, Ou R-B et al (2008) Protective effect of ganoderan on renal damage in rats with chronic glomerulonephritis. Clin Invest Med 31:E212–E217
49. Jesenak M, Urbancek S, Majtan J et al (2016) β -Glucan-based cream (containing pleuran isolated from pleurotus ostreatus) in supportive treatment of mild-to-moderate atopic dermatitis. J Dermatol Treat 27:351–354. https://doi.org/10.3109/09546634.2015.1117565
50. Kanagasabapathy G, Malek SNA, Kuppusamy UR, Vikineswary S (2011) Chemical composition and antioxidant properties of extracts of fresh fruiting bodies of Pleurotus sajor-caju (Fr.) singer. J Agric Food Chem 59:2618–2626. https://doi.org/10.1021/jf104133g
51. Kanagasabapathy G, Chua KH, Malek SNA et al (2014) AMP-activated protein kinase mediates insulin-like and lipo-mobilising effects of β-glucan-rich polysaccharides isolated from Pleurotus sajor-caju (Fr.), singer mushroom, in 3T3-L1 cells. Food Chem 145:198–204. https://doi.org/10.1016/j.foodchem.2013.08.051
52. Zhang Y, Kong H, Fang Y et al (2013) Schizophyllan: a review on its structure, properties, bioactivities and recent developments. Bioact Carbohydr Diet Fibre 1:53–71. https://doi.org/10.1016/j.bcdf.2013.01.002
53. Leathers TD, Nunnally MS, Stanley AM, Rich JO (2016) Utilization of corn fiber for production of schizophyllan. Biomass Bioenergy 95:132–136. https://doi.org/10.1016/j.biombioe.2016.10.001
54. Asgher M, Wahab A, Bilal M, Nasir Iqbal HM (2016) Lignocellulose degradation and production of lignin modifying enzymes by *Schizophyllum commune* IBL-06 in solid-state fermentation. Biocatal Agric Biotechnol 6:195–201. https://doi.org/10.1016/j.bcab.2016.04.003
55. Singdevsachan SK, Auroshree P, Mishra J et al (2016) Mushroom polysaccharides as potential prebiotics with their antitumor and immunomodulating properties: a review. Bioact Carbohydr Diet Fibre 7:1–14. https://doi.org/10.1016/j.bcdf.2015.11.001
56. Jamshidian H, Shojaosadati SA, Mohammad Mousavi S et al (2017) Implications of recovery procedures on structural and rheological properties of schizophyllan produced from date syrup. Int J Biol Macromol 105:36–44. https://doi.org/10.1016/j.ijbiomac.2017.06.110
57. Sutivisedsak N, Leathers TD, Biresaw G et al (2016) Simplified process for preparation of schizophyllan solutions for biomaterial applications. Prep Biochem Biotechnol 46:313–319. https://doi.org/10.1080/10826068.2015.1031392
58. Ng SH, Mohd Zain MS, Zakaria F et al (2015) Hypoglycemic and antidiabetic effect of Pleurotus sajor-caju aqueous extract in normal and streptozotocin-induced diabetic rats. Biomed Res Int 2015:1–8. https://doi.org/10.1155/2015/214918
59. Mao CF, Hsu MC, Hwang WH (2007) Physicochemical characterization of grifolan: thixotropic properties and complex formation with Congo red. Carbohydr Polym 68:502–510. https://doi.org/10.1016/j.carbpol.2006.11.003
60. Suzuki I, Takeyama T, Ohno N et al (1987) Antitumor effect of polysaccharide grifolan NMF-5N on syngeneic tumor in mice. J Pharmacobiodyn 10:72–77
61. El Enshasy HA, Hatti-Kaul R (2013) Mushroom immunomodulators: unique molecules with unlimited applications. Trends Biotechnol 31:668–677. https://doi.org/10.1016/j.tibtech.2013.09.003
62. Jayachandran M, Xiao J, Xu B (2017) A critical review on health promoting benefits of edible mushrooms through gut microbiota. Int J Mol Sci 18:1934. https://doi.org/10.3390/ijms18091934
63. Zhou Y, Yang X, Yang Q (2006) Recent advances on triterpenes from ganoderma mushroom. Food Rev Int 22:259–273. https://doi.org/10.1080/87559120600694739
64. Sliva D (2003) *Ganoderma lucidum* (Reishi) in cancer treatment. Integr Cancer Ther 2:358–364. https://doi.org/10.1177/1534735403259066
65. Min BS, Nakamura N, Miyashiro H et al (1998) Triterpenes from the spores of *Ganoderma lucidum* and their inhibitory activity against HIV-1 protease. Chem Pharm Bull (Tokyo) 46:1607–1612

66. Min BS, Gao JJ, Nakamura N, Hattori M (2000) Triterpenes from the spores of *Ganoderma lucidum* and their cytotoxicity against meth-A and LLC tumor cells. Chem Pharm Bull (Tokyo) 48:1026–1033
67. Cheng P-G, Phan C-W, Sabaratnam V et al (2013) Polysaccharides-rich extract of *Ganoderma lucidum* (M.A. Curtis:Fr.) P. Karst accelerates wound healing in streptozotocin-induced diabetic rats. Evid Based Complement Alternat Med 2013:1–9. https://doi.org/10.1155/2013/671252
68. Zapata P, Rojas D, Atehortúa L (2012) Production of biomass, polysaccharides, and ganoderic acid using non-conventional carbon sources under submerged culture of the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt.:Fr.) P. Karst. (Higher Basidiomycetes). Int J Med Mush 14:197–203
69. el-Mekkawy S, Meselhy MR, Nakamura N et al (1998) Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum*. Phytochemistry 49:1651–1657
70. You B-J, Tien N, Lee M-H et al (2017) Induction of apoptosis and ganoderic acid biosynthesis by cAMP signaling in *Ganoderma lucidum*. Sci Rep 7:318. https://doi.org/10.1038/s41598-017-00281-x
71. Zhang X, Ip F, Zhang D et al (2011) Triterpenoids with neurotrophic activity from *Ganoderma lucidum*. Nat Prod Res 25:1607–1613
72. Chi B, Wang S, Bi S et al (2017) Effects of ganoderic acid A on lipopolysaccharide-induced proinflammatory cytokine release from primary mouse microglia cultures. Exp Ther Med 15:847–853. https://doi.org/10.3892/etm.2017.5472
73. Su HJ, Fann YF, Chung MI et al (2000) New lanostanoids of *Ganoderma tsugae*. J Nat Prod 63:514–516. https://doi.org/10.1021/np990367l
74. González AG, León F, Rivera A et al (2002) New lanostanoids from the fungus *Ganoderma concinna*. J Nat Prod 65:417–421. https://doi.org/10.1021/np010143e
75. Mothana RAA, Awadh Ali NA, Jansen R et al (2003) Antiviral lanostanoid triterpenes from the fungus *Ganoderma pfeifferi*. Fitoterapia 74:177–180. https://doi.org/10.1016/S0367-326X(02)00305-2
76. El Dine RS, El Halawany AM, Ma CM, Hattori M (2008) Anti-HIV-1 protease activity of lanostane triterpenes from the Vietnamese mushroom *Ganoderma colossum*. J Nat Prod 71:1022–1026. https://doi.org/10.1021/np8001139
77. Baby S, Johnson AJ, Govindan B (2015) Secondary metabolites from Ganoderma. Phytochemistry 114:66–101. https://doi.org/10.1016/j.phytochem.2015.03.010
78. Weng C-J, Fang P-S, Chen D-H et al (2010) Anti-invasive effect of a rare mushroom, *Ganoderma colossum*, on human hepatoma cells. J Agric Food Chem 58:7657–7663. https://doi.org/10.1021/jf101464h
79. Chen X-Q, Chen L-X, Zhao J et al (2017) Nortriterpenoids from the fruiting bodies of the mushroom *Ganoderma resinaceum*. Molecules 22:1073. https://doi.org/10.3390/molecules22071073
80. Tohtahon Z, Xue J, Han J et al (2017) Cytotoxic lanostane triterpenoids from the fruiting bodies of *Piptoporus betulinus*. Phytochemistry 143:98–103. https://doi.org/10.1016/J.PHYTOCHEM.2017.07.013
81. Pleszczyńska M, Lemieszek MK, Siwulski M et al (2017) Fomitopsis betulina (formerly *Piptoporus betulinus*): the Iceman's polypore fungus with modern biotechnological potential. World J Microbiol Biotechnol 33:1–12. https://doi.org/10.1007/s11274-017-2247-0
82. Peintner U, Pöder R, Pümpel T (1998) The iceman's fungi. Mycol Res 102:1153–1162. https://doi.org/10.1017/S0953756298006546
83. Vunduk J, Klaus A, Kozarski M et al (2015) Did the iceman know better? Screening of the medicinal properties of the Birch Polypore medicinal mushroom, *Piptoporus betulinus* (Higher Basidiomycetes). Int J Med Mush 17:1113–1125. https://doi.org/10.1615/IntJMedMushrooms.v17.i12.10

84. Pleszczyńska M, Wiater A, Siwulski M et al (2016) Cultivation and utility of *Piptoporus betulinus* fruiting bodies as a source of anticancer agents. World J Microbiol Biotechnol 32:151. https://doi.org/10.1007/s11274-016-2114-4
85. Phosri C, Watling R, Suwannasai N et al (2014) A new representative of star-shaped fungi: *Astraeus sirindhorniae* sp. nov. from Thailand. PLoS One 9:e71160. https://doi.org/10.1371/journal.pone.0071160
86. Isaka M, Palasarn S, Srikitikulchai P et al (2016) Astraeusins A – L, lanostane triterpenoids from the edible mushroom *Astraeus odoratus*. Tetrahedron 72:1–2. https://doi.org/10.1016/j.tet.2016.04.057
87. Srisurichan S, Piapukiew J, Puthong S, Pornpakakul S (2017) Lanostane triterpenoids, spiro-astraodoric acid, and astraodoric acids E and F, from the edible mushroom *Astraeus odoratus*. Phytochem Lett 21:78–83. https://doi.org/10.1016/j.phytol.2017.05.020
88. Gargano ML, van Griensven LJLD, Isikhuemhen OS et al (2017) Medicinal mushrooms: valuable biological resources of high exploitation potential. Plant Biosyst 151:548–565. https://doi.org/10.1080/11263504.2017.1301590
89. Homer JA, Sperry J (2017) Mushroom-derived indole alkaloids. J Nat Prod 80:2178–2187. https://doi.org/10.1021/acs.jnatprod.7b00390
90. Tsujikawa K, Kanamori T, Iwata Y et al (2003) Morphological and chemical analysis of magic mushrooms in Japan. Forensic Sci Int 138:85–90. https://doi.org/10.1016/j.forsciint.2003.08.009
91. Kovacic P, Somanathan R, Abadjian M-C (2015) Natural monophenols as therapeutics, antioxidants and toxins; Electron transfer, radicals and oxidative stress. Nat Prod J 5:142–151. https://doi.org/10.2174/2210315505031510161538 37
92. Wurst M, Kysilka R, Flieger M (2002) Psychoactive tryptamines from basidiomycetes. Folia Microbiol (Praha) 47:3–27
93. Holloway T, González-Maeso J (2015) Epigenetic mechanisms of serotonin signaling. ACS Chem Neurosci 6:1099–1109. https://doi.org/10.1021/acschemneuro.5b00033
94. Lee H-M, Roth BL (2012) Hallucinogen actions on human brain revealed. Proc Natl Acad Sci 109:1820–1821. https://doi.org/10.1073/pnas.1121358109
95. Lenz C, Wick J, Hoffmeister D (2017) Identification of ω- *N*-Methyl-4-hydroxytryptamine (norpsilocin) as a *Psilocybe* natural product. J Nat Prod 80:2835–2838. https://doi.org/10.1021/acs.jnatprod.7b00407
96. Carhart-Harris RL, Bolstridge M, Rucker J et al (2016) Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. Lancet Psychiat 3:619–627. https://doi.org/10.1016/S2215-0366(16)30065-7
97. Carhart-Harris RL, Roseman L, Bolstridge M et al (2017) Psilocybin for treatment-resistant depression: FMRI-measured brain mechanisms. Sci Rep 7:1–11. https://doi.org/10.1038/s41598-017-13282-7
98. Wittstein K, Rascher M, Rupcic Z et al (2016) Corallocins A–C, nerve growth and brain-derived neurotrophic factor inducing metabolites from the mushroom *Hericium coralloides*. J Nat Prod 79:2264–2269. https://doi.org/10.1021/acs.jnatprod.6b00371
99. Geissler T, Brandt W, Porzel A et al (2010) Acetylcholinesterase inhibitors from the toadstool *Cortinarius infractus*. Bioorg Med Chem 18:2173–2177. https://doi.org/10.1016/j.bmc.2010.01.074
100. Brondz I, Ekeberg D, Høiland K et al (2007) The real nature of the indole alkaloids in *Cortinarius infractus*: evaluation of artifact formation through solvent extraction method development. J Chromatogr A 1148:1–7. https://doi.org/10.1016/j.chroma.2007.02.074
101. Steglich W, Kopanski L, Wolf M et al (1984) Indolalkaloide aus dem blätterpilz (agaricales). Tetrahedron Lett 25:2341–2344. https://doi.org/10.1016/S0040-4039(01)80250-1
102. Bröckelmann MG, Dasenbrock J, Steffan B et al (2004) An unusual series of thiomethylated canthin-6-ones from the North American mushroom *Boletus curtisii*. Eur J Org Chem 2004:4856–4863. https://doi.org/10.1002/ejoc.200400519

103. Vieira Torquato HF, Ribeiro-Filho AC, Buri MV et al (2017) Canthin-6-one induces cell death, cell cycle arrest and differentiation in human myeloid leukemia cells. Biochim Biophys Acta, Gen Subj 1861:958–967. https://doi.org/10.1016/j.bbagen.2017.01.033
104. Barros-Filho BA, de Oliveira MCF, Mafezoli J et al (2012) Secondary metabolite production by the basidiomycete, *Lentinus strigellus*, under different culture conditions. Nat Prod Commun 7:771–773
105. Barros-Filho BA, de Oliveira M d CF, Lemos TLG et al (2009) *Lentinus strigellus*: a new versatile stereoselective biocatalyst for the bioreduction of prochiral ketones. Tetrahedron Asymmetry 20:1057–1061. https://doi.org/10.1016/j.tetasy.2009.02.008

Bioactive Molecules of *Spirulina*: A Food Supplement

54

Meeta Mathur

Contents

M. Mathur (✉)
Department of Botany, Mithibai College, University of Mumbai, Mumbai, India
e-mail: meet.mat2000@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_97

Abstract

Spirulina is a nature's gift as super food to mankind. It is a photosynthetic filamentous microalga which has emerged as a potent food supplement because of its rich micro- and macronutrient contents. The body of *Spirulina* is smooth and with weak cell wall that makes it easily digestible. It is a valuable source of proteins, vitamins, minerals, β-carotene, fatty acids, etc. which makes it perfect as food and fodder. NASA has stated that the nutritional value of 1000 kg of fruits and vegetables equals to 1 kg of *Spirulina.* In 1992 WHO has declared *Spirulina* as "Best food for future" to redress malnutrition especially in children. Apart from being a food supplement, *Spirulina* has gained considerable popularity and paramount importance due to the presence of certain pigments and secondary metabolites. It shows pharmacognosic properties like immuno-protective, anti-cancer, antidiabetic, antiviral, anti-obesity, etc. it is the most nutritionally concentrated compact whole food known which owe a potential to drastically lower the chances of developing cancer, heart disease, or stroke or of contracting a life-threatening virus such as HIV and prevent eyes from cataract formation. Many animal studies in vivo and in vitro and human trials have proved *Spirulina* to be commercialized and sold for therapeutic purposes. It appears to have a considerable potential for developing a key crop in coastal and alkaline regions where traditional agriculture struggles. Thus, looking at its global nutritional significance, more should be done in culture isolation, purification, and quality control of *Spirulina* and its products.

Keywords

Spirulina platensis · IIMSAM · C-phycocyanin · β-carotene · Nutraceutical · Hepatoprotective · Anticancer · Mass cultivation · Biofertilizer · Nephrotoxicity

Abbreviations

AIDS	acquired immunodeficiency syndrome
C-PC	C-phycocyanin
EFA	essential fatty acid
GLA	gamma linolenic acid
HIV	human immunodeficiency virus
IIMSAM	intergovernmental institution for the use of micro-algae *Spirulina* against malnutrition
NASA	National Aeronautics and Space Administration

NPU	net protein utilization
PER	protein efficiency ratio
PUFA	polyunsaturated fatty acids
WHO	World Health Organization

1 Introduction to *Spirulina*

1.1 Historical Background and Rediscovery as Food and Animal Feed

In sixteenth century a German algae scientist Dr. Darwin discovered the existence of spiral-shaped algae and called it *Spirulina.* Later Dr. Christopher Hills rediscovered these spiral blue-green algae in Lake Chad, Africa, and popularized it as a food supplement. He was called "The father of Spirulina." According to him, *Spirulina* contains billions of years of successful evolutionary wisdom coded in its DNA [1].

In Africa, *Spirulina* has served as sole of nutrition in certain communities in the times of famine, and the entire population has existed eating only *Spirulina* for over a month [2]. The National Aeronautics and Space Administration (NASA) used *Spirulina* predominantly as a food supplement for astronauts on space missions. NASA has stated that the nutritional value of 1000 kg of fruits and vegetables equals to 1 kg of *Spirulina* [3]. The United Nations World Health Organization (WHO) has accepted *Spirulina* as an interesting food for multiple reasons and can be administered to even children without any risk. WHO also confirmed it is effective in cancer prevention, hay fever, herpes, HIV, high cholesterol, liver protection, weight loss, etc.

A worldwide *Spirulina* campaign "Intergovernmental Institution for the Use of Micro-Algae *Spirulina* Against Malnutrition" (IIMSAM) was started in the United Nations by Sarah Obama for improvement of public health. IIMSAM works to promote the use of *Spirulina* as a humanitarian instrument in fighting against severe malnutrition worldwide. IIMSAM also maintains communication channels with entities around the world. *Spirulina* is also said to be highly nutritious potential feed resource for agriculturally important animal species, by improving growth, fertility, and nutritional product quality, and it is emerging as a cost-effective means of improving animal productivity for a sustainable and viable food security future [4].

1.2 Taxonomic Position and Characters

Spirulina is a 300 million years old Cyanophycean spiral-shaped filamentous microalgae (Oscillatoriaceae family) found naturally in alkaline, mineral-rich, pollution free waters with high pH. It thrives well in alkaline lakes where it is difficult or impossible for other microorganisms to survive [5]. Its name comes from a Latin word meaning tiny spiral.

It is microscopic but may attain a size of 0.5 mm in length, which makes some individuals visible to naked eyes. The helical shape of the filaments is characteristic of the genus and is maintained always. This helical shape of the filament and presence of gas-filled vacuoles in the cells result in floating mats. *Spirulina* has a prokaryotic organization, pluri-stratified cell wall, photosynthetic lamellar system, ribosomes and fibrils of DNA region, and numerous inclusions (Fig. 1).

Its main photosynthetic pigment is C-phycocyanin (C-PC). It is also rich in chlorophyll, carotenoids, and phycocyanin. *Spirulina* is an obligate photoautotroph, i.E., it cannot grow in the dark. It reduces carbon dioxide in the light and assimilates mainly nitrates. The main assimilation product is glycogen. It grows well in temperature ranging from 37 °C to 40 °C. Also the resistance of *Spirulina* toward ultraviolet radiations is high than any other algae [6]. The body surface of *Spirulina* is smooth and without covering so it is easily digestible by simple enzymatic systems. There are several species of *Spirulina*, but the most widely used species as food supplement are *S. platensis* and *S. maxima* [7] (Fig. 2).

2 Bioactive Molecules of *Spirulina*

By the past many decades, *Spirulina* has been of enormous interest by nutritional researchers because of its high micro- and macronutrient contents. Its concentrated nutrition makes it ideal for people of all ages and lifestyles. *Spirulina* is an incredible natural source of nutrients which has been used since ancient times. Its nutritional composition has been monitored and analyzed since 1970.

Fig. 1 Floating mats of *Spirulina*

Fig. 2 *Spirulina platensis**

It has been an excellent source of proteins, vitamins, fatty acids, minerals, photosynthetic pigments, and many secondary metabolites [8]. Its composition is discussed below (Fig. 3).

2.1 Proteins

Proteins are building blocks of life; they are essential for living a healthy life. The body uses proteins to build and repair muscles and other tissues. Proteins are broken down to amino acids which are necessary components in enzymes and hormones. Proteins are crucial component of every single cell in human body and that is why are vital to be included in one's diet.

Spirulina contains usually high amount of protein between 55 and 70% by dry weight [9]. *Spirulina* consists of essential amino acids mainly leucine, valine, isoleucine, tryptophan, methionine, phenylalanine, theanine, and lysine [10]. Aspartate and glutamate are the two nonessential amino acids present in *Spirulina* (Table 1). Unlike other plant-derived proteins, *Spirulina* is a perfect protein as it contains all the essential amino acids. Its status as a plant-based source of complete protein makes it an ideal dietary supplement choice for vegetarians.

Spirulina cells do not have cellulose walls but relatively fragile envelope of murein which is one of its kinds in plant kingdom. This explains the very high digestibility of its proteins [11]. The net protein utilization (NPU) is calculated by knowing the percentage of nitrogen retained when source of proteins under studying the only limiting nutritional factor. The NPU value of *Spirulina* is estimated between 55 and 92% more in comparison to casein [12]. While, the weight gained by the

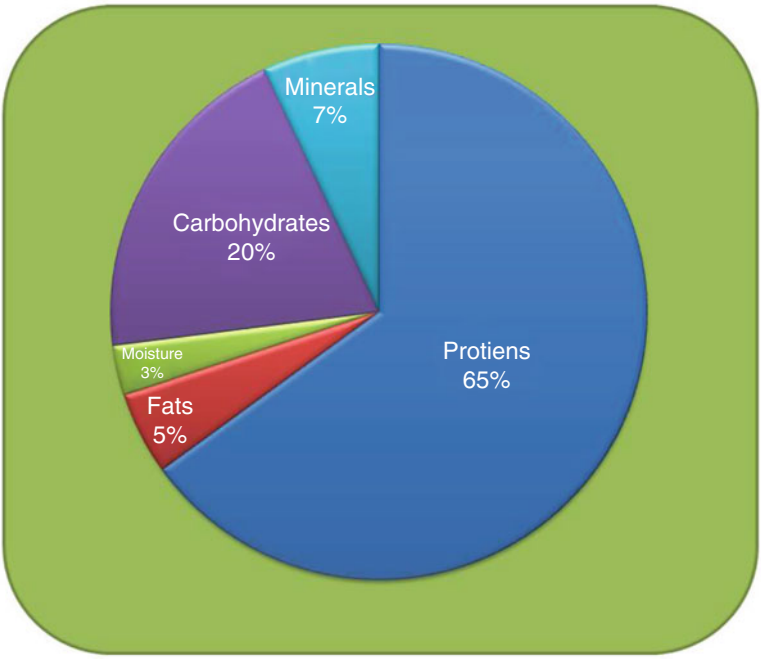


Fig. 3 Composition of biomolecules in *Spirulina*

Table 1 Protein composition of *Spirulina*

S.No	Amino acids	Concentration ($g100g^{-1}$)
1	Leucine	4.94
2	Isoleucine	3.20
3	Valine	3.51
4	Tryptophan	0.93
5	Theanine	2.97
6	Lysine	3.02
7	Methionine	1.15
8	Phenylalanine	2.78
9	Total	22.5

individual divided by the weight of protein ingested is called protein efficiency ratio (PER). The PER values for *Spirulina* was found out to be double than casein [13].

2.2 Vitamins and Minerals

Vitamins are organic compounds having diverse biochemical functions. These are essential nutrients that an organism needs in smaller amounts as it cannot make them by itself.

Table 2 Vitamin composition of *Spirulina*

S.No.	Vitamins	Concentration ($mg100g^{-1}$)
1	Vitamin B_1	3.5
2	Vitamin B_{12}	0.32
3	Vitamin K	2.2
4	Carotene	140
5	Riboflavin	4
6	Niacin	14
7	Folic acid	0.01
8	Biotin	0.005
9	Vitamin E	100
10	Total	264.035

Spirulina contains vitamin B_1 (thiamine), B_2 (riboflavin), B_3 (nicotinamide), B_6 (pyridoxine), B_9 (folic acid), B_{12} (cyanocobalamin), vitamin C, vitamin D and vitamin E (tocopherol), and most important provitamin a (β-carotene) (Table 2). *Spirulina* is the only vegetable source of vitamin B_{12} having two and half times more than meat. It contains the highest amount of beta-carotene much more than carrots, which is a precursor of vitamin a and is not dose dependent. It is also a very good source of vitamin E, similar to wheat germ.

Minerals are inorganic essential nutrients that the body needs to carry out various functions and processes for healthy living. *Spirulina* is an adequate source of many minerals like iron, zinc, sodium, potassium, phosphorous, manganese, magnesium, copper, and calcium (Table 3). Very high iron content makes *Spirulin*a economically important. The calcium, phosphorous, and magnesium occur in quantities comparable to those found in milk [14].

2.3 Carbohydrates and Essential Fatty Acids

Around 15–20% of dry weight of *Spirulina* is carbohydrate. It contains glucose, fructose, sucrose, glycerol, mannitol, and sorbitol. Mesoinositol is a carbohydrate which is an excellent source of organic phosphorous. *Spirulina* is eight times richer in mesoinositol than beef and very much more than vegetables. Recent studies indicate calcium spirulan (Ca-SP) is a novel sulfated polysaccharide found in *Spirulina* [14] (Table 4).

Seven percent of the total volume of *Spirulina* is lipids. It has a high amount of polyunsaturated fatty acids (PUFA). Vitamin-like substances which cannot be synthesized in the human body also called essential fatty acids (EFA) are essential for healthy living; they include linoleic, linolenic, and arachidonic acids. EFA help to reduce total cholesterol and triglyceride levels (associated with arteriosclerosis and heart disease). Their cell membranes are largely made up of lipids. When present with vitamins E and A, they protect the cell membranes against antioxidant and free radical attacks. Such attacks can alter the absorption of nutrients through the cell

Table 3 Minerals composition of *Spirulina*

S.No.	Minerals	Concentration ($mg100g^{-1}$)
1	Iron	100
2	Copper	1.2
3	Calcium	700
4	Zinc	3
5	Sodium	900
6	Potassium	1400
7	Phosphorous	800
8	Manganese	5
9	Magnesium	400
10	Total	4309.2

Table 4 Carbohydrate composition of *Spirulina*

S.No.	Carbohydrate	Concentration ($mg100g^{-1}$)
1	Glucose	54.4
2	Rhamnose	22.3
3	Mannose	9.3
4	Xylose	7
5	Galactose	3
6	Total	96

membranes. Since membrane damage may alter antigens, this could cause the immune system to fail. *Spirulina* with a special fatty acid called gamma linolenic acid (GLA) has reportedly shown to be an effective immunoprotector. *Spirulina* is also nature's highest available source of GLA. It also contains stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA) (Table 5).

2.4 Photosynthetic Pigments or Phytonutrients

Spirulina is of special interest because of its color and presence of variety of pigments with specific functional properties. It contains mainly chlorophyll a, xanthophylls, beta (β)-carotene (both isomers), echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, beta cryptoxanthin, oscillaxanthin, phycobiliproteins, 3-hydroxyechinenone, c-phycocyanin (C-PC), and allophycocyanin [15] (Table 6).

It has predominance of phycocyanins and beta-carotenes which makes it an ideal nutraceutical worldwide. Different strains of *Spirulina* depicted variable influence on pigments with changed environmental parameters. Growth and pigment production was recorded to be most efficient under optimized conditions of light intensity (70 μmol m^{-2} s^{-1}), temperature (30 °C), CO_2 concentration (550 ppm and 750 ppm), pH (10.5), and NaCl level (2 g L^{-1}) [16].

Table 5 Lipid composition of *Spirulina*

S.No.	Lipids	Concentration ($g100g^{-1}$)
1	GLA	1
2	Palmitic	2
3	Arachidic	0.05
4	Oleic	0.017
5	Myristic	0.04
6	Total	3.1

Table 6 Phytonutrient composition of *Spirulina*

S.No.	Phytonutrients	Concentration ($g100g^{-1}$)
1	*Cis* β-carotene	0.07
2	*Trans* β-carotene	0.3
3	Chlorophyll a	1
4	C-phycocyanin (C-PC)	12
5	Total	13.37

3 *Spirulina* a Therapeutic and Nutraceutical Agent

Spirulina has emerged as a wonder drug because of its various medicinal properties for improvement of health in humans [17]. It boosts immunity and increases resistance power of individual. *Spirulina* is gaining more attention from medical science as "nutraceutical" and as source of potential medicine. Experimental data have suggested the following medicinal uses:

3.1 Used Against Cancer

Recent research findings show that the antioxidant and immune modulation characteristics of *Spirulina* have a possible mechanism of tumor destruction and hence play a role in cancer prevention especially stomach cancer [18]. It also contains pigment C-phycocyanin (C-PC) which inhibits liver cancer cells through an apoptotic mechanism [19]. According to a finding, combination of *Spirulina* and selenium inhibited breast cancer by growth arrest and apoptosis [20]. *Spirulina* is also chemoprotective and induced lesion regression in tobacco chewers with oral leukoplakia [21].

3.2 Used Against Diabetes

The presence of gamma linolenic acid (GLA), high fiber content, and peptides in *Spirulina* makes it a potent hypoglycemic agent; as a result the blood glucose levels are lowered [22]. Clinical studies on thousands of diabetes patients around the world

have shown a number of benefits and powerful effects to improve their condition. C-phycocyanin(C-PC) in *Spirulina* stimulates the body's insulin function; it also improves insulin resistance of patients and manages their lipid metabolism. These effects of *Spirulina* supplement in type 2 diabetes showed improvement in fasting blood sugar and lipid profiles [23].

3.3 Used Against AIDS and as Antiviral Drug

Calcium spirulan (Ca-SP) isolated from *Spirulina* has shown antiviral activities against different viruses such as (human immunodeficiency virus) HIV-I, herpes simplex virus type-I, and influenza virus. The antiviral activity is suggested to be due to the effect of chelation of calcium ion to sulfate groups [24]. A recent study on the antiviral activity of *Spirulina* has resulted in the isolation of cyanovirin-N (CV-N), a novel cynobacterial carbohydrate-binding protein that inhibits HIV-I and other enveloped viral particles [25].

3.4 Used Against Allergic Rhinitis and Asthma

The pigment present in *Spirulina* called C-phycocyanin (C-PC) can selectively inhibit release of histamine from mast cells and prevent increase in immunoglobulin E (IgE). IgE stimulated the immune system and causes the airways to become narrow and makes asthma symptoms worse. Thus reduction in IgE causes considerable improvement in allergic rhinitis and asthma. 1gm/day dose of *Spirulina* produced improvement in lung parameters [26].

3.5 Used Against Hypertension and Hyperlipidemia

Spirulina decreases lipoperoxidation products and show hepatoprotective activity. C-phycocyanin pigment present in *Spirulina* exhibits hypocholesterolemic action [27]. It is also suggested that the gamma linolenic acid (GMA) content also helps in the mechanism of action [28]. The high potassium and low sodium contents of *Spirulina also* have positive effects on blood pressure. It is thus shown that *Spirulina* reduced systolic and diastolic blood pressure when given by oral route [29]. In a study, volunteers given 4.5 g/day of *Spirulina* for 6 weeks lowered blood pressure and total cholesterol LDL and increased HDL [30].

3.6 Used Against Heart Strokes

The gamma linolenic acid (GLA) found in *Spirulina* works with the heart to improve a robust healthy system which helps to prevent heart diseases, cardiac arrest, and stroke. Another compound beta-carotene present in it also guards the cardiac system. According to a study, the degree of strokes and heart attack reduced to 50% in heart patients given

Spirulina supplement for more than 4 weeks. *Spirulina* is therefore beneficial in preventing atherosclerosis and reduced risk for cardiovascular diseases [31].

3.7 Used Against Anemia

Spirulina contains the pigment phycocyanin which is shown to stimulate the bone marrow to produce blood cells more effectively. It also modulates the production of cytokines by human blood mononuclear cells and increases flavonoids and sulfolipids [32]. *Spirulina* thus enhances red blood cell's production and function. Its intake has shown a steady increase in average values of mean corpuscular hemoglobin. Older women were benefitted more rapidly from *Spirulina* supplements [33]. Level of anemia was also decreased in children given *Spirulina* supplements for 12 weeks [34].

3.8 Used Against Eye Diseases

Spirulina contains ten times more beta-carotene than carrots. Beta-carotene gets converted to vitamin A which helps to protect the cornea, which is the outer surface of the eye. This is essential for having good vision. If it is not protected, then it may cause blurry vision, eye pain, eye redness, etc. it also protects the retina from clump deposits. Thus *Spirulina* provides the daily dietary dose of beta-carotenes which prevent blindness and eye diseases [35].

3.9 Used as Immunity Booster

Spirulina helps in building immunity and improving resistance to infections. Several experiments have shown that it has a favorable regulatory effect on the immune system [36]. It enhances the components of the mucosal and systemic immune system as it activates the cells of innate immune system. It also activates macrophages T and B cells [37]. *Spirulina* use leads to higher level of natural killer cells, interferon gamma, and more potent production of interleukins – The cytokines of low molecular weight that are produced by lymphocytes and macrophages and that function especially in regulation of the immune system [38].

3.10 Used as Antioxidant

Antioxidants are substances which neutralize the unstable free radicals generated due to oxidative stress. This beneficial antioxidant property of *Spirulina* is because of the presence of tocopherols, phenolic acids, and beta-carotene. This causes prevention of oxidative stress and inflammation and their associative damages [39].

Geriatric patients administered *Spirulina* for 16 weeks showed a remarkable improvement in antioxidant potential, as measured by increased levels of antioxidants in plasma of the individuals [40].

3.11 Used as Radioprotective Agent

The incredible radioprotective effect of *Spirulina* is due to its ability to bind to heavy metals and radioisotopes. Numerous studies have found that it protects the body against and even heals it from damaging harmful radiations. It was confirmed be a research in the early nineties that *Spirulina* effectively decreases the radioactive load received by the body when consuming radiation-contaminated food. After just 20 days, children fed 5gm doses of *Spirulina* every day for 2 weeks showed an average 50% reduction in urine radioactivity levels. *Spirulina* works so well at attenuating the damage caused by radiation that it was actually awarded a Russian patent in 1995 for improving the immunity of children affected by radiation from the Chernobyl disaster. Many exposed children became stricken with chronic radiation sickness and elevated immunoglobulin E (IgE) levels, and they also tested positive for high allergy sensitivity. On consuming *Spirulina* for 45 days, the children's IgE levels and allergic sensitivities were restored back to normal [41]. Later in 2001 a study showed *Spirulina* extracts effectively protect against both the damage caused by chemotherapy drugs and gamma radiation exposure. It was thus also prescribed to cancer patient undergoing chemotherapy [42]. S*pirulina* polysaccharides are believed to have a stimulating effect on DNA repair mechanisms which might explain the radioprotective effect mentioned several times in relation to *Spirulina*. Conclusively, *Spirulina* offers remarkable radioprotective benefits and offers a surefire way to mitigate the damaging effects of harmful radiation in addition to its many other health-promoting benefits.

3.12 Used as Trace Metal Supplement

Spirulina also contains selenium which is one of the important trace metals involved in immune function, reproduction, cardiovascular disease, cancer, viral infection control, and metal toxicity. Another essential trace element is iodine, whose deficiency affects thyroid function, cardiovascular function, and other brain disorders [43]. These trace elements help in preserving bone health since they reduce decalcification risk. *Spirulina* is an oxalate-free plant food; thus as with iron it provides calcium with high availability; thus it improves its absorption [44].

4 *Spirulina* a Food Supplement

4.1 *Spirulina* Value as Super Food and Feed

Spirulina is a miracle food that nourishes our body providing most of the proteins needed for healthy living; it helps to develop resistance against allergies, reinforce the immune system, help to control high blood pressure and cholesterol, and protect us from deadly diseases like cancer and AIDS. *Spirulina* is being developed as "food of the future" because of the presence of active biomolecules and their beneficial effects (Table 7).

Table 7 Beneficial effects of major bioactive molecules in *Spirulina*

Bioactive compounds in *Spirulina*	Functions in human body
β-Carotene, GLA	Protection and maintenance
Chlorophyll, lutein	Cleansing (removal of free radicals)
C-phycocyanin (C-PC), proteins	Repair
Zeaxanthin	Antioxidant damage repair
Arginine	Hormonal balance

Spirulina has three benefits – cleansing, restoring, and fortifying. It promotes the body natural cleansing processes, compensates for deficiencies in the diet, and stimulates metabolism. It boosts resistance and activates body's natural defense mechanisms. It was then adopted as a super food to overcome malnutrition.

It has gained considerable popularity in the human health food industry, and in many countries of Asia, it is used as protein supplement and as health food. *Spirulina* has also been used as a protein and vitamin-supplemented feed for fish, shrimp, and poultry. China is using this microalga as a partial substitute of imported forage to promote the growth, immunity, and viability of shrimp. In Japan, research on using Spirulina as aquaculture feed additives is accomplished [45].

4.2 Use as Supplement in Humanitarian Emergencies

As discussed in the chapter earlier, *Spirulina* can serve as a supplementary cure for many diseases and to cure malnutrition. The aftermath of malnutrition (lack of essential vitamins, iron, zinc, essential amino acids, etc.) leads to irreversible damages such as mental retardation, growth problems, blindness, and fatal infections. Malnutrition plays a role in more than half of children death over the world; its ravages get extended to the million survivors who will be disabled and chronically vulnerable to diseases. Malnutrition can only be overcome by allowing extremely poor populations to cover their essential nutritional needs. *Spirulina* local production system emerges as a micronutrient complement, responding to overcome malnutrition. This paved a way in the development of *Spirulina* producing industries.

A study on 28 children suffering from manifest protein-energy diseases was carried out from January to November 1989 in Zaire [46]. The parameters measured during this study show the generally positive effects of *spirulina* on patients' nutritional status, regardless of the inevitable hazards associated with studies in the field. Researchers in China analyzed the impact of a daily portion of 1.5 grams of *Spirulina* on the health status of the children and found it to be very beneficial [47].

4.3 Food Safety Aspects Related to Human Consumption

Before being adopted as super food and nutraceutical, it is very important to clarify the specific species of *Spirulina* used for human consumption is safe. It is known that

many of the existing blue-green algae species are known to produce toxin (microcystins, in particular MCYST-LR). It is particularly important in countries where no such regulation exists on this type of products.

The results of recent studies on risk management showed that only products from *Spirulina platensis* have so far been cleared for consumption (United States of America, Australia, Canada), under specific conditions, by public health authorities. In Canada, it was found that no microcystins was detected in blue-green algal products containing only *Spirulina* [48], while a study conducted for the Oregon Department of Agriculture (ODA) published in 2000 found MCYST-LR in all the 15 *Spirulina* samples (dietary supplements) analyzed [49]. *Spirulina* has been recognized as GRAS (generally recognized as safe) under the "indented conditions of use" implying that it is "for use as an ingredient in foods, at levels ranging from 0.5 to 3.0 grams per serving." This means in relatively small amounts. Special precautionary measures would be necessary on the consumption of spirulina products to some segments of the population at risk to include pregnant women, nursing mothers, and people in dialysis and immune-compromised.

5 *Spirulina* Industry and Products

Edible blue-green algae like *Nostoc, Chlorella*, and *Aphanizomenon* species have been used for food for thousands of years. Recently *Spirulina* has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquaculture diets. *Spirulina* grows well in alkaline waters, but its production for mass cultivation is to be done in areas with suitable climatic conditions. It is difficult to have an ideal growth due to different environmental factors like solar radiation, rain, wind, temperature fluctuation, etc.

5.1 Culturing *Spirulina*

Spirulina cultivation takes place either by natural production, laboratory cultivation small-scale commercial production, and commercial and mass cultivation.

5.1.1 Natural Production

Commercial production systems set for natural production of *Spirulina* are shallow ponds mixed by a paddle wheel. However, there are still some examples of *Spirulina* being harvested commercially from naturally occurring populations. In 1967 Mexico has the largest single plant for the production of *Spirulina* biomass at 2200 m at 18 °C. After filtration, the algal biomass is spray-dried after homogenization and pasteurization. The first pilot plant which produced 150 tons of dry *Spirulina* biomass per year started production in 1973; its production capacity was thereafter raised to 300 tons of medium-grade product per year from 12.0 hectares of natural ponds. The toxicological tests were performed before marketing the product [50]. Another seminatural lake in Myanmar has been reported to be used as a production

site for *Spirulina*. During the blooming season in the summer, when *Spirulina* forms thick mats on the lake, people in boats collect it. *Spirulina* is harvested on parallel-inclined filters, washed with fresh water, dewatered, and pressed again. This paste is extruded and dried in the sun on transparent plastic sheets. Dried chips are taken to a pharmaceutical factory, pasteurized, pressed into tablets, and marketed.

5.1.2 Laboratory Cultivation

For laboratory cultivation of *Spirulina*, the factors that should be taken care are luminosity (photoperiod 12/12, 4 luxes), temperature (30 °C), inoculation size, stirring speed, dissolved solids (10–60 g/liter), pH (8.5–10.5), water quality, and macro- and micronutrient presence (C, N, P, K, S, Mg, Na, Cl, Ca and Fe, Zn, Cu, Ni, Co, Se) [51].

Luminosity: In a culture of *Spirulina platensis* grown in a flat-plate photo bioreactor, cell concentration and productivity of biomass were obtained at the highest light intensity. It was concluded that the higher the light intensity, the higher optimal culture density, highest algal concentrations, and productivity of biomass will be obtained. But too high a rate of mixing resulted in cell damage and reduced output rate [52].

Nutritional media: *Spirulina* can be cultured on different media with inorganic and decomposed organic nutrients. Different types of *Spirulina* were cultured to evaluate growth and biochemistry under similar controlled conditions [53]. Out of the three species cultured, viz., *Spirulina platensis*, *S. laxissima*, and *S. lonar*, *S. platensis* showed highest growth rate, biomass, and pigment concentration. Thus *S. platensis* reached highest growth in shortest doubling time and could be the strain for large-scale cultivation. The most favorable growth rates of *S. platensis* occurred in the presence of 2.57 g/liter KNO3 with growth rate of 0.3 –0.4/day.

Spirulina can also be cultured in different agro-industrial wastes such as sugar mill waste effluent, poultry industry waste, fertilizer factory waste, and urban waste and organic matter. The growth parameters of *Spirulina platensis* were higher than other cultures on the supernatant of 2.0 and 6.0 g/liter digested poultry waste which might be due to appropriate nutrient content and other environment parameters [54]. Growth performance of *Spirulina platensis* was also studied in three different concentrations of banana leaf ash added with 0.4 g/liter jackfruit seed powder and 0.2 g/liter with urea in the laboratory which showed positive results [55].

5.1.3 Small-Scale Production

The small-scale production of *Spirulina* has emerged as a potential income-generating activity for village collectives. *Spirulina* produced could be used for local consumption by the poor farmers or for animal or aquatic feeds. *Spirulina* cultivation may be carried out in unlined ditches through which flow is low (e.g., 10 cm/s). Stirring may be provided by a simple device driven by wind energy or harnessed to humans. Harvesting may be readily performed using some suitable cloth, and the biomass dehydrates in the sun. The quality of the *Spirulina* product extracted by this process would not be as high as what is attained in "clean cultures," but product could serve well as animal feed. From early eighties, Bangladesh is

producing *Spirulina* through a pilot project using paddle wheel under transparent shade in the campus of BCSIR (Bangladesh Council of Scientific and Industrial Research). In India, the Murugappa Chettiar Research Centre in Chennai has developed the technology, and this has been successfully propagated on a large scale in the rural areas of Pudukkottai District of Tamil Nadu [56].

5.1.4 Mass Cultivation

The main commercial large-scale culture of *Spirulina* started in the early 1960s in Japan at Lake Texcoco, Mexico. *Spirulina* is produced in at least 22 countries: Benin, Brazil, Burkina Faso, Chad, Chile, China, Costa Rica, Côte d'Ivoire, Cuba, Ecuador, France, India, Madagascar, Mexico, Myanmar, Peru, Israel, Spain, Thailand, Togo, United States of America, and Vietnam. The total industrial production of *Spirulina* was about 3000 tons in 2004 [57]. Mass cultivation of *Spirulina* is usually carried out in shallow ponds, equipped with paddle wheels to mix the culture. Two types of open raceway ponds are typically used: the first is lined by concrete and is therefore expensive; the second is a shallow earthen tunnel lined with polyvinyl chloride (PVC) or some other durable plastic material. The surface of commercial raceways varies from 0.1 to 0.5 hectares, and culture depth is usually maintained at 15–18 cm. The paddle wheel, large (with a diameter up to 2.0 m and a speed of 10 rpm) or small (with a diameter of 0.7 m and a speed two to three times faster than 2.0 m diameter paddle wheel), is the most common stirring device [58]. The first successful culture of *Spirulina maxima* using untreated seawater in laboratory condition was reported in Italy in 1984 by Materassi et al. (1984). The culture technique developed in the laboratory has been successfully applied to outdoor mass culture of *S. maxima*. The United States of America has a number of the largest intensive farms in the world, mainly based in Hawaii and California (Fig. 4).

5.2 Extraction of Bioactive Molecules

Spirulina platensis is harvested directly from the cultivation area and is dried directly by using oven at temperature 40 °C for 10 h to get final water content below 10%. It is then extracted by using soxhletation (for dried *Spirulina*) and refluxion combined with sonication (for fresh *Spirulina*) and analyzed for the nutritional and bioactive compound of proximate analysis, flavonoid, phenolic, and antioxidant activity. 100 mL extract is added with ethanol with ratio of 2:1 and is used for further analysis [59].

5.3 *Spirulina* and Agriculture

Used as biofertilizer: In 1981 the FAO documented the possibilities of blue-green algae replacing chemical fertilizers and rebuilding the structure of depleted soils (FAO, 1981). *Spirulina* is used by rice farmers as this natural nitrogen source is only one-third the cost of chemical fertilizer, and it increases annual rice yield in India by

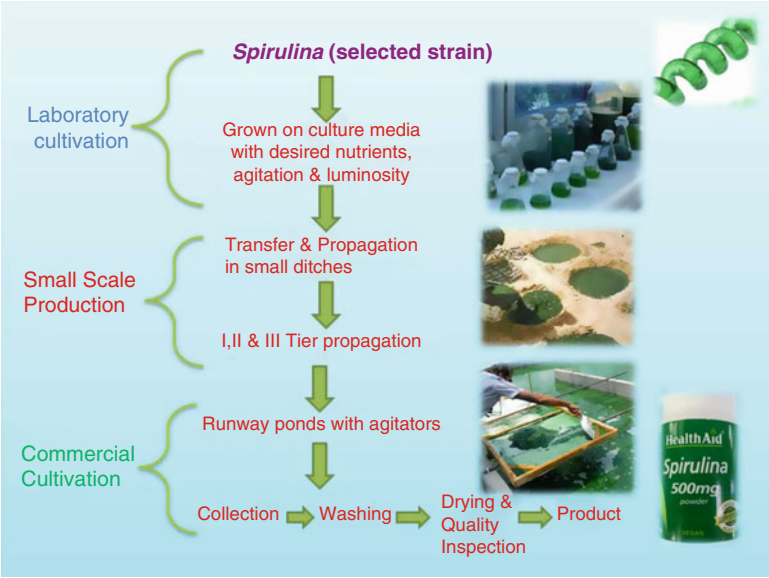


Fig. 4 Cultivation of *Spirulina*

an average of 22 percent. The use of *Spirulina*-based biofertilizers is impeded by the low cost, ready availability, and preferred use of inorganic fertilizers. Studies have proved Spirulina used in combination with other fertilizers also gave good yield of tomato [60]. *Spirulina* contains 10 percent N w/w (high percentage), and other macro- and micronutrients which are slowly released under normal soil conditions, and increases fertility.

Used as a protein supplement in poultry and livestock feeds: *Spirulina* can be used as an excellent feed for poultry. It has been studied and proved by researchers that the redness of muscles of broiler chickens reaches maximum when fed with 40 g *Spirulina* kg^{-1} diet [61].

Used as a natural colorant: *Spirulina* is rich in pigments; two of its pigments, viz., phycocyanin (blue) and chlorophyll (green), are combined with another group of pigments known as carotenoids (red, orange, and yellow) and used as food colorant. This phycocyanin extracted from *Spirulina* was first marketed in 1980 and was mainly used as a food colorant, as an edible dye in ice creams, and as a natural dye in the cosmetics industry. However, as the pigment is light sensitive, special care must be taken in protecting it from bleaching [62].

5.4 Recent Developments and Future Outlook

With the increasing demand of *Spirulina* as super food, transgenic manipulation is adopted to improve its quality in different countries of the world mainly China

[63]. But, s gene transfer system has not been established. Though conjugation has been widely used for gene manipulation in microalgae, very little lead was obtained in *Spirulina*. Electroporation was the method used transferring foreign genes in *S. platensis*. At present, *Spirulina* production is restricted to either countries with a high demand (i.e., the United States of America and China) or to a few countries that have specifically focused on small-scale production to supplement human diets or to integrate animal and fish production.

Spirulina has always been in the limelight as a nutraceutical, especially from the last few years. Recent studies have shown its development as a "super food for women," and it is used worldwide to improve women health. As discussed earlier, it has a novel compound GLA which reduces inflammation and eases the premenstrual syndrome (PMS). Studies done very recently have proved *Spirulina* diet when given to lactating mothers can protect against neuroinflammation and decreased antioxidant defense in the brain, possibly via decreased activation of p38 and high levels of the antioxidant miRNA-146a [64]. Nephrotoxicity is another disease which can be defeated by consuming *Spirulina* with camel milk [65].

6 Conclusions and Recommendations

Preventive healthcare says "let your food be your medicine." *Spirulina* truly comes in such category of food supplement. As discussed in the chapter, it has a range of bioactive molecules with extremely high amount of digestible protein, nine essential amino acids, high levels of β-carotene, vitamin B_{12}, iron and trace minerals, rare essential fatty acid γ-linolenic acid (GLA), and pigments like C-phycocyanin (C-PC) and phycobilin which makes it extremely desirable. In addition, it has no obvious negative cultural and religious issues associated with its consumption.

Spirulina also has considerable potential in industry, especially as small-scale crop in alkaline areas where other crops fail to grow. Its production occupies only a small environmental footprint, with considerable efficiency in terms of energy consumption. Its production can be done at different scales, from pot cultures to commercial bioreactors or natural large reservoirs. It also has potential for amalgamation with rural organic waste treatment process to ameliorate both environmental condition energy transfer efficiency in ecosystems.

With the developing need to move toward natural products, agriculture is also getting modernized, and the use of chemical fertilizers is minimized. As discussed earlier *Spirulina* is an excellent biofertilizer; recent studies have shown *Spirulina* increases rice growth and seed yield productivity [66]. This makes us to realize we need to pave a way toward new industries dealing with quality products. Thus, optimization of bioactive molecules of *Spirulina* can be a future prospect of research which may provide a direct link from laboratory to mass cultivation of this super food. One such study was conducted recently where the biomass and phycocyanin content of *Spirulina platensis* was enhanced [67].

But, despite of knowing the benefits of *Spirulina*, it has not received serious consideration it deserves to be a potential key crop in alkaline areas where traditional

agriculture struggles. An examination of the literature and research on S*pirulina* also suggests that interest in its development has dwindled over recent years. Much of the work was conducted over the 1980s and 1990s with relatively little over the last decade. Most modern articles available on the internet appear to highlight it as relatively little internationally recognized scientific material.

It is now the allegiance of both national governments and intergovernmental organization to re-evaluate the potential of *Spirulina* to fulfill both their own food security needs and a tool for their overseas development and emergency response efforts. International organizations working against famine victims and malnutrition should develop improved technical and economic solutions to *Spirulina* production as well as prepare tested production packages for rapid use in emergency situations where a sustainable supply of high protein and high vitamin food is required. An urgent need is there to develop clear guidelines on food safety aspects of *Spirulina* so that human health risk can be managed and all products in the market pass the toxicology test. And, the reputation loss which *Spirulina* is facing due to mixed cheap marketed products is prevented.

References

1. Desai K, Sivakami S (2004) *Spirulina*: the wonder food of the 21th century. Asia Pac Biotech News 8:1298–1302
2. Capelli B, Cysewski GR (2010) Potential health benefits of *Spirulina* microalgae: a review of existing literature. Nutra Foods 9(2):19–26
3. Ravi M, De SL, Azharuddin S, Paul SFD (2002) The beneficial effects of *Spirulina* focusing on its immunomodulatory and antioxidant properties. Nutr Diet Suppl 2:73–83
4. Mohan A, Mishra N, Srivastav D, Umapathy D, Kumar S (2014) *Spirulina* – the nature's wonder. SJAMS 2(4C):1334–1339
5. Kebede E, Ahlgren G (1996) Optimum growth conditions and light utilization efficiency of Spirulina platensis (Cyanophyta) from Lake Chitu, Ethiopia. Hydrobiol 332:99–109
6. Richmond AE (1986) Microalgae. CRC Crit Rev Biotechnol 4(4):349–438 Boca Raton, FL
7. Thomas S (2010) The role of parry organic Spirulina in health management
8. Sánchez M, Bernal-Castillo J, Rozo C, Pyne PK, Bhattacharjee P, Srivastav PP (2017) Microalgae (*Spirulina Platensis*) and its bioactive molecules: review. Indian J Nutr 4(2):160. Rodríguez I (2003) Spirulina (Arthrospira): an edible microorganism: a review. Univ Sci 8:7–24.
9. Phang SM, Chu WL (1999) University of Malaya Algae Culture Collection (UMACC). Catalogue of strains. Institute of Postgraduate Studies and Research. University of Malaya, Kuala Lumpur
10. Colla LM, Reinehr OC, Reichert C, Costa JA (2007) Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioresour Technol 98:1489–1493
11. Cifferi O (1983) *Spirulina*. The edible microorganism. Microbial Rev 47(4):551–578
12. Belay A (2008) Spirulina (Arthrospira): production and quality assurance. In: Gershwin ME, Belay A (eds) Spirulina in human nutrition and health. CRC Press, Taylor and Francis Group, New York, pp 1–25
13. Becker W (2004) Microalgae in human and animal nutrition. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science, Blackwell Publishing Company, pp 312

14. Pyne PK, Bhattacharjee P, Srivastav PP (2017) Microalgae (*Spirulina platensis*) and its bioactive molecules: review. Indian J Nutr 4(2):160
15. Sánchez M, Bernal-Castillo J, Rozo C, Rodríguez I (2003) Spirulina (Arthrospira): an edible microorganism: a review. Univ Sci 8:7–24
16. Kumar D, Kumar N, Pabbi S, Walia S, Dhar DW (2013) Protocol optimization for enhanced production of pigments in *Spirulina*. Indian J Plant Physiol 18(3):308–312
17. Zeng WL, Li HR, Cai ZL, Ouyang F (2001) The relationship between *Spirulina platensis* Geitler growth and its light utilization. J Plant Resour Environ 10:7–10
18. Manjit Kaur SD, Ahluwalia AS (1992) Biochemical studies on Spirulina protein. In: Spirulina ETTA national symposium MCRC, pp 78–84
19. John RP, Anisha GS, Nampoothiri KM, Pandey A (2011) Micro and macroalgae biomass: a renewable source for bioethanol. Bioresour Technol 102:186–193
20. Hirahashi T, Matsumoto M, Hazeki K, Saeki Y, Ui M et al (2002) Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. Int Immunopharmacol 2:423–434
21. Subhashini J, Mahipal SV, Reddy MC, Reddy MC, Rachamallu A et al (2004) Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562. Biochem Pharmacol 68:453–462
22. El-Sheekh MM, Daboor SM, Swelim MA, Mohamed S (2014) Production and characterization of antimicrobial active substance from *Spirulina platensis*. Iran J Microbiol 6:112–119
23. Belay A (2008) Spirulina (Arthrospira): production and quality assurance. In: Gershwin ME, Belay A (eds) Spirulina in human nutrition and health. CRC Press, Taylor and Francis Group, New York, pp 1–25
24. Ayehunie S, Belay A, Baba TW, Ruprecht RM (1998) Inhibition of HIV-replication by an aqueous extract of *Spirulina platensis* (Arthrospiraplatensis). J Acquir Immune Defic Syndr Hum Retrovirol 18:7–12
25. Sari RF (2011) Study on bioactive compounds of *Spirulina platensis* as antioxidant (in Indonesia). FPIK, UNDIP, Semarang
26. Panggabean LMG (1998) Microalgae: future food alternative and industrial material (in Indonesia). Oseana 23(1):19–26
27. Layam A, Reddy CLK (2006) Antidiabetic property of *Spirulina*. Diabetol Croat 35(2):29–33
28. Parikh P, Mani U, Iver U (2001) Role of Spirulina in the control of glycemia and lipidemia in type 2 Diabetes Mellitus. J Med Food 4(4):193–199
29. Guan Y, Zhao HY, Ding XF, Zhu YY (2007) Analysis of the contents of elements in Spirulina from different producing areas. Guang Pu Xue Yu Guang Pu Fen Xi 27(5):1029–1031
30. Hsiao G, Chou PH, Shen MY, Chou DS, Lin CH, Sheu JR (2005) C-phycocyanin, a very potent and novel platelet aggregation inhibitor from *Spirulina platensis*. J Agric Food Chem 53 (20):7734–7740
31. Cheong SH, Kim MY, Sok DE, Hwang SY, Kim JH, Kim HR (2010) *Spirulina* prevents atherosclerosis by reducing hypercholesterolemia in rabbits fed a high-cholesterol diet. J Nutr Sci Vitaminol (Tokyo) 56(1):34–40
32. Carmel R (2008) Nutritional anemias and the elderly. Semin Hematol 45(4):225–234
33. Selmi C, Leung PS, Fischer L, German B, Yang CY, Kenny TP (2011) The effects of Spirulina on anemia and immune function in senior citizens. Cell Mol Immunol 8(3):248–254
34. Schwartz J, Shklar G (1987) Regression of experimental hamster cancer by beta carotene and algae extracts. J Oral Maxillofac Surg 45(6):510–515
35. Blinkova LP, Gorobets OB, Baturo AP (2001) Biological activity of Spirulina. Zh Mikrobiol Epidemiol Immunobiol 2:114–118
36. Hirahashi T, Matsumoto M, Hazeki K, Saeki Y, Ui M, Seya T (2002) Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. Int Immunopharmacol 2 (4):423–434

37. Shklar G, Schwartz J (1988) Tumor necrosis factor in experimental cancer regression with alphatocopherol, beta-carotene, canthaxanthin and algae extract. Eur J Cancer Clin Oncol 24 (5):839–850
38. Sheahan S, Bellamy CO, Harland SN, Harrison DJ, Prost S (2008) TGF beta induces apoptosis and EMT in primary mouse hepatocytes independently of p53, p21Cip1or Rb status. BMC Cancer 8:191–201
39. Henrikson R (2000) Earth food *Spirulina*: essential fatty acids and phytonutrients. Ronore enterprises. Inc., Laguna Beach
40. Ramamoorty PK, Bono A (2007) Antioxidant activity, total phenolic and flavonoid content of Morinda citrifolia fruit from various extraction processes. J Eng Sci Technol 2:70–80
41. Gershwin ME, Belay A (2009) *Spirulina*: human nutrition and health. 21(6):747–748
42. Bodri B (2004) How to help support the body's healing after intense radioactive or radiation exposure. Top Shape Publishing, LLC, Reno
43. Suda D, Schwartz J, Shklar G (1986) Inhibition of experimental oral carcinogenesis by topical beta carotene. Carcinogenesis 7:711–715
44. Ravi M, De SL, Azharuddin S, Paul SF (2010) The beneficial effects of Spirulina focusing on its immunomodulatory and antioxidant properties. Nutr Diet Suppl 2:73–83
45. Farooq SM, Ebrahim AS, Subramhanya KH, Sakthivel R, Rajesh NG, Varalakshmi P (2006) Oxalate mediated nephronal impairment and its inhibition by c-phycocyanin: a study on urolithic rats. Mol Cell Biochem 284:95–101
46. Bhat VB, Madyastha K (2000) C-phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro. Biochem Biophys Res Commun 275:20–25
47. Hassanen MR, Mahfouz MK, Farid AS (1997). Biochemical effects of spirulina platensis against oxidative stress caused by doxorubicin. 10:8–14
48. Pang Q, Guo B, Ruan J (1988) Enhancement of endonuclease activity and repair DNA synthesis by polysaccharide of Spirulina platensis. Acta Genet Sin 15:374–378
49. Gilroy DJ, Kauffman KW, Hall RA, Huang X, Chu FS (2000) Assessing potential health risks from microcystin toxins in blue-green algae dietary supplements. Environ Health Perspect 108:435–439
50. Olguín EJ (1986) Appropriate biotechnology systems in the arid environment. In: Doelle HW, Helén CG (eds) Applied microbiology, vol 2. D Reidel Publ Com./UNESCO, Trends in Sci & Res, Dordrecht/Paris, pp 111–134
51. Ciferri O (1983) *Spirulina*, the edible organism. Microbiol Rev 47:551–578
52. Hu QA, Richmond A (1996) Productivity and photosynthetic efficiency of Spirulina platensis as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor. J Appl Phycol 8:139–145
53. Bhattacharya S, Shivaprakash MK (2005) Evaluation of three *Spirulina* species grown under similar conditions for their growth and biochemicals. J Sci Food Agric 85:333–336
54. Parvin M (2006) Culture and growth performance of *Spirulina platensis* in supernatant of digested poultry waste. Bangladesh Agricultural University, Mymensingh (M.S. Thesis)
55. Toyub MA, Rahman MM, Miah MI, Habib MAB (2005) Growth performance of *Spirulina platensis* in three different concentrations of banana leaf ash with added jackfruit seed powder and urea. J Bangladesh Agril Univ 3:303–308
56. Raoof B, Kaushika BD, Prasanna R (2006) Formulation of a low-cost medium for mass production of Spirulina. Division of Microbiology, Indian Agricultural Research Institute, New Delhi and the Centre for Conservation and Utilization of Blue–Green Algae, Indian Agricultural Research Institute, New Delhi
57. Shimamatsu H (2004) Mass production of *Spirulina,* an edible microalga. Hydrobiologia 512:39–44
58. Vonshak A, Richmond A (1988) Mass production of the blue-green alga *Spirulina*: an overview. *Biomass* 15:233–247
59. Nuhu AA (2013) Spirulina (*Arthrospira*): an important source of nutritional and medicinal compounds. J Mar Biol 2013:8

60. Zeenat R, Sharma VK, Rizvi Z (1990) Synergistic effect of cyanobacteria and DAP on tomato yield. Sci Cult 56:129–131
61. Toyomizu M, Sato K, Taroda H, Kato T, Akiba Y (2001) Effects of dietary *Spirulina* on meat color in muscle of broiler chicken. Br Poultry Sci 42:197–202
62. Vonshak A, Chanawongse L, Bunnag B, Tanticharoen M (1996) Light acclimation and photo-inhibition in three *Spirulina platensis* (Cyanobacteria). J Appl Phycol 8:35–40
63. Gaoge W, Xuecheng Z, Delin D, Chengkui T (2004) Study on recipient system for transgenic manipulation in *Spirulina platensis* (Arthrospira). Jpn J Phycol 52:243–245
64. Patil J, Matte A, Mallard C, Sandberg M (2018) *Spirulina* diet to lactating mothers protects the antioxidant system and reduced inflammation in post-natal brain after systemic inflammation. Nutr Neurosci 29:59–69
65. Hamad EM, Mousa HM, Ashoush IS, Salam AM (2018) Nephroprotective effect of camel milk and *Spirulina platensis* in gentamicin induced nephrotoxicity in rats. Int J Pharmacol 14:559–565
66. Kumar DK, Kumaravel R, Gopalsamy J, Sikder MNA, Kumar SP (2018) Microalgae as bio-fertilizers for rice growth and seed yield productivity. Wate and Biomass Valorization 9:793–800
67. Khazi M, Demirel Z, Conk Dalay M (2018) Enhancement of biomass and phycocyanin content of *Spirulina platensis*. Front Biosci (Elite Ed) 10:276–286

Bioactive Compounds from *Garcinia* Fruits of High Economic Value for Food and Health

55

Hosakatte Niranjana Murthy, Vijayalaxmi S. Dandin, Dayanand Dalawai, So-Young Park, and Kee-Yoeup Paek

Contents

H. N. Murthy (✉)
Department of Botany, Karnatak University, Dharwad, India

Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju, Republic of Korea
e-mail: nmurthy60@yahoo.co.in

V. S. Dandin · D. Dalawai
Department of Botany, Karnatak University, Dharwad, India
e-mail: vijayalaxmidandin@gmail.com; dayananddalawai@gmail.com

S.-Y. Park · K.-Y. Paek
Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju, Republic of Korea
e-mail: soypark7@chungbuk.ac.kr; cbnbio@hotmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_65

Abstract

Garcinias (Mangosteen, Brindle berry, and Kokum) are tropical fruits, and are rich source of nutrients, minerals, vitamins, and dietary fibers. They are also abundant with bioactive compounds namely xanthones, benzophenones, hydroxycitric acid, and anthocyanins. Many studies have detailed that these compounds possess antioxidant, anti-inflammatory, anticancer, antimicrobial, antiallergy, antiulcer, antiparasitic, and antihelminthic activities to aid in human health and also weight loss and appetite-reducing properties, making them good dietary supplements. Therefore, bioactive compounds extracted from Garcinia fruits could be used in the preparation of pharmaceuticals and nutraceuticals. This review presents an overview of the bioactive compounds derived from Garcinia fruits and their biological activities for promoting human health as food and drug.

Keywords

Brindle berry · *Garcinia* · Garcinol · Hydroxycitric acid · Kokum · Mangosteen

1 Introduction

Garcinias are important tropical fruits naturally occurring in Asia, Africa, South America, Australia, and Polynesia. Mangosteen (Botanical name; *Garcinia mangostana* L.), Brindle berry [Botanical name: *Garcinia gummigutta* (L.) N. Bobson. Syn. *G. cambogia* (Gaertn.) Desr.], and Kokum (Botanical name: *Garcinia indica* Choisy) are main fruit yielding trees cultivated in different regions of the world including Australia, Cuba, Dominica, Ecuador, Gabon, Ghana, Guatemala, Honduras, India, Jamaica, Liberia, Myanmar, Nepal, Philippines, Puerto Rico, Singapore, Sri Lanka, Thailand, Trinidad and Tobago, United States of America, Vietnam, and Zanzibar [1]. The fruits of Garcinias with medicinal and neutraceutical properties have been used since ancient times in traditional medicinal practices. The progress and promise in advanced technology for isolation of the bioactive compounds from plants was vital in preferring the synthetic products from the fruits of these plants for pharmacological treatment as it could help in structural modifications of the plant-derived compounds to produce potentially more active and safer drugs and also in improving the economy of pharmaceutical industries. The rind and fruit pulp of mangosteen, brindle berry, and kokum are the rich sources of compounds like xanthones, benzophenones, anthocyanins, and hydroxycitric acid which are proved to be the plant drugs demonstrating a large array of biological activities. Fruit pulp of these plants is rich in nutrients, minerals, vitamins, and dietary fibers. Hence, the Garcinia fruits serve as pools of nutrients as well as medicinal drugs to aid in human health.

2 Fruit Description and Traditional Medicinal Uses of *Garcinia* Fruits

Mangosteen, brindle berry, and kokum fruits are used in traditional medicine in Asian countries as folk medicine or herbal medicine to treat various ailments. Juice extracted from the entire fruit or extract obtained from the pericarp (rind) along with arils (pulp) or dried powdered rind (Fig. 1) is used in the preparation of drugs in Indian, Chinese, Thai, and Malaysian system of medicine.

2.1 Fruit Description and Traditional Uses of Mangosteen

Mangosteen fruits are round, red to purplish in color, soft, juicy with sweet flavor, and pleasant aroma, therefore, mangosteen fruits are popularly known as "Queen of fruits" (Fig. 2a) [2]. The pericarp or rind is rich in pigments especially anthocyanins (Fig. 2b). The arils (edible parts) are white, juicy, sweet, and acidic (Fig. 2c) [3]. Mangosteen fruit rinds are used in the treatment of dysentery, ulcers, skin infections, wound and as an astringent, antimicrobial, and antiparasitic agent in China, India, and Thailand [4–9]. The rind decoction is utilized to relieve diarrhea, cystitis, gonorrhea, and gleet [4, 7, 10].

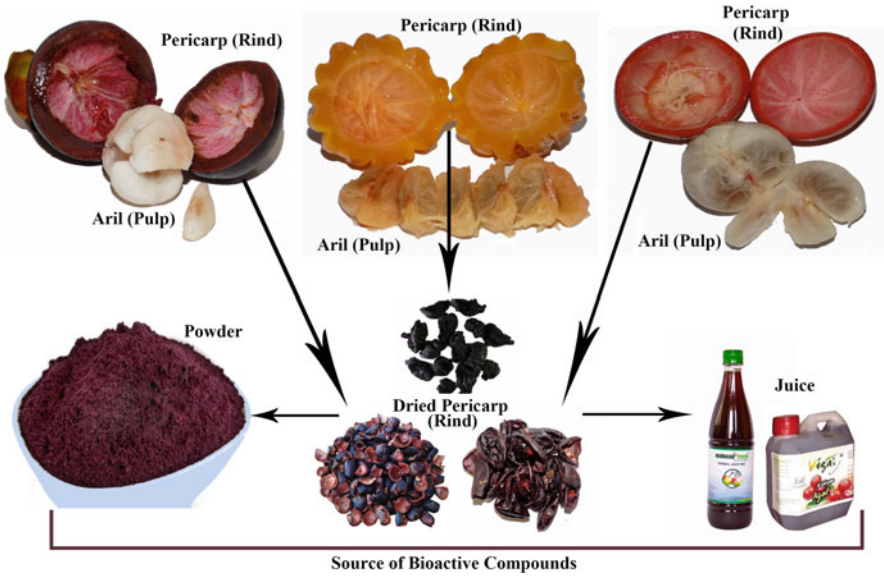


Fig. 1 Fruits, dried pricarp (rind), powdered pericarp and juice of mangosteen, bridle berry, and kokum used as a source of bioactive compounds

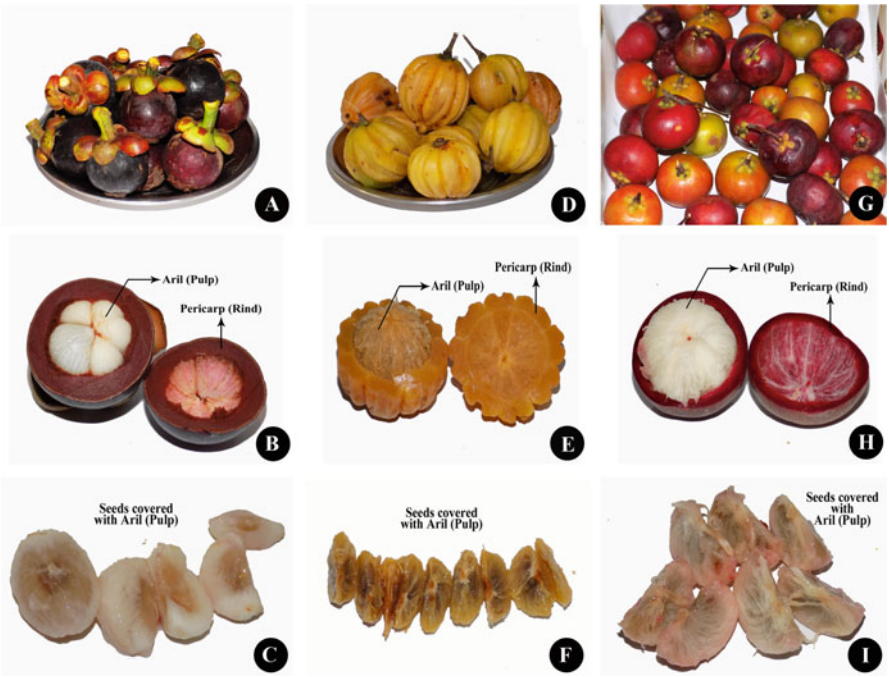


Fig. 2 Fruits of *Garcinia* species. (**a**) Fruits of mangosteen; (**b**) Cross section of mangosteen fruit showing aril and pericarp; (**c**) Arils of mangosteen; (**d**) Fruits of brindle berry; (**e**) Cross section of brindle berry fruit showing aril and pericarp; (**f**) Arils of brindle berry; (**g**) Fruits of kokum; (**h**) Cross section of kokum fruit showing aril and pericarp; (**i**) Arils of kokum

2.2 Fruit Description and Traditional Uses of Brindle Berry

Brindle berry fruits are small, about 5 cm in diameter with 6–8 grooves yellow or red in color (Fig. 2d, e). Arils are whitish to yellow (Fig. 2f). The fruits are edible, acidic and dried fruit rind is used as condiment. Fruits are also rich source of hydroxycitric acid which is an antiobesity drug [11]. Brindle berry fruits are edible; the rind is dried and used as condiment in India and Sri Lanka [1, 11]. The juice or powdered rind is used in traditional medicine to treat rheumatism and bowel problems. Rind preparations are used as purgative, hydragogue, antihelminthic and emetic [12].

2.3 Fruit Description and Traditional Uses of Kokum

Kokum fruits are round or oval, yellow to purple in color (Fig. 2g–i). The fruits are used in the preparation of juice which is used as a coolant and dried rinds are used as condiment (Fig. 1). Mangosteen, brindle berry, and kokum fruits are rich in nutrients, minerals, vitamins, and dietary fibers [13–15]. Kokum fruits are edible, delicious, and have a pleasant flavor and sour taste. Fruits are used in making health beverages

or squash and jellies. The dried rinds of Kokum are used as acidulant and preservative in Indian dishes [16]. The fruit juice is used as a coolant, and is beneficial to cure stomach and liver disorders. Kokum is also found to be effective in treatment of dysentery, tumors, and heart complaints [4, 17].

3 Bioactive Compounds Isolated from *Garcinia* Fruits

3.1 Bioactive Compounds Isolated from Mangosteen Fruits

The major bioactive compounds in mangosteen, brindle berry, and kokum fruits are xanthone derivatives [2, 18]; benzophenone derivatives [4, 19]; and anthocyanins [4]. Xanthones are secondary metabolites which belong to the polyphenolic group consisting of tricyclic aromatic ring. Xanthones are classified into five groups namely simple oxygenated xanthones, xanthone glycosides, prenylated xanthones, xanthonolignoids, and miscellaneous xanthones [20]. Mangosteen fruits consist of xanthones which have substituted isoprene, phenolic, and methoxy groups. α-mangostin was first isolated by Schmid [21] from mangosteen fruits and its chemical nature was elucidated by Dragendorff [22]. More than 54 xanthone derivatives have been isolated from mangosteen fruit till now (Table 1). Major benzophenones isolated are garcimangosone D, maclurin, kolanone [4]. Chrysanthemin, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-sophoroside are the anthocyanins extracted from fruits of mangosteen (Table 1) [4].

3.2 Bioactive Compounds Isolated from Brindle Berry Fruits

The important phytochemicals reported from brindle berry fruits are organic acids, xanthones, and benzophenones (Table 2). Hydroxycitric acid (HCA) is the major organic acid isolated from the fruit of brindle berry and the concentration of HCA ranges from 10% to 13% [11, 48]. HCA lactone or Garcinia lactone was also obtained along with HCA from fruits [51]. Major xanthones isolated from fruits of brindle berry are polyisoprenylated xanthones, i.e., oxy-guttiferone I, oxy-guttiferone K, oxy-guttiferone K2, and oxy-guttiferone M (Table 2) [52]. Guttiferone I, guttiferone N, guttiferone J, guttiferone K, and guttiferone M were the polyisoprenylated benzophenones isolated from fruits of brindle berry [52].

3.3 Bioactive Compounds Isolated from Kokum Fruits

The principal phytochemicals isolated from kokum fruits with proven biological activity are hydroxycitric acid, benzophenone derivatives, and anthocyanins (Table 3). Kokum fruit contains polyisoprenylated benzophenone derivatives such as garcinol (camboginol/guttiferone E), isogarcinol (cambogin), xanthochymol,

Table 1 Compounds isolated from fruit of mangosteen

Xanthones	References
α-mangostin	[2, 19, 23–35]
β-mangostin	[19, 24, 25, 27, 30, 31, 35]
γ- mangostin	[2, 19, 23, 24, 27, 30, 31, 33, 34, 36, 37]
1,2-dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2-enyl) furo[3,2-a]xanthen-11-one	[32]
1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl) xanthone	[9]
1,3,6,7-tetrahydroxy-8-(3 methyl-2-butenyl)-9H–xanthon-9-one	[19]
1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl) xanthone	[9]
1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl) xanthone	[31, 32]
1,5-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxy-xanthone	[19, 30]
1,5-dihydroxy-2-isopentyl-3-methoxy xanthone	[4]
1,6-dihydroxy-7-methoxy-8-isoprenyl-6,6-dimethylpyrano (2,3:3,2) xanthone	[25, 26]
1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxy-xanthone	[19, 24, 26, 30]
1,7-dihydroxy-2-isopentyl-3-methoxy xanthone	[4]
11-hydroxy-1-isomangostin	[25]
1-isomangostin hydrate	[31]
1-isomangostin	[2, 19, 31]
2-(γ, γ-dimethylallyl)-1,7-dihydroxy-3-methoxyxanthone	[31, 33]
2,7-di-(3-methy-but-2-enyl) -1,3,8-trihydroxy 4-methylxanthone	[38]
2,8-di-(3-methy-but-2-enyl)-7-carboxy-1,3 dihydroxyxanthone	[38]
3-isomangostin hydrate	[31]
3-isomangostin	[19, 31]
5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2H,6H–pyrano-[3,2,6]-xanthene-6-one	[19, 33, 39]
6-deoxy-7-demethylmangostanin	[32]
8-deoxygartanin	[2, 19, 25, 27, 33, 40]
8-hydroxycudraxanthone G	[2]
BR-xanthone A	[41]
BR-xanthone B	[41]
Calabaxanthone	[31]
Cudraxanthone G	[2]
Demethylcalabaxanthone	[26, 31]
Garcimangosone A	[19]
Garcimangosone B	[2, 19]
Garcimangosone C	[19]
Garcinone A	[42]
Garcinone B	[19, 25, 26, 35, 42]
Garcinone C	[25, 42]
Garcinone D	[2, 19, 25, 27, 43]
Garcinone E	[2, 19, 24, 25, 30, 33, 34, 44]
Gartanin	[2, 19, 25, 27, 30, 31, 33, 40]

(*continued*)

Table 1 (continued)

Xanthones	References
Mangostanin	[25, 26, 32]
Mangostanol	[19, 25, 26, 33, 35]
Mangostenol	[26, 35]
Mangostenone A	[26, 35]
Mangostenone B	[35]
Mangostenone C	[25]
Mangostenone D	[25]
Mangostenone E	[25]
Mangostingone	[2]
Mangostinone	[2, 24–26, 30, 35]
Smeathxanthone A	[2]
Thwaitesixanthone	[25]
Tovophyllin A	[2, 19, 34]
Tovophyllin B	[19, 26, 35]
Trapezifolixanthone (Toxyloxanthone A)	[26, 35]
Benzophenones	
Garcimangosone D	[19, 45]
Kolanone	[4]
Maclurin	[4]
Anthocyanins	
Chrysanthemin	[4]
Cyanidin-3-*O*-glucoside	[46]
Cyanidin-3-*O*-sophoroside	[46]
Pelargonidin 3-glucoside	[47]

Table 2 Compounds isolated from fruit of brindle berry

Organic Acids	References
Hydroxycitric acid	[48–50]
Garcinia lactone (HCA lactone)	[51]
Xanthones	
Oxy-guttiferone I	[52]
Oxy-guttiferone K	[53]
Oxy-guttiferone K2	[52]
Oxy-guttiferone M	[52]
Benzophenones	
Garcinol	[54]
Guttiferone I	[53]
Guttiferone J	[53]
Guttiferone K	[53–55]
Guttiferone M	[53, 55]
Guttiferone N	[53]

Table 3 Compounds isolated from fruit of kokum

Organic Acids	References
Hydroxycitric acid	[56]
Garcinia lactone (HCA lactone)	[56]
Benzophenones	
Garcinol	[57]
Isogarcinol	[57]
Xanthochymol	[58]
Isoxanthochymol	[58]
Anthocyanins	
Cyanidin-3-*O*-glucoside	[15, 59, 60]
Cyanidin-3-sambubioside	[15, 59, 60]

isoxanthochymol [56, 61]. Kokum fruit is also rich in anthocyanin namely cynidin-3-glucoside and cynidin-3-sambubioside [15].

4 Biological Activities of Compounds Obtained from *Garcinia* Fruits

4.1 Biological Activities of Xanthones

The most abundant xanthones in the mangosteen fruits are α-mangostin and γ-mangostin, and among these α-mangostin is reported to have antioxidant, anti-inflammatory, anticancer, and antimicrobial activities [62–65].

4.1.1 Antioxidant Activity

α-mangostin was reported to be a potent antioxidant and it was demonstrated to reduce copper/peroxyl radical induced oxidation of human low density lipoproteins [66, 67]. Jung et al. [2] showed optimal $ONOO^-$ scavenging activity of α-mangostin in mouse mammary organ cultures. Sampath and Vijayaraghavan [68] studied the impact of α-mangostin on the antioxidant defiance system and lipid peroxidation against isoproterenol-induced myocardial infarction in rats. Induction of rats with isoproterenol (150 mg kg^{-1}) for 2 days resulted in marked elevation of lipid peroxidation enzymes in the serum namely creatine phosphokinase (CPK), lactate dehydrogenase (LDH), glutamate pyruvate transaminase (GPT), and glutamate oxaloacetate transaminase (GOT) and remarkable decrease in various antioxidant enzymes such as glutathione-S-transferase (GST), glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH). Pretreatment of α-mangostin (200 mg kg^{-1}) orally for 6 days prior to isoproterenol administration and 2 days along with isoproterenol administration substantially attenuated such changes.

The renoprotective effect of α-mangostin on cisplatin-induced nephrotoxicity in rats was reported by Perez-Rojas et al. [69]. For 10 days 12.5 mg kg^{-1} day^{-1} of α-

mangostin was administered to experimental rats and on the seventh day the rats were treated with a single dose of cisplatin (7.5 mg kg^{-1}). After 3 days, the rats were killed and studied for the impact of α-mangostin. The attenuation of renal dysfunction, oxidative/nitrosative stress, and structural damages was recorded. The preventive effect of α-mangostin on cisplatin-induced apoptic death is attributed to the inhibition of p53 expression and reactive oxygen species generation. Similarly, the protective effect of α-mangostin on cardiac reperfusion damage was investigated by Buelna-Chontal et al. [70]. Their findings show that α-mangostin could maintain the mechanical work of the heart, decrease the area of infarct, and even prevent the decrease in cardiac ATP and phosphocreatine levels in reperfused myocardium. The defensive effect of α-mangostin was related with reduction in oxidative stress. α-mangostin treatment was found to prevent reperfusion injury-induced protein oxidation, and reduction of glutathione content and lipid peroxidation.

4.1.2 Anti-Inflammatory and Antiallergic Activity

Alpha-mangostin was reported to have potent anti-inflammatory and antiallergic effects [23, 71–73]. Chen et al. [23] studied the effect of α-mangostin on the murine macrophage cell line RAW 264.7 and reported the inhibition of nitric oxide (NO) and prostaglandin E2 (PGE_2) production. At 3–25 μM α-mangostin, the amount of NO production was measured continuously and the IC_{50} value was 12.4. The production of PGE_2 in lipopolysaccharide-activated RAW 264.7 cell was also significantly reduced by α-mangostin (IC_{50} value of 11.08 μM). Chen et al. [23] also verified induction of nitric oxide synthase (iNOS) and expression of cyclooxygenase (COX) enzyme to investigate the effect of α-mangostin. α-mangostin concentration was found to reduce iNOS induction in a concentration-dependent manner. 1 μg ml^{-1} lipopolysaccharide was used to activate the RAW 264.7 cells for 12 h and nitric oxide synthase activity in the activated RAW 264.7 macrophages was inhibited following 24-h treatment of 5 μg ml^{-1} α-mangostin.

A study conducted by Chae et al. [73] to investigate the effect of α-mangostin and γ-mangostin on the bone marrow-derived mast cell (BMMC) mediated allergy mechanism induced by phorbol 12-myristate 13-acetate (PMA) plus calcimycin A23187. Both α-mangostin and γ-mangostin were shown to inhibit the production of interleukin-6 (IL-6), prostaglandin D2 (PGD2), and leukotriene C4 (LTC4) and degranulation of BMMC induced by PMA plus calcimycin A23187. In addition, both α-mangostin and γ-mangostin were found to repress cyclooxygenase (COX-2) expression as assessed by reverse transcription polymerase chain reaction (RT-PCR) analysis. These results advocate the usefulness of α-mangostin and γ-mangostin in reduction of allergic inflammatory responses.

4.1.3 Anticancer Activity

The α-mangostin (as well as β- and γ-mangostin) was reported to possess inhibition of cell proliferation of human cancer cells [74]. The antiproliferative effects of α-, β-, γ-mangostin were associated with cell-cycle arrest by affecting the expression of cyclins, cdc2, and p27. α- and γ-mangostin were found to induce apoptosis of cancer cells through G1 and S arrest by the activation of intrinsic pathway following the

downregulation of signaling pathways involving MAP kinases and serine/threonine kinase activities.

Matsumoto et al. [24] conducted a study on the inhibitory effects of α-mangostin, β-mangostin, γ-mangostin, mangostinone, garcinone E, and 2-isoprenly-1, 7-dihydroxy-3-methoxyxanthone on cell growth of the human leukemia cell line HL60, K562, NB4, and U937. α-mangostin was found to be potent at 10 μM concentration and exhibited the highest inhibitory activity compared to other xanthones. Matsumoto et al. [75] also studied antiproliferative effects of α-, β-, γ-mangostin and methoxy-β-mangostin on human cancer DLD-1 cells. Their results showed that α-mangostin strongly suppressed cell growth at 20 μM, and these effects were associated with cell-cycle arrest by affecting cyclins, cdc2, and p27 expression.

Sato et al. [10] examined the effect of α-mangostin on PC12 rat pheochromocytoma cells and the results showed apoptosis of cells through DNA fragmentation and caspase-3 cleavage. α-mangostin also exhibited features of the mitochondrial apoptotic pathway, including mitochondrial membrane depolarization. Besides, α-mangostin inhibited the endoplasmic reticulum Ca^{2+}-ATPase and activated c-Jun NH2 terminal kinase (JNK) which depicts endoplasmic reticulum stress. These results suggest that α-mangostin inhibits Ca^{2+}-ATPase to accomplish apoptosis of PC12 cells through the mitochondrial pathway.

Suksamrarn et al. [25] studied the effect of 19 xanthones on human breast cancer (BC-1), epidermoid carcinoma of the mouth (KB), and small cell lung cancer (NCI-H187) cell lines and reported that α-mangostin was found to be the most potent biochemical with an IC_{50} value (0.92 μg ml^{-1}) followed by gacinone E and γ-mangostin. Similarly, Kurose et al. [76] conducted a study on the effect of α-mangostin on the human breast cell line MDA-MB231 and described the apoptosis of these cell lines. They also reported significant cytochrome-c release with α-mangostin treated cell lines which suggested that MDA-MB231 cell line apoptosis occurred through the mitochondrial pathway. Their study also revealed that α-mangostin treatment induces cell cycle arrest though upregulation of the cyclin-dependent kinase (CDK) inhibitor $p21^{cip1}$ and cell cycle checkpoint regulator CHEK2 [76].

Hung et al. [77] evaluated the antimetastatic effect of α-mangostin against human prostrate carcinoma cell line PC-3 and reported the decreased expression of multiple matrix degrading proteinases including matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), and urokinase-plasminogen activator (u-PA). α-mangostin also inhibited the phosphorylation of c-JUN N-terminal kinase 1 and 2 (JNK1/2) as well as activation of nuclear factor kappa B (NF-kB), oncogene c-Fos and c-Jun, which are associated with invasion and metastasis of cancer cells. Similar results were also recorded when human melanoma cell line SK-MEL-28 and squamous cell carcinoma cell line A-431 were treated with α-mangostin [78].

In a study, Aisha et al. [79] verified the effect of α-mangostin and γ-mangostin on colon cancer cell line HCT 116, and their study revealed that these xanthones showed strong cytotoxicity through induction of the mitochondrial apoptosis pathway. In addition, α-mangostin and γ-mangostin was found to inhibit cell

migration, invasion, and clonogenicity, which are the major steps in tumor metastasis.

4.1.4 Antimicrobial Activity

Xanthones isolated from fruits of *Garcinia* species especially α-mangostin and γ-mangostin have been shown to exhibit antimicrobial activity against a range of pathogens including bacteria, fungi, and viral species [26, 80–84]. Sundaram et al. [85] studied the effect of α-mangostin on majority of bacterial and fungal species including *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Bacillus subtilis, Klebsiella* sp., *Proteus* sp., *Escherichia coli*, *Epidermophyton floccosum*, *Alternaria solani*, *Mucor* sp., *Rhizopus* sp., *Cunninghamella echinulata*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Aspergillus niger*, *Aspergillus, flavus*, *Penicillium sp*., *Fusarium roseum*, and *Curvularia lunata*. The minimum inhibitory concentration of α-mangostin was between 1.25–50 μg ml^{-1} for bacteria and 1–5 μg ml^{-1} for fungi, respectively. Various scientists [62, 81] evaluated the effect of α-mangostin against Methicillin-resistant *Staphylococcus aureus* (MRSA) and results revealed that minimum inhibitory values ranging between 1.57–12.5 μg ml^{-1}. Gopalakrishnan et al. [27] demonstrated the potentiality of α-mangostin against phytopathogenic fungi, *Fusarium oxysporum vasinfectum*, *Alternaria tenuis*, and *Drechslera oryzae*.

HIV-1 protease activity of α-mangostin was demonstrated by Chen et al. [86] by using pepstatin-A as a positive control and α-mangostin exhibited an IC_{50} value of 5.12 μM. Kaomongkolgit et al. [87] discovered the inhibitory activity of α-mangostin against microorganism involved in oral-candidiasis, *Candida albicans*. They showed that α-mangostin was effective (at a minimum inhibitory concentration of 1000 μg ml^{-1}) when compared to clotrimazole and nystatin (antifungal medicines). Therefore, α-mangostin could be promising agent for the treatment of oral candidiasis. All the above investigations indicated the antimicrobial properties of α-mangostin.

4.1.5 Antiparasitic and Antihelminthic Activity

Various studies have shown that α-mangostin has insecticidal properties against dipteran, coleopteran, and hemipteran pests [88–90]. Ee et al. [88] discovered the inhibitory effect of α-mangostin on *Aedes aegypti* larval growth [lethal concentration (LC_{50}) was found to be 19.4 μg ml^{-1} for 24 h]. Kim and Lan [91] studied the larvicidal activities of α-mangostin using larvae and adults of the Colorado potato beetle, *Leptinotarsa decemlineata*. Their results reveal that α-mangostin had larvicidal activity at LC_{50} concentration of 63.33, 6.27, and 4.09 mM for 7-, 14-, and 23-day treatment, respectively. In addition, Bullangpoti et al. [92, 93] demonstrated the efficacy of α-mangostin against weevil (*Sitophilus oryzae*) and the brown plant hopper (*Nilaparvata lugens*), suggesting that α-mangostin inhibits esterase, acetyl cholinesterase, and glutathione *S*-transferase activities. Larson et al. [90] also demonstrated the larvicidal activities of α-mangostin in *Anopheles stephensi, Anopheles gambiae,* and *Culex pipiens, Anopheles aegypti* (Orlando strain), *Anopheles quadrimaculatus* Say, and *Culex quinquefasciatus* Say.

Keiser et al. [94] studied antihelmenthic effects of α-mangostin against trematodes *Schistosoma mansoni*, *Echinostoma caproni*, *Fasciola hepatica*, and the nematodes *Heligmosomoides polygyrus*, *Ancylostoma ceylanicum*, and *Trichuris muris*. Lack of activity of α-mangostin was recorded against *Heligmosomoides polygyrus* (third-stage larvae), *Ancylostoma ceylanicum* (third-stage larvae), and *Trichuris muris* (adults). A low activity was observed against *Ancylostoma ceylanicum* (adults; IC_{50} of 91 μg ml^{-1}), whereas promising activities were revealed against *Schistosoma mansoni*, *Echinostoma caproni*, *Fasciola hepatica* in vitro (IC_{50} value of 2.9–15.6 μg ml^{-1}). Worm burden reductions, ranging from 0% to 38% against *Schistosoma mansoni* and 11 to 54% against *Echinostoma caproni* were attained by single oral dose of the drug (400 mg kg^{-1} and 800 mg kg^{-1}) in vivo. The above investigations suggest that α-mangostin could be used as organic larvicidal and antihelminthic agent.

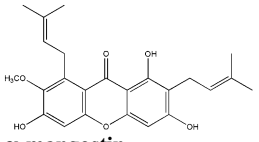
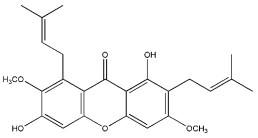
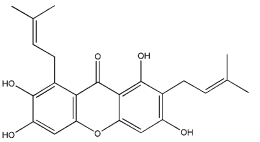
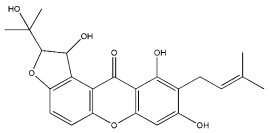
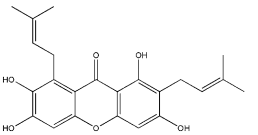
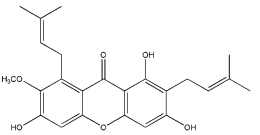
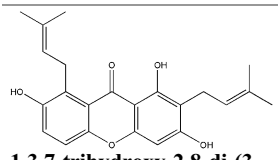
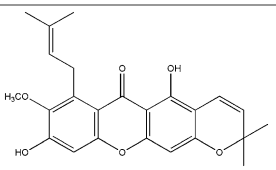
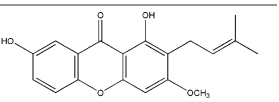
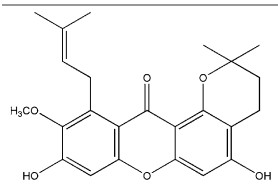
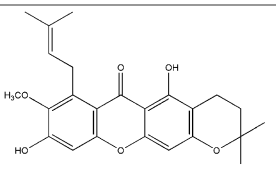
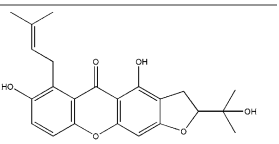
4.2 Biological Activity of Benzophenones

Natural benzophenones are a class of compounds having phenol-carbonyl-phenol skeleton. The A-ring is benzene ring which is derived from shikimic acid pathway, whereas B-ring is derived from acetate-malonate pathway, and undergoes prenylation and cylization producing a variety of compounds. Various numbers of –OH, OMe, prenyl, and geranyl groups are added as side chains [95]. Various polyisoprenylated benzophenones are reported from fruits of *Garcinia* species. Garcimangosone D, kolanone, and maclurin were isolated from fruits of mangosteen (Table 1) [4, 19]. Garcinol, guttiferone I, guttiferone J, guttiferone K, guttiferone M, guttiferone N were isolated from fruits of brindle berry (Table 2) [53–55]. Whereas fruits of kokum possessed garcinol, isogarcinol, xanthochymol, isoxanthochymol compounds (Table 3) [57, 58]. All the benzophenones isolated from the fruits of mangosteen, brindle berry, and kokum were reported to possess strong biological activities (Tables 2, 3, and 4) and garcinol is a well-known compound in terms of its pharmacological properties (Tables 5 and 6).

4.2.1 Antioxidant Activity

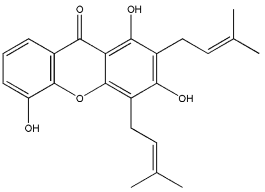
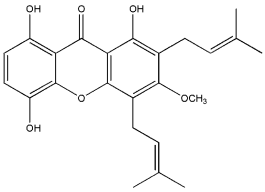
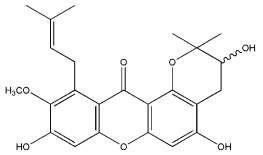
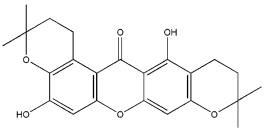
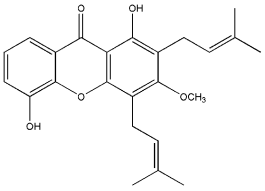
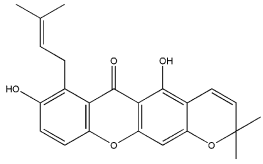
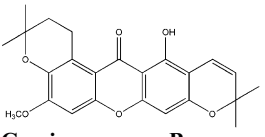
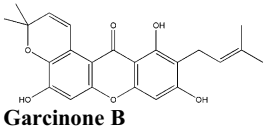
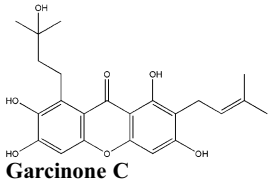
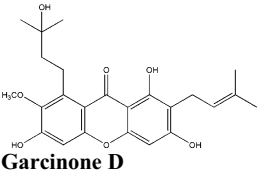
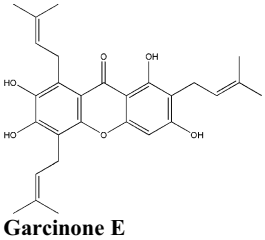
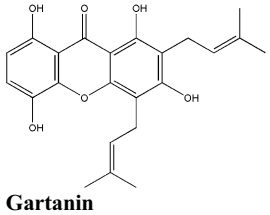
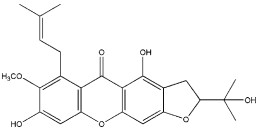
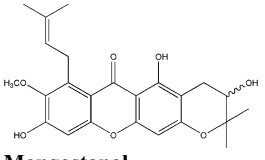
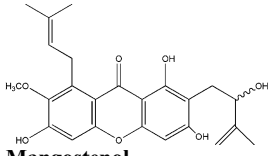
Garcinol exhibited a strong antioxidant activity against superoxide anion, hydroxyl radical, and methyl radicals. Yamaguchi et al. [96, 97] studied antioxidative, chelating, and free radical scavenging activities of garcinol and reported a moderate antioxidative activity in the micellar linoleic acid peroxidation system, while it exhibits nearly three times greater free radical scavenging activity against 2, 2, diphenyl-1-picrylhydrazyl (DPPH) radicals than the DL-α tocopherol (used standard chemical) by weight. These authors also recorded superoxide anion scavenging activity of garcinol and suppression of protein glycation in bovine serum/fructose system. Hong et al. [98] investigated a possible mechanism of antioxidant action of garcinol and its derivatives (cambogin, gracim-1, and gracim-2) on arachidonic acid metabolism and nitric oxide (NO) synthesis in lipoploysaccharide (LPS)-stimulated RAW264.7 cells. Results of this evaluation revealed that there was a significant

Table 4 Structure and biological activity of compounds obtained from mangosteen fruit

Xanthones		
 **α-mangostin** Biological activity: Anticancer, Anti-inflammatory, Antioxidant, Anti-obesity, Antimicrobial, Antihistamine, CNS depressant Activity, Antiulcer, Antituberculosis, Anti-allergy	 **β-mangostin** Biological activity: Anticancer, Antimicrobial, Antituberculosis	 **γ- mangostin** Biological activity: Anti-inflammatory, Antioxidant, Antimicrobial, Anticancer, Antihistamine, Antituberculosis, Anti-allergy
 **1, 2-dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methylbut-2 enyl)furo[3,2-a] xanthen-11-one** Biological activity: Antioxidant	 **1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl) xanthone** Biological activity: Antioxidant	 **1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl) xanthone** Biological activity: Antioxidant
 **1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl)-xanthone** Biological activity: Antioxidant	 **1,6-dihydroxy-7-methoxy-8-isoprenyl-6,6-dimethylpyrano(2,3:3,2) xanthone** Biological activity: Anticancer	 **1,7-dihydroxy-2-(3-methylbut-2-enyl)-3-methoxy-xanthone** Biological activity: Anticancer
 **1-isomangostin** Biological activity: Anti-inflammatory, Antimicrobial, Antioxidant, Anticancer	 **3-isomangostin** Biological activity: Antimicrobial	 **6-deoxy-7-demethylmangostanin** Biological activity: Antioxidant

(*continued*)

Table 4 (continued)

 **8-deoxygartanin** Biological activity: Anticancer, Antioxidant	 **8-hydroxycudraxanthone G** Biological activity: Antioxidant, Anticancer	 **11-hydroxy-1-isomangostin** Biological activity: Anticancer
 **BR-xanthone A** Biological activity: Antifungal	 **Cudraxanthone G** Biological activity: Anticancer, Antioxidant	 **Demethylcalabaxanthone** Biological activity: Antibacterial, Anticancer, Antituberculosis
 **Garcimangosone B** Biological activity: Antioxidant	 **Garcinone B** Biological activity: Antituberculosis	 **Garcinone C** Biological activity: Anticancer
 **Garcinone D** Biological activity: Antimicrobial, Anticancer, Antioxidant, Antituberculosis	 **Garcinone E** Biological activity: Anticancer, Antioxidant	 **Gartanin** Biological activity: Antioxidant, Antimicrobial, Anticancer
 **Mangostanin** Biological activity: Antioxidant, Antituberculosis, Anticancer	 **Mangostanol** Biological activity: Antituberculosis, Anticancer	 **Mangostenol** Biological activity: Antituberculosis

(*continued*)

Table 4 (continued)

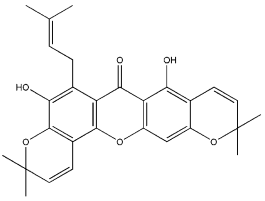
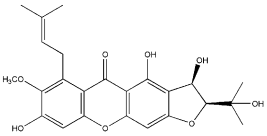
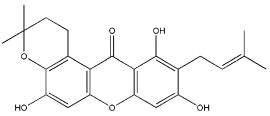
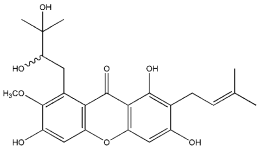
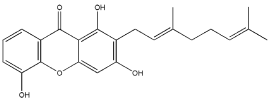
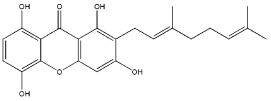
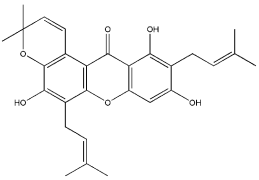
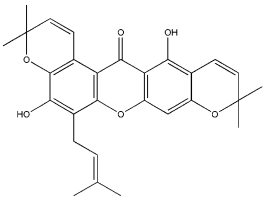
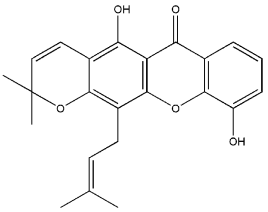
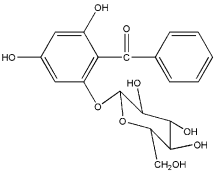
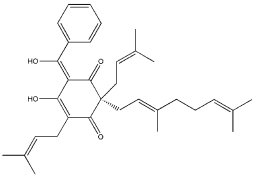
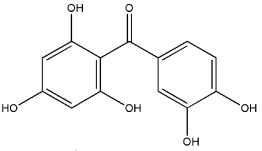
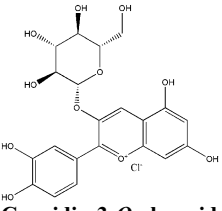
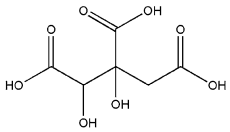
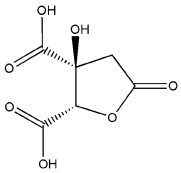
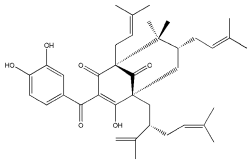
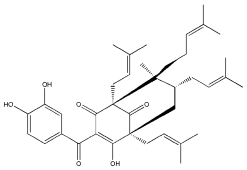
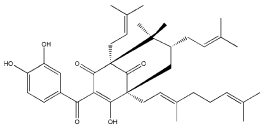
 **Mangostenone A** Biological activity: Antituberculosis	 **Mangostenone C** Biological activity: Anticancer	 **Mangostenone D** Biological activity: Anticancer
 **Mangostenone E** Biological activity: Anticancer	 **Mangostinone** Biological activity: Anticancer, Antituberculosis	 **Smeathxanthone A** Biological activity: Antioxidant
 **Tovophyllin A** Biological activity: Anticancer	 **Tovophyllin B** Biological activity: Antituberculosis	 **Trapezifolixanthone** Biological activity: Antituberculosis
Benzophenones		
 **Garcimangosone D** Biological activity: Inhibitor of pentosidine formation	 **Kolanone** Biological activity: Antimicrobial	 **Maclurin** Biological activity: Antioxidant
Anthocyanins		
 **Cyanidin-3-*O*-glucoside** Biological activity: Anti-inflammatory, Apoptosis inducer		

Table 5 Structure and biological activity of compounds obtained from brindle berry fruit

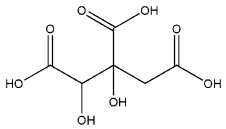
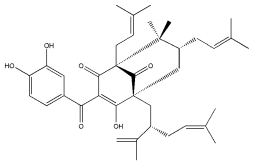
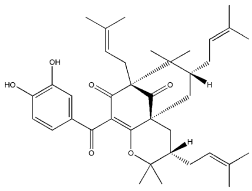
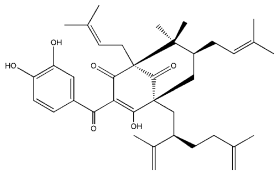
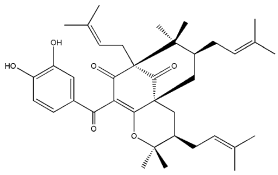
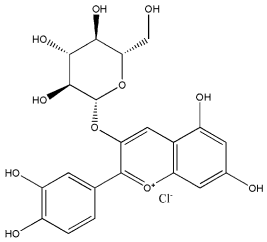
Organic Acids	
 **Hydroxycitric acid** Biological activity: Anti-obesity	 **Garcinia lactone (HCA lactone)** Biological activity: Anti-obesity
Benzophenones	
 **Garcinol** Biological activity: Antioxidant, Anticancer	 **Guttiferone K** Biological activity: Antioxidant, Anticancer
 **Guttiferone M** Biological activity: Anticancer	

inhibition to the release of arachidonic acid and it metabolizes in macrophages with the treatment of 1 mM garcinol and its derivatives to cell cultures after 1 h of LPS stimulation. Similar inhibitory activity of garcinol was also recorded by Hong et al. [98] in intestinal cell lines (HT-29, HCT-116, and IEC-6). Garcinol remarkably decreased inducible nitric oxide synthase (iNOS) express and nitric oxide (NO) release from LPS-stimulated macrophages. In another study, Sang et al. [99, 100] assessed the mechanism of antioxidant reactions of garcinol with a stable radical DPPH and characterized the reaction products. Depending on the position of hydroxyl group (C-3 or C-5) which initiates the reaction, different reaction products were formed (GDPPH-1 and GDPPH-2). Their study revealed that garcinol reacts with peroxyl radicals by a single electron transfer followed by deprotonation of the hydroxyl group from the enolized 1,3-diketone to form a resonance pair. The above investigations suggested that garcinol has potentiality as a free radical scavenger. Similar to garcinol, other benzophenones like xanthochymol, isoxanthchymol, guttiferone K, and maclurin are reported to possess excellent antioxidant activities [95].

4.2.2 Anti-Inflammatory Activity

Aberrant arachidonic acid metabolism and generation of nitric oxide were reported in lipopolysaccharide (LPS)-induced/stimulated inflammation in rat neuron cultures [98]. Archidonic acid is released by phospholipase A2 (cPLA2) from membrane phospholipids and is further metabolized by cyclooxygenase (COX), lipooxygenase (LOX) enzymes, and cytochrome P450 pathways. Cell cultures treated with garcinol (5 μM) showed modulation of archidonic acid metabolism through suppression of cytosolic PLA2 (cPLA2) and inhibition of extracellular ERK1/2 kinase activation

Table 6 Structure and biological activity of compounds obtained from kokum fruit

Organic Acids	
 **1. Hydroxycitric acid** Biological activity: Anti-obesity, Anti-inflammatory	
Benzophenones	
 **Garcinol** Biological activity: Antioxidant, Anti-inflammatory, Anticancer, Antiulcer, Antimicrobial, Gastroprotective	 **Isogarcinol** Biological activity: Anti-inflammatory, Anticancer, Antiulcer, Antiobesity, Antimicrobial
 **Xanthochymol** Biological activity: Antibacterial	 **Isoxanthochymol** Biological activity: Antibacterial
Anthocyanin	
 **Cyanidin-3-*O*-glucoside** Biological activity: Cardioprotective	

and suppression of iNOS expression through modulation of the janus kinase (JAK) pathway [98], and the results suggested the potent anti-inflammatory effects of garcinol. Similarly, Hung et al. [101] demonstrated the inhibitory effect of garcinol against 12–0-tetradecanoylphorbol 13-acetate (TPA)-induced skin inflammation in mice. Topical pre-treatment of mouse skin with garcinol remarkably reduced TPA-induced expression of inducible nitric oxide synthase and cyclooxygenase-2. In addition, garcinol markedly reduced TPA-induced activation of extracellular signal-regulated kinases (ERK), c-Jun-N-terminal kinases (JNK), p38 mitogen-

activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K)/Akt, which are upstream of NF-κB. In addition, Koeberle et al. [102] demonstrated the significant effect of garcinol and its interference with two enzymes that play crucial role in inflammation, namely, 5-lipoxygense and microsomal prostaglandin PGE2 synthase (mPGES)-1. Garcinol was found to suppress 5-lipoxygense product formations in intact human neutrophils and reduced PGE2 formation of interleukin-1β-stimulated A549 human lung carcinoma cells and in human whole blood stimulated by lipopolysaccharide. Garcinol also hindered with isolated COX-1 enzyme (IC_{50} of 12 μM) and with formation of COX-1-derived 12(S)-hydroxy-5-cis-8, 10-trans-heptadecatrienoic acid and thromoxane B2 in human platelets [102].

4.2.3 Anticancer Activity

Various studies have examined the potential of benzophenones, especially garcinol, against different cancer types including breast cancer, colon cancer, pancreatic cancer, prostate cancer, lung cancer, leukemia, hepatocellular carcinoma. Tanaka et al. [103] carried out studies on the effect of garcinol on the development of azoxymethane (AOM)-induced colinic aberrant crypt foci (ACF) in F344 rats. In addition, this group also studied the effect of garcinol on proliferating cell nuclear antigen (PCNA) index in ACF and activities of detoxifying enzymes namely glutathione S-transferase (GST) and quinone reductase (QR) in liver. It was noticed that garcinol administration significantly reduced PCNA index in ACF and considerably elevated liver GST and QR activities. Further, garcinol was also found to suppress superoxide anion (O_2^-) and nitric oxide (NO) generation and expression of inducible nitric oxide synthase and cyclooxygenase-2 proteins. Liao et al. [104] studied the effects of garcinol in human colorectal cancer cell line HT-29 and showed the beneficial effects of tumor prevention. The cell lines treated with 10 μM garcinol inhibited cell invasion and decreased the tyrosine phosphorylation of focal adhesion kinase (FAK). Western blot analysis revealed that garcinol inhibits activation of the Src, MAPK/ERK, and P13K/Akt signaling pathways. In addition, the study also indicated that decreased metalloproteinase-7 (MMP-7) protein levels in HT-29 cells result in sensitization to garcinol and that the compound significantly inhibits the expression of metalloproteinase-7 (MMP-7) in IL-beta-induced HT-29 cells. Hong et al. [105] conducted a study to examine the effects of garcinol and its derivatives, cambogin, gracim-1, gracim-2, on the growth of HT-29 and HCT-116 colon cancer cells, as well as IEC-6 and INT-407 which are the normal immortalized intestinal cells. Garcinol and its derivatives showed strong growth-inhibitory effects on all intestinal cells, with IC_{50} values in the range of 3.2–21.4 μM after 72-h treatment. Garcinol was found to be more effective in inhibiting growth of cancer cells than normal immortalized cells. These observations suggest the possible chemopreventive role of garcinol.

Garcinol reported to possess a strong growth inhibitory activity in human leukemia HL-60 cells (IC_{50} of 9.42 μM) through the induction caspase-3 activity in a dose- and time-dependent manner and including degradation of poly (ADP-ribose) polymerase (PARP) protein [106]. Matsumoto et al. [24] examined the effects of garcinol, isogarcinol, and xanthochymol on cell growth in human leukemia cell

lines, U937, K562, NB4, and HL60 and all the compounds exhibited strong growth suppression due to apoptosis mediated by the activation of capsase-3. Ahmad et al. [107, 108] has demonstrated beneficial effects of garcinol in suppression of breast, prostate, and pancreatic cancer cell growth by induction of apoptosis which was mediated by caspase-3 followed by downregulation of the NFkB pathway.

4.2.4 Antibacterial and Antiulcer Activity

Iinuma et al. [81] evaluated garcinol, isogarcinol, and xanthochymol for their antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and results revealed high efficacy of all the compounds with minimum inhibitory concentration values ranging between 3.1 and 12.5 μg ml^{-1}. Various physical and psychological stresses cause gastric ulceration in human and experimental animals [109]. Recently oxygen-derived free radicals have been shown to play a role in experimental gastric damage induced by ischemia and reperfusion [110]. Yamaguchi et al. [96] have reported a significant free radical scavenging activity against hydroxyl radicals and it was vigorous than that of α-tocopherol. Therefore, garcinol is expected to be useful for preventing gastric ulcers. Yamguchi et al. [97] demonstrated that garcinol suppressed the gastric injury in rats induced by indomethacin and water immersion stress. These investigations suggest that garcinol may have potential as an antiulcer drug.

4.3 Biological Activity of Hydroxycitric Acid

Hydroxycitric acid (HCA) is the major organic acid found in the fruits of brindle berry and kokum. HCA exist in free form as well as in the lactone form, and HCA in free form is reported to possess potent biological activities [111]. HCA is an antiobesity drug and its activity is through regulation of serotonin and food intake suppression, decreased de novo lipogenesis, and enhanced fat oxidation [112]. Various in vivo studies have been carried out to understand these effects of HCA. HCA has been shown to be a strong inhibitor of ATP citrate lyase (EC 4.1.3.8) which catalyzes the cleavage of citrate to oxaloacetate and acetyl-Co-A, results in limitation of acetyl-Co-A required for fatty acid biosynthesis [113, 114]. As a consequence of this, the consumed carbon source is diverted to glycogen synthesis in liver, which results in signaling brain cells and increased production of serotonin and concomitant with a reduced appetite. Preuss et al. [115] reported that HCA generated a significant reduction in appetite, weight loss, and plasma leptin level, accompanying with an increase in the serum serotonin level and a favorable lipid profile in human clinical trials. Asghar et al. [116] conducted experiments in obese Zucker rats which were fed with HCA and recorded decrease in body weight combined with increased serotonin levels. Another possible consequence of HCA effect is the depletion of the acetyl-Co-A which is the precursor of fatty acid and cholesterol biosynthesis. Various in vitro and in vivo studies conducted in rodent models by Sullivan et al. [113, 114, 117, 118] established the inhibition of lopogenesis by HCA. Several experimental evidences suggest that HCA intake is also responsible for increased fat

oxidation. Ishihara et al. [119] carried out a study on acute and chronic effects of HCA on energy metabolism. Acute administrations of HCA (10 mg μl^{-1}) per mice significantly increased serum free fatty acid levels and levels of glycogen in the muscle; however, respiratory exchange was normal. In contrast, chronic administration of HCA (10 mg μl^{-1} twice a day) significantly lowered the respiratory quotient during resting and exercised conditions in mice. Lim et al. [120, 121] also reported that short-term administration of HCA led to fat oxidation in human volunteers.

4.4 Biological Activity of Anthocyanins

Chrysanthemin, cynidin-3-*O*-glucoside, and cynidin-3-*O*-sophoroside are the major anthocyanins isolated from fruits of mangosteen (Table 1) [4], whereas, kokum fruits were rich in anthocyanins namely cynidin-3-*O*-glucoside and cynidin-3-sambubioside [15]. The anthocyanins from kokum has a high prospective as a natural colorant and they are used in the production of confectionery, jellies, jams, health beverages, and deserts [59, 122]. Anthocyanins are having become more important in the food industry because of their bright and attractive shades and water solubility, which allows their incorporation into aqueous food systems [59, 123]. Various studies have also proved that anthocyanins are having possible health benefits as antioxidant, anti-inflammatory, anticancer, antidiabetic, cardio-protective, and neuroprotective agents [124–126]. In a study Min et al. [127] demonstrated neuroprotective effects cynidin-3-*O*-glucoside in a mouse model of permanent middle cerebral artery occlusion (pMCAO) even when delivered 3 h after the onset of ischemia, which is a clinically relevant time point in stroke. Cynidin-3-*O*-glucoside decreased cerebral superoxide levels, inhibited apoptosis-inducing (AIF) release from mitochondria, but did not influence the cytochrome-c related cell death pathway. Wang et al. [128] conducted a study to examine the role of cynidin-3-*O*-glucoside in the prevention of triple-negative breast cancer (TNBC). It was discovered that cynidin-3-*O*-glucoside preferentially promotes the apoptosis of TNBC cells, which co-express the estrogen receptor alpha 36 (ERα36) and the epidermal growth factor receptor (EGFR). Cynidin-3-*O*-glucoside binds to the legend-binding receptor of ERα36, inhibited EGFR/AKT signaling, and promotes EGFR degradation. In summary, all of the above, results indicate that cynidin-3-*O*-glucoside is an important anthocyanin possessing potent biological activities, and mangosteen and kokum fruits are rich in cynidin-3-*O*-glucoside; therefore, these fruits could be used as a potential source of cynidin-3-*O*-glucoside and other anthocyanins.

5 Conclusion

Latterly, Garcinia fruits especially mangosteen, brindle berry, and kokum are used in the preparation of nutraceuticals, dietary supplements, and other health foods because of their nutrient richness and chemical compounds with potential health

promoting properties. All the three fruits are rich in bioactive phytochemicals such as xanthone derivatives and benzophenone derivatives. Brindle berry and kokum are abundant with hydroxycitric acid. While, mangosteen and kokum fruits are rich in anthocyanin derivatives. Various in vitro and in vivo studies have shown that xanthone derivatives, benzophenone derivatives, and anthocyanins obtained from mangosteen, brindle berry, and kokum fruits possess antioxidant, anti-inflammatory, anticancer, antimicrobial, antiallergy, antiobesity, antiulcer, antiparasitic, and anti-helminthic properties. At the same time, hydroxycitric acid has been recognized as a potential antiobesity drug. In addition, various studies have suggested the safety of these natural compounds for human consumption and utilization [65, 112, 129–132]. Nevertheless, further preclinical and post-clinical studies are warranted to prove the efficacy and safety of these natural phytochemicals.

Acknowledgments This work was partially supported by DST-PURSE-PHASE-II program.

References

1. Lim TK (2012) Edible medicinal and non-medicinal plants. Fruits, vol 2. Springer, Heidelberg
2. Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD (2006) Antioxidant xanthones from the pericarp of *Garcinia mangostana* (Mangosteen). J Agric Food Chem 54:2077–2082. https://doi.org/10.1021/jf052649z
3. Fu C, Loo AEK, Chia FPP, Huang D (2007) Oligomeric proanthocyanidins from mangosteen pericarps. J Agric Food Chem 55:7689–7694. https://doi.org/10.1021/jf071166n
4. Farnsworth RN, Bunyapraphatsara N (1992) *Garcinia mangostana* Linn. In: Thai medicinal plants. Prachachon Co Ltd, Bangkok, pp 160–162
5. Ji X, Avula B, Khan IA (2007) Quantitative and qualitative determination of six xanthones in *Garcinia mangostana* L. by LC-PDA and LC-ESI-MS. J Pharm Biomed Anal 43:1270–1276. https://doi.org/10.1016/j.jpba.2006.10.018
6. Nakatani K, Atsumi M, Arakawa T, Oosawa K, Shimura S, Nakahata N, Ohizumi Y (2002) Inhibitions of histamine release and prostaglandin E2 synthesis by mangosteen, a Thai medicinal plant. Biol Pharm Bull 25:1137–1141. https://doi.org/10.1248/bpb.25.1137
7. Moongkarndi P, Kosem N, Luanratana O, Jongsomboonkusol S, Pongpan N (2004) Anti-proliferative activity of Thai medicinal plant extracts on human breast adenocarcinoma cell line. Fitoterapia 75:375–377. https://doi.org/10.1016/j.fitote.2004.01.010
8. Saralamp P, Chuakul W, Temsirirkkul R, Clayton T (1996) Medicinal plants in Thailand. Department of Pharmaceutical Botany, Faculty of Pharmacy, Bangkok, p 98
9. Yu L, Zhao M, Yang B, Zhao Q, Jiang Y (2007) Phenolics from hull of *Garcinia mangostana* fruit and their antioxidant activities. Food Chem 104:176–181. https://doi.org/10.1016/j.foodchem.2006.11.018
10. Sato A, Fujiwara H, Oku H, Ishiguro K, Ohizumi Y (2004) Alpha-mangostin induces Ca^{2+}-ATPase-dependent apoptosis via mitochondrial pathway in PC12 cells. J Pharmacol Sci 95:33–40. https://doi.org/10.1254/jphs.95.33
11. Jena BS, Jayaprakasha GK, Singh RP, Sakariah KK (2002) Chemistry and biochemistry of (−) hydroxycitric acid from *Garcinia*. J Agric Food Chem 50:10–22. https://doi.org/10.1021/jf010753k
12. Abraham Z, Malik SK, Rao GE, Narayanan SL, Biju S (2006) Collection and characterisation of Malabar tamarind [*Garcinia cambogia* (Gaertn.) Desr.] Genet Resour Crop Evol 53:401–406. https://doi.org/10.1007/s10722-004-0584-y

13. Ketsa S, Paull RE (2011) Mangosteen (*Garcinia mangostana* L.) In: Yahia EM (ed) Postharvest biology and technology of tropical and subtropical fruits, vol 4. Woodhead Publishing, Cambridge
14. Nazarudeen A (2010) Nutritional composition of some lesser-known fruits used by ethnic communities and local folks of Kerala. Indian J Tradit Knowl 9:398–402
15. Krishnamurthy N, Lewis YS, Ravindranath B (1982) Chemical constituents of kokam fruit rind. J Food Sci Technol 19:97–100
16. Nayak CA, Rastogi NK, Raghavarao KSMS (2010) Bioactive constituents present in *Garcinia indica* Choisy and its potential food applications: a review. Int J Food Prop 13:441–453. https://doi.org/10.1080/10942910802626754
17. Mishra A, Bapat MM, Tilak JC, Devasagayam TPA (2006) Antioxidant activity of *Garcinia indica* (kokam) and its syrup. Curr Sci 91:90–93
18. Peres V, Nagem TJ, De Oliveira FF (2000) Tetraoxygenated naturally occurring xanthones. Phytochemistry 55:683–710. https://doi.org/10.1016/S0031-9422(00)00303-4
19. Huang YL, Chen CC, Chen YJ, Huang RL, Shieh BJ (2001) Three xanthones and a benzophenone from *Garcinia mangostana*. J Nat Prod 64:903–906. https://doi.org/10.1021/np000583q
20. Pinto MMM, Sousa ME, Nascimento MSJ (2005) Xanthone derivatives: new insights in biological activities. Curr Med Chem 12:2517–2538. https://doi.org/10.2174/092986705774370691
21. Schmid W (1855) Ueber das mangostin. Liebigs Ann Chem 93:83–88
22. Dragendorff O (1930) Uber das harz von *Garcinia mangostana* L. Liebigs Ann 482:280–301
23. Chen LG, Yang LL, Wang CC (2008) Anti-inflammatory activity of mangostins from *Garcinia mangostana*. Food Chem Toxicol 46:688–693. https://doi.org/10.1016/j.fct.2007.09.096
24. Matsumoto K, Akao Y, Kobayashi E, Ohguchi K, Ito T, Tanaka T, Iinuma M, Nozawa Y (2003) Induction of apoptosis by xanthones from mangosteen in human leukemia cell lines. J Nat Prod 66:1124–1127. https://doi.org/10.1021/np020546u
25. Suksamrarn S, Komutiban O, Ratananukul P, Chimnoi N, Lartpornmatulee N, Suksamrarn A (2006) Cytotoxic prelylated xanthones from the young fruit of *Garcinia mangostana*. Chem Pharm Bull 54:301–305
26. Suksamrarn S, Suwannapoch N, Phakhodee W, Thanuhiranlert J, Ratananukul P, Chimnoi N, Suksamrarn A (2003) Antimycobacterial activity of prenylated xanthones from the fruits of *Garcinia mangostana*. Chem Pharm Bull (Tokyo) 51:857–859. https://doi.org/10.1248/cpb.51.857
27. Gopalakrishnan G, Banumathi B, Suresh G (1997) Evaluation of the antifungal activity of natural xanthones from *Garcinia mangostana* and their synthetic derivatives. J Nat Prod 60:519–524. https://doi.org/10.1021/np970165u
28. Yates P, Stout GH (1958) The structure of mangostin. J Am Chem Soc 80:1691–1700
29. Stout GH, Krahn MM, Yates P, Bhat HB (1968) The structure of mangostin. Chem Commun 4:211–212
30. Asai F, Tosa H, Tanaka T, Iinuma M (1995) A xanthone from pericarps of *Garcinia mangostana*. Phytochemistry 39:943–944. https://doi.org/10.1016/0031-9422(95)00042-6
31. Mahabusarakam W, Wiriyachitra P, Taylor WC (1987) Chemical constituents of *Garcinia mangostana*. J Nat Prod 50:474–478
32. Chin YW, Jung HA, Chai H, Keller WJ, Kinghorn AD (2008) Xanthones with quinone reductase-inducing activity from the fruits of *Garcinia mangostana* (Mangosteen). Phytochemistry 69:754–758. https://doi.org/10.1016/j.phytochem.2007.09.023
33. Chairungsrilerd N, Takeuchi K, Ohizumi Y, Nozoe S, Ohta T (1996) Mangostanol, a prenyl xanthone from *Garcinia mangostana*. Phytochemistry 43:1099–1102. https://doi.org/10.1016/S0031-9422(96)00410-4
34. Ho CK, Huang YL, Chen CC (2002) Garcinone E, a xanthone derivative, has potent cytotoxic effect against hepatocellular carcinoma cell lines. Planta Med 68:975–979. https://doi.org/10.1055/s-2002-35668

35. Suksamrarn S, Suwannapoch N, Ratananukul P, Aroonlerk N, Suksamrarn A (2002) Xanthones from the green fruit hulls of *Garcinia mangostana*. J Nat Prod 65:761–763. https://doi.org/10.1021/np010566g
36. Jefferson A, Quillinan AJ, Scheinmann F, Sim KY (1970) Isolation of γ-mangostin from *Garcinia mangostana* and preparation of the natural mangostins by selective demethylation. Aust J Chem 23:2539–2543
37. Jinsart W, Ternai B, Buddhasukh D, Polya GM (1992) Inhibition of wheat embryo calcium-dependant protein kinase and other kinases by mangostin and γ-mangostin. Phytochemistry 31:3711–3713
38. Gopalakrishnan G, Balaganesan B (2000) Two novel xanthones from *Garcinia mangostana*. Fitoterapia 71:607–609. https://doi.org/10.1016/S0367-326X(00)00199-4
39. Sen AK, Sarkar KK, Mazumder PC, Banerji N (1980) A xanthone from *Garcinia mangostana*. Phytochemistry 19:2223–2225
40. Govindachari TR, Kalyanaraman PS, Muthukumaraswamy N, Pai BR (1971) Xanthones of *Garcinia mangostana* Linn. Tetrahedron 27:3919–3926
41. Balasubramanian K, Rajagopalan K (1988) Novel xanthones from *Garcinia mangostana*, structures of BR-xanthone-A and BR-xanthone-B. Phytochemistry 27:1552–1554
42. Sen AK, Sarkar KK, Mazumder PC, Banerji N, Uusvuori R, Hase TA (1982) The structures of garcinones A, B and C: three new xanthones from *Garcinia mangostana*. Phytochemistry 21:1747–1750. https://doi.org/10.1016/S0031-9422(82)85052-8
43. Sen AK, Sarkar KK, Mazumder PC, Banerji N (1986) Garcinone-D, a new xanthone from *Garcinia mangostana* Linn. Indian J Chem 25B:1157–1158
44. Sakai S, Katsura M, Takayama H, Aimi N, Chokethaworn N, Suttajit M (1993) The structure of Garcinone E. Chem Pharm Bull 41:958–960
45. Ohno R, Moroishi N, Sugawa H, Maejima K, Saigusa M, Yamanaka M, Nagai M, Yoshimura M, Amakura Y, Nagai R (2015) Mangosteen pericarp extract inhibits the formation of pentosidine and ameliorates skin elasticity. J Clin Biochem Nutr 57:27–32
46. Du CT, Francis FJ (1977) A research note anthocyanins of mangosteen, *Garcinia mangostana*. J Food Sci 42:1667–1668
47. Zarena AS, Udaya Sankar K (2012) Isolation and identification of pelargonidin 3-glucoside in mangosteen pericarp. Food Chem 130:665–670. https://doi.org/10.1016/j.foodchem.2011.07.106
48. Lewis YS, Neelakantan S (1965) (−)-Hydroxycitric acid – the principal acid in the fruits of *Garcinia cambogia* Desr. Phytochemistry 4:619–625. https://doi.org/10.1016/S0031-9422(00)86224-X
49. Jayaprakasha GK, Sakariah KK (1998) Determination of organic acids in *Garcinia cambogia* (Desr.) by high- performance liquid chromatography. J Chromatogr A 806:337–339. https://doi.org/10.1016/S0021-9673(98)00054-5
50. Jayaprakasha GK, Sakariah KK (2000) Determination of (−) hydroxycitric acid in commercial samples of *Garcinia cambogia* extract by liquid chromatography with ultraviolet detection. J Liq Chromatogr Relat Technol 23:915–923. https://doi.org/10.1081/JLC-100101498
51. Mahapatra S, Mallik SB, Rao GV, Reddy GC, Row TNG (2007) *Garcinia* lactone. Acta Crystallogr E63:o3869. https://doi.org/10.1107/S160053680703838X
52. Masullo M, Bassarello C, Bifulco G, Piacente S (2010) Polyisoprenylated benzophenone derivatives from the fruits of *Garcinia cambogia* and their absolute configuration by quantum chemical circular dichroism calculations. Tetrahedron 66:139–145. https://doi.org/10.1016/j.tet.2009.11.034
53. Masullo M, Bassarello C, Suzuki H, Pizza C, Piacente S (2008) Polyisoprenylated benzophenones and an unusual polyisoprenylated tetracyclic xanthone from the fruits of *Garcinia cambogia*. J Agric Food Chem 56:5205–5210. https://doi.org/10.1021/jf800416j
54. Kolodziejczyk J, Masullo M, Olas B, Piacente S, Wachowicz B (2009) Effects of garcinol and guttiferone K isolated from *Garcinia cambogia* on oxidative/nitrative modifications in blood platelets and plasma. Platelets 20:487–492. https://doi.org/10.3109/09537100903165182

55. Masullo M, Menegazzi M, Di Micco S, Beffy P, Bifulco G, Dal Bosco M, Novelli M, Pizza C, Masiello P, Piacente S (2014) Direct interaction of garcinol and related polyisoprenylated benzophenones of *Garcinia cambogia* fruits with the transcription factor STAT-1 as a likely mechanism of their inhibitory effect on cytokine signaling pathways. J Nat Prod 77:543–549. https://doi.org/10.1021/np400804y
56. Jayaprakasha GK, Sakariah KK (2002) Determination of organic acids in leaves and rinds of *Garcinia indica* (Desr.) by LC. J Pharm Biomed Anal 28:379–384. https://doi.org/10.1016/S0731-7085(01)00623-9
57. Krishnamurthy N, Lewis YS, Ravindranath B (1981) On the structures of garcinol, isogarcinol and camboginol. Tetrahedron Lett 22:793–796
58. Chattopadhyay SK, Kumar S (2006) Identification and quantification of two biologically active polyisoprenylated benzophenones xanthochymol and isoxanthochymol in *Garcinia* species using liquid chromatography-tandem mass spectrometry. J Chromatogr B 844:67–83. https://doi.org/10.1016/j.jchromb.2006.07.045
59. Nayak CA, Srinivas P, Rastogi NK (2010) Characterization of anthocyanins from *Garcinia indica* Choisy. Food Chem 118:719–724. https://doi.org/10.1016/j.foodchem.2009.05.052
60. Jamila N, Choi JY, Hong JH, Nho EY, Khan N, Jo CH, Chun HS, Kim KS (2016) Identification and quantification of adulteration in *Garcinia cambogia* commercial products by chromatographic and spectrometric methods. Food Addit Contam Part A Chem 33:1751–1760. https://doi.org/10.1080/19440049.2016.1244733
61. Padhye S, Ahmad A, Oswal N, Sarkar FH (2009) Emerging role of garcinol, the antioxidant chalcone from *Garcinia indica* Choisy and its synthetic analogs. J Hematol Oncol 2(38):1–13. https://doi.org/10.1186/1756-8722-2-38
62. Chin YW, Kinghorn AD (2008) Structural characterization, biological effects, and synthetic studies on xanthones from mangosteen (*Garcinia mangostana*), a popular botanical dietary supplement. Mini Rev Org Chem 5:355–364. https://doi.org/10.2174/157019308786242223
63. Pedraza-Chaverri J, Cárdenas-Rodríguez N, Orozco-Ibarra M, Pérez-Rojas JM (2008) Medicinal properties of mangosteen (*Garcinia mangostana*). Food Chem Toxicol 46:3227–3239. https://doi.org/10.1016/j.fct.2008.07.024
64. Obolskiy D, Pischel I, Siriwatanametanon N, Heinrich M (2009) *Garcinia mangostana* L.: a phytochemical and pharmacological review. Phytother Res 23:1047–1065
65. Shan T, Ma Q, Guo K, Liu J, Li W, Wang F, Wu E (2011) Xanthones from mangosteen extracts as natural chemopreventive agents: potential anticancer drugs. Curr Mol Med 11:666–677. https://doi.org/10.2174/156652411797536679
66. Williams P, Ongsakul M, Proudfoot J, Croft K, Beilin L (1995) Mangostin inhibits the oxidative modification of human low density lipoprotein. Free Radic Res 23:175–184. https://doi.org/10.3109/10715769509064030
67. Mahabusarakam W, Proudfoot J, Taylor W, Croft K (2000) Inhibition of lipoprotein oxidation by prenylated xanthones derived from mangostin. Free Radic Res 33:643–659. https://doi.org/10.1080/10715760000301161
68. Sampath PD, Vijayaraghavan K (2007) Cardioprotective effect of α-mangostin, a xanthone derivative from mangosteen on tissue defense system against isoproterenol-induced myocardial infarction in rats. J Biochem Mol Toxicol 21:336–339
69. Pérez-Rojas JM, Cruz C, García-López P, Sánchez-González DJ, Martínez-Martínez CM, Ceballos G, Espinosa M, Meléndez-Zajgla J, Pedraza-Chaverri J (2009) Renoprotection by α -mangostin is related to the attenuation in renal oxidative/nitrosative stress induced by cisplatin nephrotoxicity. Free Radic Res 43:1122–1132. https://doi.org/10.1080/10715760903214447
70. Buelna-Chontal M, Correa F, Hernández-Reséndiz S, Zazueta C, Pedraza-Chaverri J (2011) Protective effect of α-mangostin on cardiac reperfusion damage by attenuation of oxidative stress. J Med Food 14:1370–1374. https://doi.org/10.1089/jmf.2010.0238
71. Shankaranarayan D, Gopalakrishnan C, Kameswaran L (1979) Pharmacological profile of mangostin and its derivatives. Arch Int Pharmacodyn Ther 239:257–269

72. Deschamps JD, Gautschi JT, Whitman S, Johnson TA, Gassner NC, Crews P, Holman TR (2007) Discovery of platelet-type 12-human lipoxygenase selective inhibitors by high-throughput screening of structurally diverse libraries. Bioorg Med Chem 15:6900–6908. https://doi.org/10.1016/j.bmc.2007.08.015
73. Chae HS, Oh SR, Lee HK, Joo SH, Chin YW (2012) Mangosteen xanthones, α- and γ-mangostins, inhibit allergic mediators in bone marrow-derived mast cell. Food Chem 134:397–400. https://doi.org/10.1016/j.foodchem.2012.02.075
74. Akao Y, Nakagawa Y, Iinuma M, Nozawa Y (2008) Anti-cancer effects of xanthones from pericarps of mangosteen. Int J Mol Sci 9:355–370. https://doi.org/10.3390/ijms9030355
75. Matsumoto K, Akao Y, Ohguchi K, Ito T, Tanaka T, Iinuma M, Nozawa Y (2005) Xanthones induce cell-cycle arrest and apoptosis in human colon cancer DLD-1 cells. Bioorg Med Chem 13:6064–6069. https://doi.org/10.1016/j.bmc.2005.06.065
76. Kurose H, Shibata M-A, Iinuma M, Otsuki Y (2012) Alterations in cell cycle and induction of apoptotic cell death in breast cancer cells treated with α -mangostin extracted from mangosteen pericarp. J Biomed Biotechnol 2012:1–9. https://doi.org/10.1155/2012/672428
77. Hung S-H, Shen K-H, C-H W, Liu C-L, Shih Y-W (2009) α-Mangostin suppresses PC-3 human prostate carcinoma cell metastasis by inhibiting matrix metalloproteinase-2/9 and urokinase-plasminogen expression through the JNK signaling pathway. J Agric Food Chem 57:1291–1298
78. Wang Y, Xia Z, JR X, Wang YX, Hou LN, Qiu Y, Chen HZ (2012) α-Mangostin, a polyphenolic xanthone derivative from mangosteen, attenuates β-amyloid oligomers-induced neurotoxicity by inhibiting amyloid aggregation. Neuropharmacology 62:871–881. https://doi.org/10.1016/j.neuropharm.2011.09.016
79. Aisha AFA, Abu-Salah KM, Ismail Z, Majid AMSA (2012) In vitro and in vivo anti-colon cancer effects of *Garcinia mangostana* xanthones extract. BMC Complement Altern Med 12:104–113. https://doi.org/10.1186/1472-6882-12-104
80. Mahabusarakam W, Wiriyachitra P, Phongpaichit S (1986) Antimicrobial activities of chemical constituents from *Garcinia mangostana* Linn. J Sci Soc Thail 12:239–242
81. Iinuma M, Tosa H, Tanaka T, Asai F, Kobayashi Y, Shimano R, Miyauchi KI (1996) Antibacterial activity of xanthones from guttiferaeous plants against methicillin-resistant *Staphyloococcus aureus*. J Pharm Pharmacol 48:861–865. https://doi.org/10.1111/j.2042-7158.1996.tb03988.x
82. Chomnawang MT, Surassmo S, Wongsariya K, Bunyapraphatsara N (2009) Antibacterial activity of Thai medicinal plants against methicillin-resistant *Staphylococcus aureus*. Fitoterapia 80:102–104
83. Koh JJ, Qiu S, Zou H, Lakshminarayanan R, Li J, Zhou X, Tang C, Saraswathi P, Verma C, Tan DTH, Tan AL, Liu S, Beuerman RW (2013) Rapid bactericidal action of alpha-mangostin against MRSA as an outcome of membrane targeting. Biochim Biophys Acta Biomembr 1828:834–844. https://doi.org/10.1016/j.bbamem.2012.09.004
84. Torrungruang K, Vichienroj P, Chutimaworapan S (2007) Antibacterial activity of mangosteen pericarp extract against cariogenic *Streptococcus mutans*. CU. Dent J 30:1–10
85. Sundaram BM, Gopalakrishnan C, Subramanian S, Shankaranarayanan D, Kameswaran L (1983) Antimicrobial activities of *Garcinia mangostana*. Planta Med 48:59–60. https://doi.org/10.1055/s-2007-969882
86. Chen SX, Wan M, Loh BN (1996) Active constituents against HIV-1 protease from *Garcinia mangostana*. Planta Med 62:381–382. https://doi.org/10.1055/s-2006-957916
87. Kaomongkolgit R, Jamdee K, Chaisomboon N (2009) Antifungal activity of alpha-mangostin against *Candida albicans*. J Oral Sci 51:401–406. https://doi.org/10.2334/josnusd.51.401
88. Ee GCL, Daud S, Taufiq-Yap YH, Ismail NH, Rahmani M (2006) Xanthones from *Garcinia mangostana* (Guttiferae). Nat Prod Res 20:1067–1073. https://doi.org/10.1080/14786410500463114
89. Bullangpoti V, Visetson S, Milne J, Milne M, Sudthongkong C, Pronbanlualap S (2007) Effects of alpha-mangostin from mangosteen pericarp extract and imidacloprid on *Nilaparvata*

lugens (Stal.) and non-target organisms: toxicity and detoxification mechanism. Commun Agric Appl Biol Sci 72:431–441
90. Larson RT, Lorch JM, Pridgeon JW, Becnel JJ, Clark GG, Lan Q (2010) The biological activity of α-Mangostin, a larvicidal botanic mosquito sterol carrier protein-2 inhibitor. J Med Entomol 47:249–257
91. Kim M-S, Lan Q (2011) Larvicidal activity of α-mangostin in the Colorado potato beetle, *Leptinotarsa decemlineata*. J Pestic Sci 36:370–375. https://doi.org/10.1584/jpestics.G11-09
92. Bullangpoti V, Visetson S, Milne J, Pornbanlualap S (2004) Effects of mangosteen's peels and rambutan's seeds on toxicity, esterase and glutathione-*S*-transferase in rice weevil (*Sitophilus oryzae* L.) Kasetsart J (Nat Sci) 38:84–89
93. Bullangpoti V, Visetson S, Milne M, Milne J, Pornbanlualap S, Sudthongkongs C, Tayapat S (2006) The novel botanical insecticide for the control brown planthopper (*Nilaparvata lugens* Stal.) Commun Agric Appl Biol Sci 71:475–481
94. Keiser J, Vargas M, Winter R (2012) Anthelminthic properties of mangostin and mangostin diacetate. Parasitol Int 61:369–371. https://doi.org/10.1016/j.parint.2012.01.004
95. Wu S-B, Long C, Kennelly EJ (2014) Structural diversity and bioactivities of natural benzophenones. Nat Prod Rep 31:1158–1174. https://doi.org/10.1039/C4NP00027G
96. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H (2000) Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. J Agric Food Chem 48:180–185
97. Yamaguchi F, Saito M, Ariga T, Yoshimura Y (2000) Free radical scavenging activity and antiulcer activity of garcinol from *Garcinia indica* fruit rind. J Agric Food Chem 48:2320–2325
98. Hong J, Sang S, Park H-J, Kwon SJ, Suh N, Huang M-T, Ho C-T, Yang CS (2006) Modulation of arachidonic acid metabolism and nitric oxide synthesis by garcinol and its derivatives. Carcinogenesis 27:278–286. https://doi.org/10.1093/carcin/bgi208
99. Sang S, Pan M, Cheng X, Bai N, Stark RE, Rosen RT, Lin-Shiau SY, Lin JK, Ho CT (2001) Chemical studies on antioxidant mechanism of garcinol: analysis of radical reaction products of garcinol with their antitumor activities. Tetrahedron 57:9931–9938
100. Sang S, Liao CH, Pan MH, Rosen RT, Lin-Shiau SY, Lin JK, Ho CT (2002) Chemical studies on antioxidant mechanism of garcinol: analysis of radical reaction products of garcinol with peroxyl radicals and their antitumor activities. Tetrahedron 58:10095–10102. https://doi.org/10.1016/S0040-4020(02)01411-4
101. Hung W, Liu C, Lai C, Ho C, Pan M (2015) Inhibitory effect of garcinol against 12-*O*-tetradecanoylphorbol 13-acetate-induced skin inflammation and tumorigenesis in mice. J Funct Foods 18:65–73
102. Koeberle A, Northoff H, Werz O (2009) Identification of 5-lipoxygenase and microsomal prostaglandin E2 synthase-1 as functional targets of the anti-inflammatory and anti-carcinogenic garcinol. Biochem Pharmacol 77:1513–1521
103. Tanaka T, Kohno H, Shimada R, Kagami S, Yamaguchi F, Kataoka S, Ariga T, Murakami A, Koshimizu K, Ohigashi H (2000) Prevention of colonic aberrant crypt foci by dietary feeding of garcinol in male F344 rats. Carcinogenesis 21:1183–1189. https://doi.org/10.1093/carcin/21.6.1183
104. Liao C, Ho C, Lin J (2005) Effects of garcinol on free radical generation and NO production in embryonic rat cortical neurons and astrocytes. Biochem Biophys Res Commun 329:1306–1314. https://doi.org/10.1016/j.bbrc.2005.02.110
105. Hong J, Joo S, Sang S, Ju J, Zhou J (2007) Effects of garcinol and its derivatives on intestinal cell growth : inhibitory effects and autoxidation-dependent growth-stimulatory effects. Free Radic Biol Med 42:1211–1221. https://doi.org/10.1016/j.freeradbiomed.2007.01.016
106. Pan M, Chang W, Ho C, Lin J (2001) Induction of apoptosis by garcinol and curcumin through cytochrome *c* release and activation of caspases in human leukemia HL-60 cells. J Agric Food Chem 49:1464–1474
107. Ahmad A, Wang Z, Ali R, Maitah MY, Kong D, Banerjee S, Padhye S, Sarkar FH (2010) Apoptosis-inducing effect of garcinol is mediated by NF-κB signaling in breast cancer cells. J Cell Biochem 109:1134–1141. https://doi.org/10.1002/jcb.22492

108. Ahmad A, Wang Z, Wojewoda C, Ali R, Kong D, Maitah MY, Banerjee S, Bao B, Padhye S, Sarkar FH (2011) Garcinol-induced apoptosis in prostate and pancreatic cancer cells is mediated by NF- kB signaling. Front Biosci (Elite Ed) 3:1483–1492
109. Miller TA (1987) Mechanisms of stress-related mucosomal damage. Am J Med 83:8–14
110. Perry MA, Wadhwa S, Parks DA, Pickard WES, Granger DN (1986) Role of oxygen radicals in ischemia-induced lesions in the cat stomach. Gastroenterology 90:362–367
111. De Haar JL, Wielinga PY, Scheurink AJW, Nieuwenhuizen AG (2005) Comparison of the effects of three different (−)-hydroxycitric acid preparations on food intake in rats. Nutr Metab 2:1–9
112. Chuah LO, Ho WY, Beh BK, Yeap SK (2013) Updates on antiobesity effect of *Garcinia* origin (−)-HCA. Evid Based Complement Alternat Med 2013:1–17. https://doi.org/10.1155/2013/751658
113. Sullivan AC, Triscari J, Spiegel HE (1977) Metabolic regulation as a control for lipid disorders. I. Influence of, (−)-hydroxycitrate on experimentally induced obesity in the rodent. Am J Clin Nutr 30:767–776
114. Sullivan AC, Triscari J, Spiegel HE (1977) Metabolic regulation as a control for lipid disorders. II. Influence of (−)-hydroxycitrate on genetically and experimentally induced hypertriglyceridemia in the rat. Am J Clin Nutr 30:777–784
115. Preuss HG, Bagchi D, Bagchi M, Rao CVS, Dey DK, Satyanarayana S (2004) Effects of a natural extract of (−)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and *Gymnema sylvestre* extract on weight loss. Diabetes Obes Metab 6:171–180
116. Asghar M, Zeyssig R, Monjok E, Kouamou G, Ohia SE, Lokhandwala MF, Bagchi D (2006) Hydroxycitric acid (HCA-SX) decreases oxidative stress and insulin resistance and increases brain serotonin levels in obese Zucker rats. Exp Biol Meet 20:A655.4
117. Sullivan AC, Triscari J, Hamilton JG (1974) Effect of (−)-hydroxycitrate upon the accumulation of lipid in the rat: I. Lipogenesis. Lipids 9:121–128
118. Sullivan AC, Triscari J, Hamilton JG, Miller ON (1974) Effect of (−)-hydroxycitrate upon the accumulation of lipid in the rat: II. Appetite. Lipids 9:129–134
119. Ishihara K, Oyaizu S, Onuki K, Lim K, Fushiki T (2000) Chronic (−)-hydroxycitrate administration spares carbohydrate utilization and promotes lipid oxidation during exercise in mice. J Nutr 130:2990–2995
120. Lim K, Ryu S, Ohishi Y, Watanabe I, Tomi H, Suh H, Lee W, Kwon T (2002) Short-term (−)-hydroxycitrate ingestion increases fat oxidation during exercise in athletes. J Nutr Sci Vitaminol 48:128–133
121. Lim K, Ryu S, Nho H, Choi S, Kwon T, Suh H, So J, Tomita K, Okuhara Y, Shigematsu N (2003) (−)-Hydroxycitric acid ingestion increases fat utilization during exercise in untrained women. J Nutr Sci Vitaminol 49:163–167
122. Bhat DJ, Kamat N, Shirodkar A (2005) Compendium and proceedings of 2nd National seminar on Kokum (*Garcinia indica* Choicy), March 4–5, 2005 held at Goa University
123. Francis FJ, Markakis PC (1989) Food colorants : anthocyanins. Crit Rev Food Sci Nutr 28:273–314
124. Scazzocchio B, Varì R, Filesi C, D'Archivio M, Santangelo C, Giovannini C, Iacovelli A, Silecchia G, Volti GL, Galvano F, Masella R (2011) Cyanidin-3-*O*-β-glucoside and protocatechuic acid exert insulin-like effects by upregulating PPARγ activity in human omental adipocytes. Diabetes 60:2234–2244. https://doi.org/10.2337/db10-1461
125. Pojer E, Mattivi F, Johnson D, Stockley CS (2013) The case for anthocyanin consumption to promote human health: a review. Compr Rev Food Sci Food Saf 12:483–508. https://doi.org/10.1111/1541-4337.12024
126. Ferrari D, Cimino F, Fratantonio D, Molonia MS, Bashllari R, Busà R, Saija A, Speciale A (2017) Cyanidin-3-*O*-glucoside modulates the in vitro inflammatory crosstalk between intestinal epithelial and endothelial cells. Mediators Inflamm. 2017, 8 pages doi: https://doi.org/10.1155/2017/3454023

127. Min J, Yu SW, Baek SH, Nair KM, Bae ON, Bhatt A, Kassab M, Nair MG, Majid A (2011) Neuroprotective effect of cyanidin-3-*O*-glucoside anthocyanin in mice with focal cerebral ischemia. Neurosci Lett 500:157–161. https://doi.org/10.1016/j.neulet.2011.05.048
128. Wang L, Li H, Yang S, Ma W, Liu M, Guo S, Zhan J, Zhang H, Tsang SY, Zhang Z, Wang Z, Li X, Guo Y-D, Li X (2016) Cyanidin-3-*O*-glucoside directly binds to ERα36 and inhibits EGFR-positive triple-negative breast cancer. Oncotarget 7:68864–68882. https://doi.org/10.18632/oncotarget.12025
129. Gutierrez-Orozco F, Failla ML (2013) Biological activities and bioavailability of mangosteen xanthones: a critical review of current evidences. Forum Nutr 5:3163–3183
130. Marquez F, Babio N, Bullo M, Salas-Salvodo J (2012) Evaluation of the safety and efficacy of hydroxycitric acid or *Garcinia cambogia* extracts in humans. Crit Rev Food Sci 52:585–594
131. Saadat N, Gupta SV (2012) Potential review of garcinol as an anticancer agent. J Oncol 2012:1–8. https://doi.org/10.1155/2012/647206
132. Yousuf B, Gul K, Wani AA, Singh P (2016) Health benefits of anthocyanins and their encapsulation for potential used in food system: a review. Crit Rev Food Sci 56:2223–2230

Part VIII

Agricultural Practices and Food Quality

Factors Influencing Sweet Taste in Apple

56

Mathilde Charles, Eugenio Aprea, and Flavia Gasperi

Contents

Abstract

Sweet taste is a major determinant of apple fruit as driving a large part of consumer preferences. As a consequence, increase in sweetness is very frequently one of the targets in breeding programs and is a key parameter for evaluating apple quality. Its perception can be modulated by several factors, and it is important to understand the individual impact of each of them and the processes involved in order to interpret it better.

This chapter reviews the studies of the past two decades dealing with apple and specifically its composition and the related sweet perception. It proposes an

M. Charles (✉)
BIOFORTIS Sensory and Consumer, Saint Herblain, France
e-mail: mathildeccharles@gmail.com

E. Aprea (✉) · F. Gasperi (✉)
Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all'Adige, Trento, Italy
e-mail: eugenio.aprea@fmach.it; flavia.gasperi@fmach.it

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_80

overview of the different aspects influencing sweet taste perception. First, global and sugar compositions of apple fruit are characterized followed by the definition of the principal relative methods of measurements. Then, a part is dedicated to the input brought by sensory analysis in apple sweet taste perception. Finally, the influences of volatile compounds and then texture on sweet taste perception are exposed.

Keywords
Sugars · Sensory · Instrumental · Interactions · Prediction · Apple

Abbreviations

AOAC	Association of Official Analytical Chemists
DA	Descriptive analysis
ELS	Evaporative light scattering
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography
LC	Liquid chromatography
MS	Mass spectrometry
PA	Pulsed amperometry
SSC	Soluble solid content
TDS	Temporal dominance of sensations
TFA	Trifluoroacetates
TMS	Trimethylsilyl

1 Introduction: Preferences in Apples – The Prominent Role of Sweet Taste

Apples are one of the most frequently consumed fruits around the world. Its quality has traditionally been evaluated on the basis of external characteristics such as size, color, and the absence of surface defects. However, to improve quality evaluation, sensory attributes assessed by trained panels should also be considered.

More particularly, measuring sweet taste perception is a critical factor as sweetness is playing a significant role in food preferences. Different studies tried to investigate the apple consumer preferences in the last 20 years. For example, Daillant-Spinnler et al. [1] who studied 20 apple varieties from the southern hemisphere found that consumers could be split into two groups: those that had a preference for sweet and hard apples and on the contrary those that had a preference for juicy and acidic apples. Jaeger et al. [2] performed a study on fresh and aged apples to investigate consumer preferences. They showed that the first preference dimension was more strongly related to flavor differences (sweet taste and fruity/flowery flavors contrasting with acid taste and vegetal flavor) and the second dimension to texture differences (hard, juicy, crisp on the one side; floury and granulous on the other side). Other apple preference studies tended to take into account the full sensory variability of this fruit [3, 4]. The authors used 28 different

apple varieties in order to build more accurate preference models. They observed the segmentation of 224 consumers in three groups of which the first two, representing, respectively, 37% and 43% of the selected panel, appreciated sweet apples with high aroma intensity. These two groups were not in agreement according to the liking of acid taste and lemon aroma. The predominance of sweet taste in apple preferences was confirmed in a more recent study carried out with more than 4000 apple consumers across seven European countries [5]. Researchers used these data to develop a preference map for apple which was constructed with three main dimensions. The first one was described by the descriptors sweetness, fruitiness, and floweriness [6]. Symoneaux et al. [7] who used comment analysis of consumer's likes and dislikes as an alternative tool to preference mapping corroborated also previous results and identified sweetness along with crunchiness as main sensory preference key drivers for apple consumers.

All these studies underlined well the importance of sweetness in explaining apple preferences, but the complete understanding of it is more complex and is usually a question of equilibrium of several factors such as taste and texture as mentioned by Harker et al. [8]. It is often notable that consumers respond to a cluster of attributes, for example, sweetness and crunchiness, which reflect a particular subset of apple cultivars. From a biological perspective, these sensory attributes may be genetically associated with each other [8]. Thus, consumer preferences that link texture and acidity together may, in part, reflect the biological limitations imposed by co-location of genes on the apple chromosomes. If a consumer likes one attribute, then they are forced to also like the other. Over a period of years and of repeated experiences, consumer preferences have changed to match the biological constraints of the product.

Furthermore, food preferences are influenced by a wide range of factors such as age, gender, education, etc. [9]. For example, apple preferences associated with different age groups can be marked: children aged from 9 to 13 years tend to respond more positively to attributes of sweetness and flavor than adults who tend to respond to texture and sourness [10].

In addition, it must be considered that the acceptability of a product is the result of a combination of both intrinsic (sensory) and extrinsic attributes. Endrizzi et al. [11], for example, studied with a conjoint experiment performed on a large panel of Italian consumers how the intensity of intrinsic attributes (sweetness and crunchiness) and different information about fiber and antioxidant content (extrinsic attributes) provided immediately before tasting affect the acceptability of different apple varieties. The results confirm that high levels of sweetness and crunchiness positively influence overall liking, whereas information about nutritional components is less relevant in general but can be important for certain group of consumers which might be more aware of these aspects.

We have seen that sweetness is of great significance for apple consumers, but it is also important to consider that consumers of fresh apples take into account quality more than price [8]. Thus, postharvest research is increasingly being required to evaluate the flavor and other characteristics of fruit.

Preference for sweetness in apples and more generally in foodstuffs is depending on sugar composition of products and sweetness perception. In this chapter, we will

review the basic composition of apples, notably the different sweet taste components it contains, and how they can be measured analytically. Then, a focus will be made on the input of sensory analysis on sweet taste measurement. Sweetness perception is influenced by other sensory characteristics such as aroma and texture for which we will dedicate a part of this chapter.

2 Previous and Recent Researches Related to Sweet Taste Perception in Apple

Table 1 is an attempt to summarize research studies of the last 10 years which are related, with different degrees, to sweet taste perception in apple. The thirteen investigations reported in Table 1 dealt with various topics such as the characterization of different apple cultivars in terms of chemical/phytochemical composition, nutritional values and sensory description [12–16] and the assessment of apple quality [17–21]. Other works were more oriented on sensory science and investigated the modelization of sweet taste [22] and the impact of one sensory characteristic on the perception of another such as aroma on taste and texture perceptions [23] and mealiness on sweetness perception [24].

To achieve their goals, different investigators have used different techniques which were instrumental in some cases, sensory in others, or a combination of the two. Regarding instrumental measurements, the soluble solid content (SSC) is a frequently used index and was almost always measured. This was done by means of different kinds of refractometers. Other measurements were performed such as the quantification of single sugars (fructose, glucose, sucrose, xylose, galactose, maltose, and ribose), sugar alcohols (sorbitol, xylitol, and maltitol), and starch by means of high-pressure capillary ion chromatography [22], Luff-Schoorl technique [13], enzymatic kits [15, 20], high-performance liquid chromatography (HPLC) [14], and gas chromatography-mass spectrometry (GC-MS) [15]. See Table 1.

Concerning sensory measurements, the majority of the studies determined sweetness intensity, alone or in combination with other attributes describing apple such as sourness, crunchiness, juiciness, etc. [22, 23], with structured scales either continuous [21] or discrete [18]. Some were ranging from 0 to 9 [18], 1 to 7 [13], and 0 to 10 [19] and others from 0 to 100 [17, 22, 23] and 0 to 150 [24]. Evaluations were performed by a group of trained panelists from four [18] to nineteen panelists [22]. This variety of conditions illustrates well the fact that sweet taste measurement is complex and can be considered in different manners depending on the objective of the study. Another work that stands out from the others is the one of Charles and coauthors [23], who, in addition to conventional sensory profile to evaluate sweetness intensity, applied a dynamic method called temporal dominance of sensations (TDS) [25] to measure the evolution of perception in the time of an apple. With this method, panelists need to choose the dominant attribute at each moment of the tasting. A dominant attribute is defined as the attribute corresponding to the sensation that triggers the most your attention [25]. The attribute list was composed of

Table 1 Instrumental and sensory methodologies followed to analyze sugar composition and/or sweet perception in apples

Instrumental measurements		Sensory measurements			Refs.
Type of measurement	Technique	Type of measurement	Method	Reference standards	
SSC Sucrose, glucose, fructose, xylose, and sorbitol	Refractometer High-pressure capillary ion chromatography	Sweetness intensity	Intensity scoring on scale from 0 to 100 by 19 trained panelists	20 $g.kg^{-1}$ and 80 $g.kg^{-1}$ fructose aqueous solutions for minimum and maximum sweet intensity	[22]
SSC	n.a.	–	–	–	[12]
SSC	Refractometer	Sweetness intensity Sweetness dominance	Intensity scoring on scale from 0 to 100 by 11 trained panelists Temporal dominance of sensations with 18 trained panelists	20 $g.kg^{-1}$ and 80 $g.kg^{-1}$ fructose aqueous solutions for minimum and maximum sweet intensity no reference	[23]
–	–	Sweetness intensity	Intensity scoring on scale from 0 to 10	n.a.	[19]
SSC	Hand refractometer	Sweetness intensity	Intensity scoring on scale from 0 to 150 (150 mm) by 9 trained panelists	n.a.	[24]
Total sugar content Glucose, sucrose, and fructose	Luff-Schoorl technique (NP-1420, 1987) Enzymatic kit and spectrophotometer	Sweetness intensity	Intensity scoring on a scale from 1 to 7 by 14 selected panelists	n.a.	[13]
SSC	Portable refractometer	Sweetness intensity	Intensity scoring on a scale (100 mm) by 12 trained panelists	n.a.	[20]

(*continued*)

Table 1 (continued)

Instrumental measurements		Sensory measurements			Refs.
Type of measurement	Technique	Type of measurement	Method	Reference standards	
SSC Total sugar content	Digital refractometer Schoorl method	–	–	–	[16]
SSC	Digital refractometer	Sweetness intensity	Intensity scoring on a 0–9 point scale by 4 experts	Point 4 of the scale, 20-g sucrose/liter of fresh fresh-up crisp apple juice diluted 1:1 with distilled water	[18]
SSC	Digital refractometer	Sweetness intensity	Intensity scoring on a scale from 0 to 100 (100 mm) by 12 trained panelists	n.a.	[17]
SSC	Automatic refractometer	Sweetness intensity	Intensity scoring on a scale of 120 mm by 10 trained panelists	n.a.	[21]
Glucose, fructose, sucrose Total soluble solids (TSS)	HPLC method of Dolenc and Stampar [28] Digital refractometer at 21 °C	–	–	–	[14]
Fructose, sucrose, glucose, galactose, xylose, maltose, ribose, sorbitol, xylitol, maltitol Starch	GC-MS Starch hydrolysis by enzymatic reaction according to Jones et al. [26]	–	–	–	[15]

n.a. information not available

attributes describing the texture, the flavor and the taste, including sweetness, of apple [23].

As reviewed in this part, sweetness perception was investigated with different points of view using different techniques. It is a concept difficult to handle globally. Sweet taste perception in apple is very dependent on the composition of the fruit and of the presence of different types of sweet taste components such as sucrose, fructose, glucose, sorbitol, etc. It will be developed in the next part.

3 Basic and Sugar Composition of Apples

Chemical composition of apple fruit is very complex. It consists of numerous organic and inorganic compounds and macro-biogenic and micro-biogenic elements. Most represented are sugars, acids, pectin, tannins, starch, cellulose, and different biologically active compounds such as vitamin C and certain phenolic compounds which are known to act as natural antioxidants. Most represented chemical elements are nitrogen, phosphorus, potassium, calcium, sulfur, iron, and magnesium [12, 14, 16].

Sugars, organic acids, aromas, and phenolics determine the quality of apples and more particularly play important roles in sensory characteristics such as taste, flavor, and astringency [12, 27–30].

Different research teams investigated the composition of several apple cultivars and noted that the composition and more particularly the level of soluble solids (SSC) was varying considerably according to the cultivar [12, 14]. The cultivar harvested in summer, Delicious, showed relatively low levels of soluble solids, compared to the cultivars harvested in September and October such as Fuji and Ralls [14]. Aprea et al. [22] who collected and analyzed 40 apple batches corresponding to 17 different cultivars/accessions (harvested in North of Italy) noted a difference of soluble solid content of 6.3 Brix degrees between the extreme samples. The cultivar Pinova Roho presented the lowest value with 9.5° Brix and Fujion the highest with 15.8° Brix. They also calculated the total amount of sugars by summing the relative quantity of sucrose, fructose, glucose, and xylose and observed a range from 74.7 to 142.9 $g.kg^{-1}$, with an average concentration of 116.8 $g.kg^{-1}$. Moreover, other factors influence positively the level of sugars in fruit notably the maturity stage [28, 30] and the storage [31]. Different studies examined fruit sugars and showed increasing levels of fructose, glucose, and sucrose at advanced stages [28, 30].

Fructose and glucose were identified as the principal monosaccharides in the apple fruit [14, 22, 32, 33]. Fructose level was, with few exceptions, always higher than glucose level [14, 22, 34]. Sucrose is nevertheless the most abundant sugar (41.8%) followed by fructose (39.1%) and glucose (18.3%) [22]. Xylose, a major component of xyloglucans [35], was measured in several works and represents less than 1% of the total sugars [15, 22, 36]. A summary of apple sugar composition is presented in Table 2.

Table 2 Sugars in apple fruit: composition and relative sweetness index of individual sugars and sorbitol, soluble solid content, and total amount of sugars

	Concentration range[a]	Relative sweetness index compared to sucrose[b]
Sucrose	22–91.0 $g.kg^{-1}$	1
Fructose	27–61.0 $g.kg^{-1}$	1.08–1.33 [37–41]
Glucose	11–30.2 $g.kg^{-1}$	0.54–0.69 [37–39][41][d,e,f,h]
Xylose	n.d.–1.8 $g.kg^{-1}$	0.59 [41]
Sorbitol	1.3–13 $g.kg^{-1}$	0.54 [41]
Soluble solids	9.5–16°Brix	n.a.
Total sugars[c]	75–143 $g.kg^{-1}$	n.a.

[a]Values extracted from Aprea et al. [22] built on the analysis of 17 cultivars/accessions
[b]In water solution, at suprathreshold concentration (50 $g.kg^{-1}$ sucrose)
[c]Sum of sucrose, fructose, glucose, and xylose
n.d. not detectable, *n.a.* not available

Sorbitol is also an important compound in apple fruit as being a precursor of fructose and starch. It belongs to another class of compounds, the sugar alcohols, of which it is the most abundant in apples [36]. Sorbitol and glucose, formed from the products of photosynthesis in leaves, are the translocation sugars flowing through the phloem to reach fruit tissue, where they are converted, depending on the developmental stage, into fructose, glucose, malic acid, or starch [42]. Sorbitol is preferentially converted into fructose, while glucose is preferentially incorporated into starch [43]. In apples, only a small fraction of fructose is incorporated into starch; fructose instead accumulates in the vacuoles of apple cells [43]. As a consequence, fructose is always present in larger quantity than glucose in fruit tissue. Some researchers found that sorbitol content correlates with perceived sweetness better than any other single sugar or total sugar content [22] showing the appropriateness to take into account this compound when studying sweet perception in apples.

As just seen, apple contains a variety of sweet components that need to be measured to better understand sweet perception in apple but more generally apple quality. Different methods will be developed in the following part.

4 How to Measure Sugars by Analytical Measurements

With the goal to make the information from preference maps relevant to industrial players for use in grade or quality standards, it is necessary to translate sensory descriptors (e.g., sweet, crisp) into instrumental measurements that can be used in routine quality control protocols [8]. Currently, there exist standards defining eating apple quality in different production regions of the world including Italy, England, New Zealand, and the USA. These standards specify for the products lower and upper limits for several criteria notably for sugar content [44–46]. These specifications are strongly related to fruit ripeness.

Sugar measurements are crucial at several points of the production chain: before and at the harvest in order to pick the fruit on the right date at the desired maturity

and after harvest when fruit will be sold, transformed, or stored. The presence of sugars is an indicator of fruit ripeness which makes consequently its measurement in fruit a key point. During fruit formation, at a plant physiological level, sugar is produced in apple leaves and then transported to the fruits where it is chemically changed to starch and stored. The stored starch is slowly changed back to sugar during the period of fruit maturation [48].

In the past, several fruit ripening indexes, more or less based on empirical knowledge, were suggested such as flesh firmness, red or green color of the skin, flesh color, seed color, soluble solid content of the juice, taste, harvest drop, fruit internal ethylene concentration, and starch-iodine index [48]. They are still used by professionals in the orchards to predict harvest date and fruit maturity. In the next paragraphs, we will do a focus on the three types of measurements which are directly related to sugars, namely, the starch-iodine test evaluating the level of starch regression, the measurement of soluble solid content using a refractometer, and single sugar quantification by chromatographic methods.

4.1 Starch Regression

The starch-iodine test measures the level of starch regression and is based on the percentage of stained tissue using a fresh iodine solution [48]. The slow disappearance of starch in favor of simple sugars can be observed by periodically (once or twice each week) collecting apples and staining the starch. Briefly, the protocol consists in cutting the apple, picked up within 24 h, on the equatorial plan and applying the fresh iodine solution to one cut surface of one of the halves. One-minute delay must be observed in order for the starch pattern to be completed. Then the obtained pattern is compared to the pictures of patterns presented on the reference chart. They are associated with index number. The average starch-iodine index is then used to estimate the advancement in fruit maturity in the sampling area. It is worth noting that the pattern of starch disappearance is not the same in all apple cultivars [48]. An example of starch-iodine reference chart for Delicious is presented in Fig. 1.

4.2 Soluble Solid Content

The soluble solid content (SSC), used as an approximation of the global level of sugars, can be measured by means of a refractometer and a drop of juice [49]. Sugars are the major soluble solids in fruit juice, but other soluble materials are also present and include organic acids and amino acids, soluble pectins, etc. The result can be expressed as SSC (%) or as Brix degree (°Brix). The latter has been in common use for many years and is intended to represent the dry substance content of solutions containing mainly sucrose [50]. Refractometer optically measures the density of a liquid and is further calibrated in terms of refractive index which indicates how much a light beam is “bent” when it passes through the fruit juice. It also usually contains a scale in terms of degrees Brix. Temperature of the solutions is a very important factor

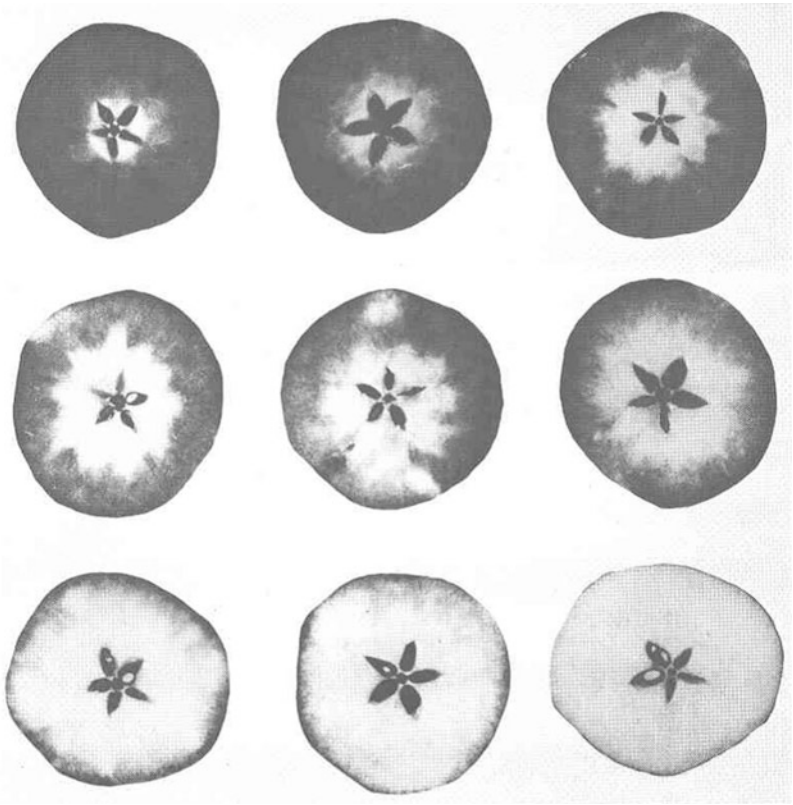


Fig. 1 Example of starch-iodine reference chart for Delicious apple cultivar. Least starch hydrolysis, top left, assigned value of 1 to the most starch hydrolysis, bottom right, assigned a value of 9 (Adapted from Smith et al. [47])

in the accuracy of reading. All materials expand when heated and become less dense. For a sugar solution, the change is about 0.5% sugar for every Celsius degree. Good-quality refractometers have a temperature compensation capability. Refractometers are often used for the measurements of concentration of solutions in the food industry [51]. The use of near-infrared ray technology as a non-destructive method is another way of predicting sugar level [44].

SSC is often included in assessments of both the preharvest ripeness and the postharvest quality of apples [52] and sometimes associated with sensory analysis tests [10, 18, 33, 44, 53–58].

4.3 Single Sugar Measurement by Chromatographic Methods

Different analytical methods, based on chromatographic separation, can be used to quantify single sugars. Chromatographic methods are rapid, sensitive, and suitable for routine analyses requiring dedicated instruments.

The method of choice in many laboratories for sugar analysis is the HPLC coupled to refractive index detectors. In the Official Methods of Analysis of AOAC International (Association of Official Analytical Chemists), the HPLC methods recommend the use of an analytical column containing a silica stationary phase with a propyl amine functionality that is very specific to the matrix. Besides the use of the stationary phase with a propyl amine functionality, there are many other separation mechanisms and chemistries for the HPLC determination of sugars. Anion exchange, cation exchange, liquid/liquid partition, and size exclusion represent a few useful chemistries. Other detectors coupled to liquid chromatography (LC) are pulsed amperometry (PA), evaporative light scattering (ELS), and mass spectrometry (MS). This allows the identification and quantification of individual carbohydrates [59].

Sugar can also be analyzed by gas chromatography after derivatization, necessary to make them volatile. Methyl ethers, acetates, trifluoroacetates (TFA), and trimethylsilyl (TMS) ethers are the most common derivatives used for carbohydrate determination. TMS ethers have a good volatility and the derivatives formed are well stable; furthermore the method is relatively simple making it one of the most popular [60].

5 Sensory Analysis: A Useful Tool to Measure Sweet Taste Perception in Apple

As seen in previous Sect. 4, the soluble solid content (SSC) expressed as °Brix is commonly used as an estimate of fruit sweetness and is included in assessments of the postharvest quality of apples [18, 61]. However, several studies showed that prediction of sweetness by °Brix in apples is inadequate [33, 62]. In general, sweetness prediction in apples is not an easy task [22, 24, 62]. Harker et al. [33] found that SSC and sweet taste are poorly correlated and recommended the use of trained sensory panels for the evaluation of sweetness in apple. Furthermore, 9 out of 13 studies, listed in Table 1, carried out sensory analysis tests to investigate questions related to fruit quality and sweetness perception.

Sensory analysis is defined as a set of methods and tools to evoke, measure, analyze, and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste, and hearing [63]. There are several advantages in using these techniques both at industrial and research levels. Firstly, it reduces uncertainty and risks in decision making. Secondly, it ensures a cost-efficient delivery of new products with high consumer acceptability. Finally, human observers are good measuring instruments. People can sometimes detect odorants at levels lower than what can be detected by an instrument and instruments cannot measure liking [63]. Moreover, on the contrary to analytical tools which allow to measure only one type of compound or characteristic (e.g., sugars, volatile compounds, texture, etc.), sensory analysis is a more holistic approach considering all sensory properties at the same time or in a sequential manner but evaluated by the same unique "instrument," the human being.

Descriptive analysis is one of the major methods used to describe the sensory characteristics of products. There are a number of variants of descriptive analysis, depending on the procedures of training and testing and on the statistical treatment of collected data. Quantitative descriptive analysis (QDA®) [64, 65] is the reference method. Descriptive analysis (DA), described as a relevant method for evaluating fruit quality, consists in rating, in terms of intensity, a certain number of attributes defined beforehand [66]. Indeed, it allows detecting fine differences of quality [49].Nevertheless most of the fruit varies at a sensory level for several properties, within a given cultivar, which makes sensory characteristic measurement difficult [67, 68]. Sensory analysis has been used in the majority of the studies presented in Table 1 and is commonly used to support breeding programs [18, 69, 70].

Sensory analysis tends to be as objective as possible in measuring various characteristics of food products. Descriptive trained panels, which can be qualitative and/or quantitative, generate data that should be equivalent in terms of quality that the one expected for a machine – to know – repeatable, reproducible, and accurate [71]. Moreover, members of a panel need to demonstrate abilities to discriminate products and to be in agreement with each other [71]. In order to avoid any bias, some particular cares need to be taken before and all along the testing phase. Hence, different aspects must be controlled such as product preparation, panelists' screening and training, tasting conditions, and environment. More details are available in methodological manuals [63, 71]. We will review below some points regarding sweet taste perception in apples specifically.

As a machine, a sensory panel should be calibrated and its own sensitivity evaluated [71]. It has been demonstrated by several studies that taste sensitivity varies a lot among the population. It is thus necessary to organize a selection of the panelists before performing sensory tests. This screening step is usually a preliminary work to an experimentation [71]. Nevertheless, this step is rarely described in the literature in the case of apples and more particularly for sweet taste. Harker and colleagues [33], who studied sweet and acid tastes in apple fruit, screened 40 panelists for the taste sensitivity during a pre-screening session. They used artificial solutions containing mixtures of sucrose (7–14 °Brix) and malic acid (0.08–0.2% wt./v), as well as whole fruit. From this step, the authors selected the 20 most taste-sensitive panelists from the group.

Another point important to take into account when performing descriptive analysis method [63] is the definition of the terms belonging to the lexicon and the associated standard references. "Sweet taste" is defined by the ISO Standard 5492 [72] as "the basic taste caused by aqueous solutions of various substances such as sucrose." It is also generally associated in the literature with a very similar definition "the intensity of the taste sensation caused by sugars." Sweet taste is a very common trait evaluated when studying apples. Among the studies listed in Table 1, 9 out of 13 measured sweet taste intensity [13, 17–24]. Sweetness discrimination threshold in an apple matrix was also determined by Harker et al. [33] and is equivalent to 1° Brix for their trained panelists.

The choice of standard references is essential as panelists will use these references to calibrate themselves for scoring the attribute on a scale. Nevertheless, these references are not frequently indicated in research articles. Two studies used 20 $g.kg^{-1}$ and 80 $g.kg^{-1}$ fructose aqueous solutions for minimum and maximum sweet intensity in apples [22, 23]. Oraguzie and colleagues [18] proposed a different reference which consists of a 20-g sucrose/liter of fresh Fresh-Up Crisp Apple juice diluted 1:1 with distilled water for the point 4 of the scale (out of 9). Different types of sugars have been used for this purpose, either fructose [22, 23] or sucrose [3]. But it is important to underline that each type of sugar has its own sweetening power. For example, fructose has a relative sweetness index of 1.08 to 1.33 compared to sucrose in a 50 $g.kg^{-1}$ aqueous solution [37–41]. In Table 2 are reported the relative sweetness indexes of the most abundant sugars present in apple fruit. However, relative sweetness varies with concentration of the sugars, and the mixture of two or more of these sugars at the proper concentration can result in a solution possessing a sweetness greater than the simple addition of the two alone (synergism) [40]. Products and concentration used for standard references are selected and determined by panelists with control of the panel leader during the training phase. References are always compared with real products relatively similar to what will be tested at the end.

As stated above, sensory analysis is a holistic method, and it is difficult to distinguish and evaluate each sensory property individually and objectively. When someone put a piece of apple in his mouth and start chewing, he will perceive in few seconds different sensations such as hardness, crunchiness, juiciness, sweet, and acid taste. As a consequence, sweetness perception is obviously influenced by other sensory characteristics: other taste sensations, aroma, and texture.

To evaluate sensory quality of fruit, sugar/acid ratios are commonly determined [14] as good relationships were found along with consumer acceptability of fruit [73–75]. Besides, the calculation of this ratio illustrates the importance of taste interactions and the masking effect of sugar on acid taste: when sugar is present or added, the sweet taste perception overcomes and reduces the sourness perception [76, 77]. This phenomenon is also described as a contrast effect between sweetness and acidity [18] and can be observed in different conditions, both in water solutions and in complex matrices (see [78] for a review).

6 Importance of Aroma/Volatile Compounds

Sweet taste perception is modulated by other taste perceptions, but it is also influenced by the presence of certain molecules responsible for aroma perception.

Aroma perception is induced by volatile compounds released by the food in the mouth during eating or drinking. They reach the olfactory receptors located in the roof of the nose after swallowing through the exhaled breath. It is also called retronasal perception.

Fruit aroma is a complex combination of numerous volatile compounds that contribute to the overall sensory quality of fruit specific to species and varieties [79]. More particularly, apple aroma is made of more than 350 volatile compounds that have been identified [80, 81]. These compounds can be classified into two key categories: the vegetal/herbaceous category (reported with the sensory attributes of green/sharp, cut grass, green apple, cucumber, and pumpkin) and, the fruity category (banana, pear, pineapple, red apple, strawberry, and ripe) [82, 83].

Taste perception is closely linked to aroma perception as both of them enter in the combination of sensations which is called flavor. Flavor was first defined as the association of taste and aroma sensations [84, 85]. Since then, its definition has evolved thanks to various and numerous researches on this topic. Nowadays, under this term are brought together different stimuli: aroma, taste, somesthetic sensations, and texture but also sound and visual cues [86]. Furthermore, Keast and coauthors [87] described flavor perception of the food/beverage as a result of complex stimulus-response interactions between a food matrix and human sensory, perceptual, and cognitive processes.

Taste and smell (ortho- or retronasal), perceived through gustatory and olfactory receptors, respectively, can modulate the perception of each other, and this phenomenon is called interaction. The study on taste-smell interactions started about 40 years ago and was initiated by the works of Murphy et al. [88] and Murphy and Cain [89]. Different types of interactions between these sensory properties occur and have been demonstrated. Taste can be modified by an aroma in different ways, either by decreasing/masking or by increasing its intensity. This will depend on the congruency of the pair of the stimuli.

Sweet taste was the most studied basic taste. Several studies showed that fruity odor and aroma enhance sweet taste perception [23, 90, 91]. For example, strawberry flavor increased perceived intensity of sweet taste, but on the contrary, peanut butter aroma had no effect. This is due to the congruence phenomenon [92]. Aromas play also a role in the perception of other tastes. For example, in a sucrose solution, caramel aroma masked sourness and on the contrary leads to a rise in sweetness intensity [93] (see [94] for a review).

However, the majority of the studies dealing with sensory interaction have generally been performed using model matrices. It allowed the perfect control of the matrix in terms of composition but leads to conclusions based on something different from a real product and relatively distant from consumer perception [94]. To date, very few studies investigating the impact of smell on taste perceptions have used real products in general and more specifically fruit and vegetables. Studies on tomatoes and strawberries have highlighted the effect of volatile compounds on the perceived sweetness intensity of these fruits [95, 96].

One recent work investigated the effect of aroma on other sensory modalities in apple fruit [23]. They modified apple discs with different aromas either fruity or vegetal and/or sweet taste. Researchers showed that, thanks to TDS dynamic method, banana aroma increased logically fruity attribute dominance but also enhanced sweet taste dominance. More interestingly, it anticipated in the evaluation the period of dominance of sweet taste attribute (Fig. 2). They observed similar

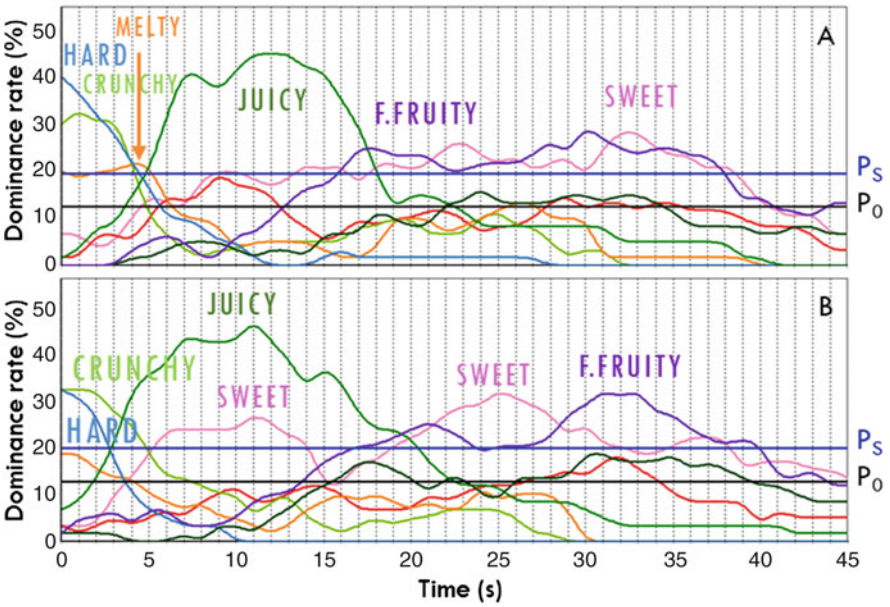


Fig. 2 **TDS curves of apples in "normal taste conditions"**. (**a**) sample without aroma addition, (**b**) sample with banana aroma. P_0 represents the chance level and P_s is the significance level (Adapted from Charles et al. [23])

results with green grass aroma. This study is the first attempt for investigating aroma-related interaction taking into account the time dimension and underlined the importance of aroma in sweetness perception in apples.

Furthermore, Aprea and co-workers [22] investigated the possible role of volatile compounds on the perception of sweetness using a statistic approach applied to a combination of instrumental and sensory data. They built different statistic models, based on OPLS regression, trying to predict sweetness perceived by sensory panel. The authors evidenced that the model including the volatile compounds (95 variables) together with single sugars (sucrose, glucose, fructose, and xylose), sorbitol, malic acid, and SSC explained 92.0% of sample sweetness variance with a good predictive ability (Q2 = 62.7%). More precisely, approximately 33% of perceived sweetness was accounted by volatile compounds. This shows once again that sensory perception is regulated by multisensory stimuli. Among the volatile compounds included in the model, some had a positive contribution of which three esters, three farnesene isomers, and benzothiazole. Furthermore, esters strongly contribute to sweet fruity descriptors in apple [97, 98] that may elicit odor-induced enhancement of sweetness perception [23, 90, 91]. On the contrary, other compounds associated with the attributes earthy fungal and green herbaceous contributed negatively to the sweetness model. A positive correlation between the intensities of sweetness with floral and fruity attributes and negative correlation with the green attributes in Fuji apples were also observed in another work [99].

7 Importance of Texture

Several authors have investigated the impact of texture properties on taste perception and specifically sweetness. These studies were mainly applied on model matrices such as solutions, juices, dairy products, and solid foods [100–105]. A common procedure consisted in adding thickening agents to modify the texture of the solutions or gels [100]. Generally, these compounds increase the viscosity of liquid systems or the hardness of gel-like systems and tend to reduce the intensity of flavor perception (taste and aroma). This is also true when using different types of sugar and with a slight different approach. Kokini [106] who studied the perceived sweetness intensity of sucrose and fructose in solutions changing the texture by varying the level of tomato solid content noted that the increase of tomato solids in solution decreased the sweetness intensity of sucrose and fructose.

The physical structure of the food matrix can impact individual physiological oral behavior (e.g., salivation, throat coating) and the strength, time, and speed of chewing. This could lead to diverse sensory interactions, which are linked to both mechanical and biochemical degradations [94]. More particularly, Gierczynski et al. [107] demonstrated that the oral behavior (i.e., chewing force, frequency and duration, as well as the opening and closing of the velum-tongue barrier) is influenced by the matrix structure. The researchers hypothesized that a firmer gel, perceived as granular and with a heterogeneous breakdown, required more attention to the texture by the subject than a softer gel did, which was perceived as smooth, spread, and thus more easily destroyed. As a consequence, for the firmer gel, less attention would be paid to other perceptions, such as taste, which would thus be perceived as less intense.

When talking about apple texture, several sensory attributes must be cited to grasp it as a whole. Juiciness, crunchiness, and hardness are usually considered as key drivers of liking, whereas mealiness is almost always rejected by consumers and seen as a defect [1–3]. Very few studies investigated the effects of specific aspects of texture on sweet taste in apples.

Harker et al. [34] studied the influence of juiciness and hardness on sweetness perception. By considering the biological understanding of apple fruit tissue, they hypothesized the existence of a relationship between perceived sweetness, hardness, and the amount of juice released during breakdown of apple flesh rather than measures of sugar and acid content. Indeed, they speculated that in mealy fruit, cell fluids are not released, and then less sugars and acids are expected to come in contact with the sensory receptors than in hard and juicy apples. Their results demonstrated that hardness had an influence on perceived juiciness; nevertheless, they did not clearly support a direct relationship between juiciness and sweetness. Thanks to the use of sensory temporal method, they showed that the sensation of sweetness persists after the sensation of juiciness has been lost. These researchers verified thus that perception of sweetness is not affected by the volume or rate of release of juice into the mouth during consumption. It seems that in this study, even if texture between samples was different (hard and crunchy vs. soft and mealy), the

viscosity of the solution (juice) was presumably unchanged. The apple system can be assimilated as a form of flavor encapsulation.

Other researchers investigated the relation between sweetness and mealiness [24]. They reported that apples displaying a high degree of mealiness are perceived as less sweet, while samples exhibiting a low degree of mealiness are perceived by the panelist as being sweeter. They also noted that this effect was not supported by a correlation between the two variables ($r = -0.15$), meaning that the effect of mealiness on the perception of sweetness is monotonous. This can be explained by the fact that in soft apples, tissue fracture occurs as a result of cell-to-cell de-bonding. Thus, when the sample is chewed, individual cells do not break and release their cellular juice avoiding sweetness perception of the dissolved sugars in the cellular fluids.

Sometimes, taste can, on the opposite, modulate texture perception. For example, a research group found that the acid taste of Boskoop apples was so strong that it tended to be more dominant than mealiness (a dry and often floury texture) in driving consumer preferences [2]. Mealiness is a negative attribute that generally causes most consumers to reject apples [108]. Furthermore, Henan et al. [109] showed interesting results about the influence of sugar composition on flavor release modulating texture in a strawberry-flavored cereal bar system by in vitro and in vivo analyses. The researchers demonstrated that the sugar composition of the cereal bars modified textural properties. More particularly, an easier structural breakdown during chewing significantly affected, as a consequence, the concentration of the flavor volatile compounds released.

8 Conclusions

Sweetness is essential in explaining preferences of apple consumers as demonstrated by various studies. However, this criterion is not the only one to be taken into account in breeding programs and when investigating apple liking. Sweet taste is always perceived in combination with the other sensory properties which thus influenced its evaluation.

Apple is composed of numerous elements of several sugars, notably sucrose, fructose, glucose, and sorbitol. Their measurements are necessary at different levels to assess fruit maturity, and quality and try to estimate its sweetness level. However, sweetness perception is a complex and multisensory process, and the consideration of gustatory stimuli solely is not sufficient to fully understand it and predict it. Other perceptions such as aroma and texture are essential. Indeed, fruity aroma tends to enhance sweetness perception contrarily to green or vegetal ones. Regarding texture, mealiness seems to decrease sweet taste intensity, but this does not mean that there is a link between juiciness degree and sweetness perception. Further researches need to be undertaken on the effects of aroma and texture on sweetness perception to better elucidate underlying mechanisms.

References

1. Daillant-Spinnler B, MacFie H, Beyts P, Hedderley D (1996) Relationships between perceived sensory properties and major preference directions of 12 varieties of apples from the southern hemisphere. Food Qual Prefer 7(2):113–126
2. Jaeger SR, Andani Z, Wakeling IN, MacFie HJ (1998) Consumer preferences for fresh and aged apples: a cross-cultural comparison. Food Qual Prefer 9(5):355–366
3. Charles M (2013) Contribution aux réflexions méthodologiques relatives à l'étude des préférences des consommateurs et à l'étude des interactions sensorielles: Application au modèle pomme. Alimentation et Nutrition. Université d'Angers, 2013.
4. Vigneau E, Charles M, Chen M (2014) External preference segmentation with additional information on consumers: a case study on apples. Food Qual Prefer 32:83–92
5. Bonany J, Brugger C, Buehler A, Carbó J, Codarin S, Donati F, Echeverria G, Egger S, Guerra W, Hilaire C (2014) Preference mapping of apple varieties in Europe. Food Qual Prefer 32:317–329
6. Bonany J, Buehler A, Carbó J, Codarin S, Donati F, Echeverria G, Egger S, Guerra W, Hilaire C, Höller I (2013) Consumer eating quality acceptance of new apple varieties in different European countries. Food Qual Prefer 30(2):250–259
7. Symoneaux R, Galmarini M, Mehinagic E (2012) Comment analysis of consumer's likes and dislikes as an alternative tool to preference mapping. A case study on apples. Food Qual Prefer 24(1):59–66
8. Harker FR, Gunson FA, Jaeger SR (2003) The case for fruit quality: an interpretive review of consumer attitudes, and preferences for apples. Postharvest Biol Technol 28(3):333–347
9. Logue A, Smith ME (1986) Predictors of food preferences in adult humans. Appetite 7(2):109–125
10. Kühn BF, Thybo AK (2001) Sensory quality of scab-resistant apple cultivars. Postharvest Biol Technol 23(1):41–50
11. Endrizzi I, Torri L, Corollaro ML, Demattè ML, Aprea E, Charles M, Biasioli F, Gasperi F (2015) A conjoint study on apple acceptability: sensory characteristics and nutritional information. Food Qual Prefer 40:39–48
12. Campeanu G, Neata G, Darjanschi G (2009) Chemical composition of the fruits of several apple cultivars growth as biological crop. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37(2):161
13. Feliciano RP, Antunes C, Ramos A, Serra AT, Figueira M, Duarte CM, de Carvalho A, Bronze MR (2010) Characterization of traditional and exotic apple varieties from Portugal. Part 1–nutritional, phytochemical and sensory evaluation. J Funct Foods 2(1):35–45
14. Wu J, Gao H, Zhao L, Liao X, Chen F, Wang Z, Hu X (2007) Chemical compositional characterization of some apple cultivars. Food Chem 103(1):88–93
15. Zhang Y, Li P, Cheng L (2010) Developmental changes of carbohydrates, organic acids, amino acids, and phenolic compounds in 'Honeycrisp'apple flesh. Food Chem 123(4):1013–1018
16. Nour V, Trandafir I, Ionica ME (2010) Compositional characteristics of fruits of several apple (Malus domestica Borkh.) cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38(3):228
17. Péneau S, Hoehn E, Roth H-R, Escher F, Nuessli J (2006) Importance and consumer perception of freshness of apples. Food Qual Prefer 17(1):9–19
18. Oraguzie N, Alspach P, Volz R, Whitworth C, Ranatunga C, Weskett R, Harker FR (2009) Postharvest assessment of fruit quality parameters in apple using both instruments and an expert panel. Postharvest Biol Technol 52(3):279–287
19. Donno D, Beccaro G, Mellano M, Torello Marinoni D, Cerutti A, Canterino S, Bounous G (2012) Application of sensory, nutraceutical and genetic techniques to create a quality profile of ancient apple cultivars. J Food Qual 35(3):169–181
20. Gatti E, Di Virgilio N, Magli M, Predieri S (2011) Integrating sensory analysis and hedonic evaluation for apple quality assessment. J Food Qual 34(2):126–132

21. Rizzolo A, Vanoli M, Spinelli L, Torricelli A (2010) Sensory characteristics, quality and optical properties measured by time-resolved reflectance spectroscopy in stored apples. Postharvest Biol Technol 58(1):1–12
22. Aprea E, Charles M, Endrizzi I, Corollaro ML, Betta E, Biasioli F, Gasperi F (2017) Sweet taste in apple: the role of sorbitol, individual sugars, organic acids and volatile compounds. Sci Rep 7:44950
23. Charles M, Endrizzi I, Aprea E, Zambanini J, Betta E, Gasperi F (2017) Dynamic and static sensory methods to study the role of aroma on taste and texture: a multisensory approach to apple perception. Food Qual Prefer 62:17–30
24. Echeverría G, Graell J, Lara I, Lopez M, Puy J (2008) Panel consonance in the sensory evaluation of apple attributes: influence of mealiness on sweetness perception. J Sens Stud 23(5):656–670
25. Pineau N, Schlich P, Cordelle S, Mathonnière C, Issanchou S, Imbert A, Rogeaux M, Etiévant P, Köster E (2009) Temporal dominance of sensations: construction of the TDS curves and comparison with time–intensity. Food Qual Prefer 20(6):450–455
26. Jones MG, Outlaw WH, & Lowry, OH (1977) Enzymic assay of 10− 7 to 10− 14 moles of sucrose in plant tissues. Plant Physiol 60(3):379–383.
27. Bengoechea ML, Sancho AI, Bartolomé B, Estrella I, Gómez-Cordovés C, Hernández MT (1997) Phenolic composition of industrially manufactured purees and concentrates from peach and apple fruits. J Agric Food Chem 45(10):4071–4075
28. Dolenc K, Stampar F (1997) An investigation of the application and conditions of analyses of HPLC methods for determining sugars and organic acids in fruits. Res Rep Biotech Fac Univ Ljubljana 69:99–106
29. Fuleki T, Pelayo E, Palabay RB (1994) Sugar composition of varietal juices produced from fresh and stored apples. J Agric Food Chem 42(6):1266–1275
30. Miller NJ, Rice-Evans CA (1997) The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chem 60(3):331–337
31. Jha SN, Rai D, Shrama R (2012) Physico-chemical quality parameters and overall quality index of apple during storage. J Food Sci Technol 49(5):594–600
32. Ackermann J, Fischer M, Amado R (1992) Changes in sugars, acids, and amino acids during ripening and storage of apples (cv. Glockenapfel). J Agric Food Chem 40(7):1131–1134
33. Harker FR, Marsh KB, Young H, Murray SH, Gunson FA, Walker SB (2002) Sensory interpretation of instrumental measurements 2: sweet and acid taste of apple fruit. Postharvest Biol Technol 24(3):241–250
34. Harker FR, Amos RL, Echeverría G, Gunson FA (2006) Influence of texture on taste: insights gained during studies of hardness, juiciness, and sweetness of apple fruit. J Food Sci 71(2):S77
35. Winisdorffer G, Musse M, Quellec S, Barbacci A, Le Gall S, Mariette F, Lahaye M (2015) Analysis of the dynamic mechanical properties of apple tissue and relationships with the intracellular water status, gas distribution, histological properties and chemical composition. Postharvest Biol Technol 104:1–16
36. Füzfai Z, Katona ZF, Kovács E, Molnár-Perl I (2004) Simultaneous identification and quantification of the sugar, sugar alcohol, and carboxylic acid contents of sour cherry, apple, and ber fruits, as their trimethylsilyl derivatives, by gas chromatography− mass spectrometry. J Agric Food Chem 52(25):7444–7452
37. Pangborn R (1963) Relative taste intensities of selected sugars and organic acids. J Food Sci 28(6):726–733
38. Cameron AT (1947) The taste sense and the relative sweetness of sugars and other sweet substances. Sugar research foundation, New York
39. Dahlberg A, Penczek E (1940) Dextrose and corn sirup for frozen desserts. NY St Agric Exp Sta Tech Bull 3–36
40. Stone H, Oliver SM (1969) Measurement of the relative sweetness of selected sweeteners and sweetener mixtures. J Food Sci 34(2):215–222

41. Yamaguchi S, Yoshikawa T, Ikeda S, Ninomiya T (1970) Studies on the taste of some sweet substances: part I. Measurement of the relative sweetness part II. Interrelationships among them. Agric Biol Chem 34(2):181–197
42. Yamaki S (2010) Metabolism and accumulation of sugars translocated to fruit and their regulation. J Jpn Soc Horticult Sci 79(1):1–15
43. Yamaki S, Ino M (1992) Alteration of cellular compartmentation and membrane permeability to sugars in immature and mature apple fruit. J Am Soc Hortic Sci 117(6):951–954
44. Harker FR, Kupferman EM, Marin AB, Gunson FA, Triggs CM (2008) Eating quality standards for apples based on consumer preferences. Postharvest Biol Technol 50(1):70–78
45. Grange G (1976) United States standards for grades of apples. United States Department of Agriculture, Washington, DC
46. WSDA (1999) Clarifying new Red Delicious inspections. Good fruit grower, 50:41
47. Smith RB, Lougheed EC, Franklin EW, McMillan I (1979) The starch iodine test for determining stage of maturation in apples. Can J Plant Sci 59(3):725–735
48. Blanpied G, Silsby KJ (1992) Predicting harvest date windows for apples. Cornell Cooperative Extension, Ithaca
49. Vaysse P, Reynier P, Codarin S, Roche L, Marti A (2006) L'analyse sensorielle sur la pomme: Un outil indispensable pour la filière. Infos-Ctifl 225:20–23
50. ICUMSA (2007) Method GS4/3–13: the determination of refractometric dry substance (RDS %) of molasses and very pure syrups (liquid sugars). International Commission for Uniform Methods of Sugar Analysis, Verlag Dr. Albert Bartens KG, Berlin
51. Dongare M, Buchade P, Awatade M, Shaligram A (2014) Mathematical modeling and simulation of refractive index based brix measurement system. Optik-Int J Light Electron Opt 125(3):946–949
52. Smith S (1985) Measurement of the quality of apples: recommendations of an EEC working group. Office for Official Publication of the European Communities, Luxembourg
53. Aaby K, Haffner K, Skrede G (2002) Aroma quality of Gravenstein apples influenced by regular and controlled atmosphere storage. LWT-Food Sci Technol 35(3):254–259
54. Lateur M, Planchon V, Moons E (2001) Evaluation par l'analyse sensorielle des qualités organoleptiques d'anciennes variétés de pommes. Biotechnol Agron Soc Environ 5(3):180–188
55. Mehinagic E, Royer G, Symoneaux R, Bertrand D, Jourjon F (2004) Prediction of the sensory quality of apples by physical measurements. Postharvest Biol Technol 34(3):257–269
56. Thybo AK, Kühn BF, Martens H (2004) Explaining Danish children's preferences for apples using instrumental, sensory and demographic/behavioural data. Food Qual Prefer 15(1):53–63
57. Varela P, Salvador A, Fiszman S (2005) Shelf-life estimation of 'Fuji' apples: sensory characteristics and consumer acceptability. Postharvest Biol Technol 38(1):18–24
58. Watada AE, Abbott JA, Hardenburg RE (1980) Sensory characteristics of apple fruit [cultivars, storage, quality]. J-Am Soc Horticult Sci (USA) 105:371–375
59. Raessler M (2011) Sample preparation and current applications of liquid chromatography for the determination of non-structural carbohydrates in plants. TrAC Trends Anal Chem 30(11):1833–1843
60. Ruiz-Matute A, Hernandez-Hernandez O, Rodriguez-Sanchez S, Sanz M, Martinez-Castro I (2011) Derivatization of carbohydrates for GC and GC–MS analyses. J Chromatogr B 879(17):1226–1240
61. Guan Y, Peace C, Rudell D, Verma S, Evans K (2015) QTLs detected for individual sugars and soluble solids content in apple. Mol Breed 35(6):135
62. Corollaro ML, Aprea E, Endrizzi I, Betta E, Demattè ML, Charles M, Bergamaschi M, Costa F, Biasioli F, Corelli Grappadelli L (2014) A combined sensory-instrumental tool for apple quality evaluation. Postharvest Biol Technol 96:135–144
63. Stone H, Sidel JL (2004) Descriptive analysis. In: Stone H, Sidel JL (eds) Sensory evaluation practices. Academic, London, pp 201–245
64. Stone H, Sidel JL (1998) Quantitative descriptive analysis: developments, applications and the future. Food Technol (USA) 52:48

65. Stone H, Sidel JL, Oliver S, Woolsey A, Singleton C (1974) Sensory Evaluation by quantitative descriptive analysis. Food Technol 28:24–28
66. Lawless HT, Heymann H (2010) Sensory evaluation of food: principles and practices. Springer Science & Business Media, New York
67. Dever MC, Cliff MA, Hall JW (1995) Analysis of variation and multivariate relationships among analytical and sensory characteristics in whole apple evaluation. J Sci Food Agric 69(3):329–338
68. Williams AA, Carter CS (1977) A language and procedure for the sensory assessment of Cox's Orange Pippin apples. J Sci Food Agric 28(12):1090–1104
69. Brookfield PL, Nicoll S, Gunson FA, Harker FR, Wohlers M (2011) Sensory evaluation by small postharvest teams and the relationship with instrumental measurements of apple texture. Postharvest Biol Technol 59(2):179–186
70. Hampson C, Quamme H, Hall J, MacDonald R, King M, Cliff M (2000) Sensory evaluation as a selection tool in apple breeding. Euphytica 111(2):79–90
71. Depledt F (ed) (2013) Evaluation Sensorielle, manuel méthodologique, 3e edn., Lavoisier / Tec&Doc, Paris
72. ISO Standard (1992) 5492. Terms relating to sensory analysis. International Organization for Standardization. Austrian Standards Institute, Vienna
73. Vangdal E (1985) Quality criteria for fruit for fresh consumption. Acta Agric Scand 35(1):41–47
74. Fellers PJ (1991) The relationship between the ratio of degrees brix to percent acid and sensory flavor in grapefruit juice. Food Technol (USA) 45:72–75
75. Mitchell GF, Mayer G, Biasi W (1991) Effect of harvest maturity on storage performance of 'Hayward'kiwifruit. Acta Hortic 297:617–626
76. Stevens JC (1996) Detection of tastes in mixture with other tastes: issues of masking and aging. Chem Senses 21(2):211–221
77. Pangborn R, Trabue IM (1964) Taste interrelationships. V. Sucrose, sodium chloride, and citric acid in lima bean puree. J Food Sci 29(2):233–240
78. Keast RS, Breslin PA (2003) An overview of binary taste–taste interactions. Food Qual Prefer 14(2):111–124
79. Sanz C, Olias JM, Perez AG (1997) Aroma biochemistry of fruits and vegetables. In: Tomas-Barberan FA, Robins RJ (eds) Phytochemistry of fruit and vegetables. Oxford University Press, New York, pp 125–155
80. Maarse H (1991) Volatile compounds in foods and beverages. CRC press, New York
81. Nijssen L, van Ingen-Visscher C, Donders J (2011) Volatile Compounds in Food (VCF) database, version 13.1. TNO Triskelion, Zeist
82. Aprea E, Corollaro ML, Betta E, Endrizzi I, Demattè ML, Biasioli F, Gasperi F (2012) Sensory and instrumental profiling of 18 apple cultivars to investigate the relation between perceived quality and odour and flavour. Food Res Int 49(2):677–686
83. Dixon J, Hewett EW (2000) Factors affecting apple aroma/flavour volatile concentration: a review. N Z J Crop Hortic Sci 28(3):155–173
84. Dalton P, Doolittle N, Nagata H, Breslin P (2000) The merging of the senses: integration of subthreshold taste and smell. Nat Neurosci 3(5):431–432
85. Prescott J (1999) Flavour as a psychological construct: implications for perceiving and measuring the sensory qualities of foods. Food Qual Prefer 10(4):349–356
86. Spence C (2012) Chapter: 10 Multi-sensory integration and the psychophysics of flavour perception. In: Food oral processing: fundamentals of eating and sensory perception. Blackwell, Oxford, p 203
87. Keast RS, Dalton PH, Breslin PA (2004) Flavor interactions at the sensory level. In: Flavor perception. Blackwell, Oxford, pp 228–255
88. Murphy C, Cain WS, Bartoshuk LM (1977) Mutual action of taste and olfaction. Sens Processes 1(3):204–211
89. Murphy C, Cain WS (1980) Taste and olfaction: independence vs interaction. Physiol Behav 24(3):601–605

90. Bonnans SR, Noble A (1995) Interaction of salivary flow with temporal perception of sweetness, sourness, and fruitiness. Physiol Behav 57(3):569–574
91. Cliff M, Noble AC (1990) Time-intensity evaluation of sweetness and fruitiness and their interaction in a model solution. J Food Sci 55(2):450–454
92. Schifferstein HN, Verlegh PW (1996) The role of congruency and pleasantness in odor-induced taste enhancement. Acta Psychol 94(1):87–105
93. Stevenson RJ, Prescott J, Boakes RA (1999) Confusing tastes and smells: how odours can influence the perception of sweet and sour tastes. Chem Senses 24(6):627–635
94. Poinot P, Arvisenet G, Ledauphin J, Gaillard J-L, Prost C (2013) How can aroma–related cross–modal interactions be analysed? A review of current methodologies. Food Qual Prefer 28(1):304–316
95. Baldwin E, Scott J, Einstein M, Malundo T, Carr B, Shewfelt R, Tandon K (1998) Relationship between sensory and instrumental analysis for tomato flavor. J Am Soc Hortic Sci 123(5):906–915
96. Schwieterman ML, Colquhoun TA, Jaworski EA, Bartoshuk LM, Gilbert JL, Tieman DM, Odabasi AZ, Moskowitz HR, Folta KM, Klee HJ (2014) Strawberry flavor: diverse chemical compositions, a seasonal influence, and effects on sensory perception. PLoS One 9(2):e88446
97. Mehinagic E, Royer G, Symoneaux R, Jourjon F, Prost C (2006) Characterization of odor-active volatiles in apples: influence of cultivars and maturity stage. J Agric Food Chem 54(7):2678–2687
98. Komthong P, Katoh T, Igura N, Shimoda M (2006) Changes in the odours of apple juice during enzymatic browning. Food Qual Prefer 17(6):497–504
99. Tanaka F, Miyazawa T, Okazaki K, Tatsuki M, Ito T (2015) Sensory and metabolic profiles of "fuji" apples (malus domestica borkh.) grown without synthetic agrochemicals: the role of ethylene production. Biosci Biotechnol Biochem 79(12):2034–2043
100. Saint-Eve A, Déléris I, Panouillé M, Dakowski F, Cordelle S, Schlich P, Souchon I (2011) How texture influences aroma and taste perception over time in candies. Chemosens Percept 4(1–2):32
101. Hollowood T, Linforth R, Taylor A (2002) The effect of viscosity on the perception of flavour. Chem Senses 27(7):583–591
102. Bult JH, de Wijk RA, Hummel T (2007) Investigations on multimodal sensory integration: texture, taste, and ortho-and retronasal olfactory stimuli in concert. Neurosci Lett 411(1):6–10
103. Lethuaut L, Brossard C, Rousseau F, Bousseau B, Genot C (2003) Sweetness–texture interactions in model dairy desserts: effect of sucrose concentration and the carrageenan type. Int Dairy J 13(8):631–641
104. de Roos KB (2003) Effect of texture and microstructure on flavour retention and release. Int Dairy J 13(8):593–605
105. Ferry A-L, Hort J, Mitchell J, Cook D, Lagarrigue S, Pamies BV (2006) Viscosity and flavour perception: why is starch different from hydrocolloids? Food Hydrocoll 20(6):855–862
106. Kokini JL, Bistany K, Poole M, Stier E (1982) Use of mass transfer theory to predict viscosity-sweetness interactions of fructose and sucrose solutions containing tomato solids. J Texture Stud 13(2):187–200
107. Gierczynski I, Laboure H, Guichard E (2008) In vivo aroma release of milk gels of different hardnesses: inter-individual differences and their consequences on aroma perception. J Agric Food Chem 56(5):1697–1703
108. Andani Z, Jaeger S, Wakeling I, MacFie H (2001) Mealiness in apples: towards a multilingual consumer vocabulary. J Food Sci 66(6):872–879
109. Heenan S, Soukoulis C, Silcock P, Fabris A, Aprea E, Cappellin L, Märk TD, Gasperi F, Biasioli F (2012) PTR-TOF-MS monitoring of in vitro and in vivo flavour release in cereal bars with varying sugar composition. Food Chem 131(2):477–484

Advances in Pseudocereals: Crop Cultivation, Food Application, and Consumer Perception

57

Natalia Manzatti Machado Alencar and
Ludmilla de Carvalho Oliveira

Contents

Abstract

Quinoa, amaranth, and buckwheat are the most important pseudocereals. Despite pseudocereals resemble in function and composition of those of the true cereals, the seeds overcome cereal properties in some aspects. Recently, the pseudocereals have attracted attention because of the proteins with high nutritive value, and their storage proteins are not toxic for celiac patients. Moreover, the seeds are an important source of dietary fiber and phenols, which are associated with health

N. M. M. Alencar (✉)
Department of Food and Nutrition, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil
e-mail: natalia.manzatti@gmail.com

L. de Carvalho Oliveira
Department of Food Technology, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil
e-mail: ludmillacoliveira@outlook.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_63

benefits. Studies have shown the pseudocereal flour suitability as cereal flour replacer in diverse food products (functional and gluten-free). At present, the food application of pseudocereals in bakery products, fermented beverages, and extruded products, among others, has shown successful. Therefore, the pseudocereals have a great potential to popularize in several countries not yet achieved, by introducing crops in human's food diet and providing new products of high quality (technological, nutritional, and sensorial). For these reasons, this chapter describes some relevant and actual information about worldwide pseudocereal crop production, nutritional and functional composition, use of pseudocereal flours in food product development, and consumer perception.

Keywords
Amaranth · Quinoa · Buckwheat · Nutritional composition · Antioxidant · Flours · Bakery products · Gluten-free · Consumer perception/

1 Introduction

Pseudocereals (also referred to as Andean grains), which include amaranth, quinoa, and buckwheat, present an attractive nutritional profile and are gluten-free grains. Quinoa and amaranth are characterized by having excellent protein quality, while buckwheat has a high level of phytochemical. In this respect, pseudocereals have been used as ingredient to increase the nutritional status in several bakery products, beverages, and gluten-free products. The use of pseudocereals in bakery product manufacturing has advantage due to the starch content inside the seeds; starch is the predominant quantitative constituent [1–5].

The regular consumption of pseudocereals promotes health benefits as reduced risk of cardiovascular disease and diabetes, anticarcinogenic effect, anti-inflammatory, and antimicrobial. This effect is due the presence of isoflavonoids and phytosterols in the grain [6]. These grains have been increasingly researched as nutritious ingredients in gluten-free formulations and as source of bioactive compounds with health-promoting effects. The regular consumption of pseudocereals promotes health benefits as reduced risk of cardiovascular disease, diabetes, anticarcinogenic effect, anti-inflammatory and antimicrobial. Some of the most attractive features of these seeds include their high-quality protein and the presence of abundant quantities of fiber and minerals such as calcium and iron [7].

Currently, new food products have been obtained from pseudocereals, including for nutrient and mineral improvement purpose. Thus, it makes contribution for enhancing grain popularity between consumers. The use of pseudocereals as ingredient can be found in different food segments: snacks [8–10], traditional breads and gluten-free breads [7, 11–13], pasta [11], porridge [13], fermented beverages [14, 15], and probiotic foods [15, 16]. The potential use of such grains in food products has been ensured by good acceptance getting in sensorial tests.

This chapter focuses on pseudocereal grains and respective flour characterization (nutrients and bioactive compounds) and technological, functional, and sensory properties of pseudocereal-based food products. The interest by Andean grains has increased as researchers showed the potential of such grains on supplying proteins, fibers, minerals, and phytochemicals. Furthermore, the sensory profile, acceptance, and perceptions of consumer are important to consider in pseudocereal-based products. This chapter was split in three sections: (1) aspects about crop cultivation, (2) discussion on recent researches with respect to pseudocereal ingredient use in food development, (3) approach in sensory aspects and consumer perception.

2 Pseudocereal Crops

In recent years, the agricultural production has been increased compared to other times. In the years between 1960 and 2011, agricultural production has tripled due to the development and widespread use of new farming technologies. Thus, agricultural technology improvement provided higher yields of major crops as cereals, roots and tubers, pulses, sugar crops, and vegetables in some potential regions around the world [17].

Cereals are the most important crop category grown across the world. The major cereal crops include wheat, maize, barley, rice, and sorghum, as showed in Table 1.

Although wheat, maize, and rice cereals are the most important crops grown, pseudocereals (amaranth, quinoa, and buckwheat) have been recognized as a notable seed, potentially used for human nutrition, whose cultivation developed in various regions of the world [19].

2.1 Morphological Definition

Pseudocereals are defined as starchy food grains excluding those currently classified as cereals, legumes, oilseeds, and nuts [20]. They are dicotyledonous plants that resemble in function and composition those of the true cereals (monocotyledonous) but do not belong to Gramineae families. According to some phylogenetic

Table 1 Total production of major cereal crops

Crop name	Production in million tons (MT)
Maize	1037.8
Wheat	729.8
Rice	741.5
Barley	144.5
Sorghum	68.9
Oats	22.7
Rye	15.2

Reference [18]

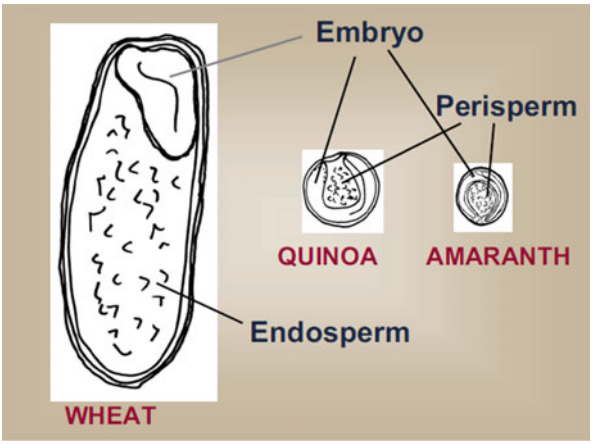


Fig. 1 Botanic structure of quinoa and amaranth seeds (Reference [1]). Copyright: Regine Schönlechner

classifications, the *Amaranthus* and *Chenopodium* genera belong together in the order Caryophyllales, whereas buckwheat (*Fagopyrum*) belongs in the Polygonales. Polygonales and Caryophyllales were closely related and are combined together in subclass Caryophyllidae, although later studies asserted that there is significant genetic distance between them [21].

The main grain amaranth species used today are *Amaranthus caudatus* L. (syn. edulis Spegazzini), *Amaranthus cruentus* L. (syn. *paniculatus* L.), and *Amaranthus hypochondriacus*. Among quinoa, sweet and bitter varieties exist, dependent on the content of saponins (saponin content <0.11% – sweet variety) [20]. Two varieties of buckwheat are commonly cultivated: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) [22].

Amaranth seeds are lentil-shaped and measure about 1 mm in diameter. Quinoa seeds are slightly larger than amaranth seeds. In contrast to cereals, the embryo surrounds the starch-rich tissue (perisperm) in the form of a ring and makes about 25% of the total seed weight. The buckwheat seed is a three-angled achene, 6–9 mm long. The fruit of *F. tataricum* is smaller (4–5 mm) and more rounded at the edges. Structurally and chemically, the endosperm resembles that of a cereal grain consisting of a non-starchy aleurone layer and large cells packed with starch granules constituting the majority of the endosperm [22] (Fig. 1).

2.2 Pseudocereals: Cultivation Around the World

Among the pseudocereals, quinoa (*Chenopodium quinoa*) and amaranth (*Amaranthus*) were an important crop for the pre-Colombian culture in Latin America, while buckwheat (*Fagopyrum esculentum*) originated from Central Asia [1].

Quinoa is a food plant of the Chenopodiaceae family, *Chenopodium* genus, native to the Andean regions of Chile, Peru, Ecuador, and Bolivia, and its cultivation dates back thousands of years, making this food, which comes from the Andean cradle, the

oldest ever recorded. Quinoa plants stand out by the considerable resistance to weather climate and soil conditions. There is a great diversity in the properties and quality of quinoa products due to the wide geographic distribution and genetic resources. The most widespread varieties of quinoa are sweet and bitter seeds. The colors of quinoa seeds are rather various, including white, yellow, red, black, and so on [20]. The world production of quinoa is estimated at around 510 tons (102,745 ha). Today, the main producers and exporters of quinoa seeds in the world are Bolivia and Peru. Together, these countries are responsible by taking over the worldwide market [23].

Amaranth is a pseudocereal native from northern higher-altitude regions of Bolivia, Ecuador, and Peru, and its species have already been cultivated in Mexico since 400 years BC [24]. Furthermore, amaranth grains are grown in different tropical regions of Africa, Central and South America, and South Asia and warm region as North America [23]. The genus *Amaranthus* L. contains more than 60 species. The main amaranth species being cultivated for their seeds and most used for human nutrition are *A. caudatus* in Peru and other Andean countries, *A. cruentus* in Guatemala, and *A. hypochondriacus* in Mexico [25]. The crop has high potential of increasing its production and consumption, especially in tropical regions.

The origin of buckwheat started in Central Asia and Siberia. However, it was cultivated in Europe and Russia and so spread to North America. As a consequence of immigration, this crop was introduced to Brazil and Chile around 1866. In 2009, the world's buckwheat production reached 3,000,000 tons/year. The main countries responsible for buckwheat production are Russia, Ukraine, Poland, the United States, Canada, and Brazil [23, 26].

As previously reported, pseudocereal cultivation has been introduced in many countries because of the importance on population diet. As the dissemination of such information, the grains have gained the world market, thus reaching more consumers and consequently more countries [19].

2.3 Nutritional Composition

Amaranth, quinoa, and buckwheat are currently relevant theme with respect to nutritional composition. Quinoa is one of the most nutritious foods used in human food, chosen by the FAO as one of the crops destined to collaborate with food security in this century [27]. Pseudocereals are mainly dietary fiber and protein source (Table 2). Amaranth contains higher protein and lipid content (ash) than wheat and other cereals [28]. Quinoa also overcomes cereals in the level of lipids and vitamins B1, B2, B6, C, E. Otherwise, buckwheat composition is most similar to wheat.

Albumins and globulins are most of the storage proteins found in pseudocereals. On the other hand, prolamin- and glutelin-like proteins are less abundant. Exceptionally, amaranth, quinoa, and buckwheat are gluten-free seeds, which enable their use by celiac patients. The absence of gliadins (gluten-forming prolamins present in wheat) and protein fractions corresponding to gliadin (found in oats, barley, rye, and malt), which are considered toxic for celiac patients, makes

Table 2 Chemical composition of amaranth, quinoa, buckwheat, and wheat (g/100)

Chemical composition	Amaranth	Quinoa	Buckwheat	Wheat
Protein	16.5	14.5	12.5	12.0–14
Fat	5.7	5.2	2.1	2.5
Total starch	61.4	64.2	58.9	63.0
Dietary fiber	20.6	14.2	29.5	17.4
Ash	2.8	2.7	2.1	1.5

Reference [28]

pseudocereals appropriate for the preparation of food products popularly referred to as "gluten-free" [29].

Celiac disease is an autoimmune enteropathy associated to genetic factors (HLA-DQ2 and HLA-DQ8). The response to disease is triggered when blind gluten peptides link to HLA-DQ2 and HLA-DQ8 and present them to T cells, which are located in the lamina propria. The T cells found gluten-sensitive $CD4^+$ helper cells, and the mechanism of the disease (immune reaction) starts, and various symptoms occur [30]. The only currently available treatment for celiac disease consists in dietary exclusion of grains containing gluten and supportive nutritional care in case of iron, calcium, and vitamin deficiencies.

Besides having great amounts of protein, amaranth is an important source of some important essential amino acids: alanine, valine, leucine, arginine, phenylalanine, pralines, methionines, α-aminobutyric acid, tryptophan, and isoleucine [31]. Owing to the similar amino acid profile, amaranth can be considered a nutritive substitute for cereals [32]. Quinoa seed has attracted attention as a new food source, because of the quality and nutritional value of proteins. Quinoa contains more protein content and balanced distribution of essential amino acids than cereals, resembling the biological value of protein in milk (casein). Thus, quinoa is one of the few plant foods that provide all essential amino acids for human life with values close to those set by the Food and Agriculture Organization (FAO), being rich in sulfur amino acids and lysine, unlike the protein content of cereals, which are especially deficient in lysine [33–35]. Buckwheat contains higher levels of lysine, arginine, and aspartic acid compared to cereals [36]. Specially, the nutritional value and the techno-functional properties of proteins found in pseudocereals make them a promising alternative for food applications [24, 37].

Quinoa has been considered an alternative oilseed crop, due to the quality and quantity of its lipid fraction. Quinoa has a fat content between 2.0 and 9.5%, being rich in unsaturated essential fatty acids (66% of fat composition). The fatty acids of quinoa oil are polyunsaturated. The most abundant unsaturated fatty acids in amaranth and quinoa are linoleic and oleic acid, whereas palmitic acid is the major saturated fatty acid reported for all pseudocereals [2, 38].

Starch is the main quantitative carbohydrate and component of pseudocereals, like in cereals. The content of carbohydrates in quinoa seeds consists mainly of starch (53.5–69.2%, dry basis) and a small percentage of sugars (maltose, D-galactose, D-ribose, fructose, and glucose). Quinoa starch has excellent freeze-

thaw stability which makes it an ideal thickener for sauces, condiments, and soups due to its low gelation temperature and storage stability at low temperatures and in other applications where resistance to retrogradation is desired, and it may also be used to produce a creamy and smooth texture similar to fats. The gelatinization of quinoa starch occurs at a relatively low temperature, between 62.6 °C and 67 °C [39, 40]. The amylose content of quinoa starch ranges from 3% to 22%, which is lower than that present in wheat or maize. The starch of amaranth species grains has amylose content ranging from 2.0% to 65.2% [31]. Lastly, buckwheat starch amylose represents 20–28% content, similar to cereals [41]. The amylose content differs between botanical sources and has significant effect on physical-chemical properties and functional characteristics of starch. Generally, the content of starch in pseudocereals and their properties, such as viscosity, provide some opportunities in food applications as baked products.

Studies have shown that the pseudocereals amaranth, quinoa, and buckwheat represent good sources of dietary fiber. In particular, dietary fiber content is significantly higher in buckwheat seeds in comparison with amaranth and quinoa, which have fiber levels comparable to those found in common cereals (Table 2) [28]. Taking into account the inadequate intake of dietary fiber by celiacs, the experts have recommend a higher consumption of whole grain pseudocereals to alleviate, at least in part, the deficit in fiber intake in this sector of the population.

Pseudocereals contain high level of minerals compared to wheat (Table 3). Amaranth and quinoa contribute with 50% of Dietary Reference Intakes (DRIs) for copper, iron, manganese, magnesium, and phosphorus. These seeds are also a good calcium source, which are relevant for celiac, since some patients present osteopenia and osteoporosis [42]. However, potassium, magnesium, and phosphorus may dominate in some quinoa genotypes [43]. Buckwheat is considered a mineral source compared to cereal of levels of magnesium, zinc, potassium, phosphorus, copper, and manganese [26, 36].

Amaranth is a good source of vitamin C and riboflavin (B_2) [31], whereas quinoa has significant concentrations of pyridoxine (B6) and folic acid in quinoa. Furthermore, both amaranth and quinoa are important source of tocopherol (vitamin E) [44]. Buckwheat exhibits high levels of vitamins B and C and tocopherols [2, 36].

In short, the valuable nutritional composition of pseudocereals, similar or greater than cereals, makes the ancient grains adequate for food industry. Also,

Table 3 Mineral content in pseudocereals and wheat

Minerals	Amaranth	Quinoa	Buckwheat	Wheat
Ca	180.1 ± 6.1	32.9 ± 3.3	60.9 ± 3.3	34.8 ± 0.0
Mg	279.2 ± 1.1	206.8 ± 6.4	203.4 ± 8.8	96.4 ± 3.7
Zn	1.6 ± 0.0	1.8 ± 0.0	1.0 ± 0.0	1.2 ± 0.1
Fe	9.2 ± 0.2	5.5 ± 0.5	4.7 ± 0.1	3.3 ± 0.1

Reference [2]. Data presented as mg/100 g dry-weight basis ± standard deviation

pseudocereals seem a good solution for nutritional problems as celiac disease, especially in less developed countries.

2.4 Phenolic Compounds and Antioxidant Activity

Pseudocereals are good sources of phenolic compounds such as phenolic acids. Phenolics are relatively hydrophilic and may include phenolic acids, flavonoids, and tannins, and they make up the majority of the secondary metabolites of plants that contribute to diverse physiological effects. Such bioactive compounds have shown a positive correlation with antioxidant activity and total phenols in pseudocereals. Recently, much attention has been focused on natural antioxidants, which can play an important role in inhibiting free radicals and oxidation chain reactions within tissues, in particular for the protection of cell membranes, with proven success in neural functions, reducing the risk of several degenerative diseases associated with oxidative stress such as cancer, cardiovascular disease, and osteoporosis [45].

Among cereals and pseudocereals, buckwheat is one of the best sources of polyphenols, being quercetin, apigenin, and luteolin as the main flavonoid glycosides found. The polyphenol's content in the pericarp is significantly higher than in the grains [43]. Buckwheat bran and hulls have two to seven times higher antioxidant activity than barley, triticale, and oats.

Quinoa seeds are also an abundant source of flavonoids, which consist mainly of quercetin and kaempferol glycosides [46]. Quinoa grains contain other various phenolics such as rutin and their derivatives, vanillic acid, ferulic acid, and ferulic acid-4-glucoside [47]. Quinoa has higher antioxidative power than amaranth [48]. Recent findings have suggested that quinoa phenolics may have the potential to prevent hyperglycemia and its associated complications by means of their ability to slow both oxidation-related damage to various organs and intestinal digestion of carbohydrates [45].

Amaranth is a rich source of polyphenols (flavonoids) with relative high antioxidant activity. The major phenolics found in amaranth seeds are caffeic acid, p-hydroxybenzoic acid, and ferulic acid [46]. Ferulic acid was investigated in amaranth-insoluble fiber, and the results showed it is predominantly bound to pectic arabinans and galactans [49]. Other bioactive compounds are present in amaranth grain including flavonoids, phenolic acids, anthocyanins, tannins, and phytosterols [46].

Despite the high proportion of total phenols, other non-phenolic compounds, such as ascorbic acid, phytic acid, tocopherols, sterols, carotenoids, saponins, and ecdysteroids, among others, may be the most likely contributors to the antioxidant activity [50].

3 Food Application

The nutritional composition (protein, dietary fiber, and minerals) and antioxidant properties of pseudocereals have exerted great contribution to its popularity in human diet. In addition to presenting high nutritional quality, they are also characterized by being gluten-free, a characteristic that enables greater offer and variety of more nutritious and suitable food products for patients with celiac disease. Finally,

Table 4 The use of pseudocereals in food

	Pseudocereals		
Food use	Quinoa	Amaranth	Buckwheat
Snacks	[51, 54]	[28, 31, 37, 51, 54]	[51, 54]
Bread	[10]	–	–
Breakfast cereals	[51, 55, 56]	[51, 55]	[51, 55]
Pasta	[13]	[57, 58]	–
Gluten-free products	[52]	[52]	[52]
Gluten-free breads	[59]	[59]	[60]
Brewing	[53, 61, 62]	[53]	[53]
Desserts	–	[54]	–
Noodles	–	[63, 64]	[65, 66]
Baked goods	[51]	[51]	[51]
Fat replacement	–	[54]	[41]
Films	–	[67, 68]	–
Oil	–	[69]	–
Supplementation of *Lactobacillus* strains	–	[70]	–
Dough	[44, 71]	–	–
Soups	[44, 71]	–	[26]
Cake	[72]		
Sourdough	[73, 74]	–	[74]
Vinegar	–	–	[24]
Fat replacers	–	–	
Porridge	–	–	[75]
Probiotic fermented milk	–	–	[15]
Fortified tea	–	–	[76]

amaranth, quinoa, and buckwheat can be an alternative food in order to reduce malnutrition diseases.

Thereby, pseudocereals have been used to produce breakfast cereal, baked goods, crackers, infant foods, and gluten-free products, among others (Table 4) [51]. One of the most common uses of seeds and respective by-products is for gluten-free diets. Therefore, pseudocereals are increasingly used in gluten-free breads, cookies, and cakes [52]. Quinoa is usually used to enhance baking flours in the preparation of biscuits, noodles, and pastries and for the preparation of baked foods to maintain the moisture and give an agreeable flavor [50]. Recently, amaranth, quinoa, and buckwheat have also been used for brewing purpose. This versatility on beer production might become possible to produce gluten-free beer with high quality [53].

3.1 Bakery Products

3.1.1 Gluten-Free Breads

Currently, gluten-free breads are an emerging topic in the literature. The quality of the gluten-free products available on the market, and food choices, may represent

major determinants in the deficiencies in macronutrients and micronutrients of celiac patients [50]. Gluten-free cereal foods are frequently rich in carbohydrates and fats, and they are made using refined gluten-free flour or starch not enriched or fortified. In this way, studies are mainly focused on gluten-free ingredients or technologies aiming to produce breads with similar quality (physical-chemical and sensorial) to those wheat flour based [77–79].

Pseudocereal flours can be used for protein enrichment of gluten-free formulations. Besides prolamins' absence, pseudocereals are rich in nutrients and source of bioactive compounds with antioxidant properties [80], which have importance for recovering the nutritional status of celiac patients when used in gluten-free products [19]. In this section, the recent researches about this subject will be approached.

Different studies have revealed great results about pseudocereal use in gluten-free breadmaking. Breads containing amaranth, quinoa, or buckwheat flour have shown similar baking characteristics compared to rice-based bread. Incorporation of pseudocereal flours led to a considerable improvement in nutritional and texture properties of bread [60]. The use of 50% of amaranth and quinoa flours in gluten-free bread provided a final product of increased loaf volume and softer crumb. The effect on volume and crumb texture could be attributed to complexes formed between fat globules and starch especially in pseudocereal presence, which contributes to stabilize gas cells [81]. Reduced percent of amaranth and quinoa flour (20%) gluten-free bread formulations showed similar technological quality with reference to specific volume and firmness. The calories at these breads were similar to gluten-free bread produced with rice flour and starches (from cassava and potatoes). Notably, the breads produced with pseudocereals had greater amounts of proteins, lipids, and minerals [59].

An improved effect of sourdough fermentation on gluten-free bread quality of quinoa or buckwheat has been reported. Such breads have shown high quality and extend shelf life [74]. Other investigation showed that the presence of milk and buckwheat proteins, as well as hydrocolloids, was responsible for improving CO_2 retention in sourdough bread of buckwheat flour (37%) [82]. Hydrocolloids and guns have effect on dough rheology and bread quality parameters in gluten-free formulation and therefore are usually necessary in this case. However, results have shown that pseudocereals flours and linseed mucilage provided a water-binding capacity in gluten-free breads which combined with starch improved the final quality of bakery products [11].

Quinoa and amaranth have also attracted attention because of having hypoglycemic effect. Consequently, gluten-free breads elaborated with such pseudocereals can also contain low glycemic index compared to traditional formulations with starches and rice flour [7].

3.1.2 Biscuit, Cookie, and Cake

Cookies are widely consumed bakery products due to their long shelf life and strong consumer preference. Although the structure-forming ability of gluten influences the rheological properties of dough and affects overall appearance of bakery products, the development of a gluten network in biscuit and cookie dough is minimal and

undesirable [83]. Therefore, gluten-free cookie production is most easily to proceed rather than loaf bread. Generally, cookies are prepared from wheat flour, which are deficient in some essential amino acids like lysine and tryptophan. To increase its nutritive value, cookies are prepared with fortified or composite flour. Pseudocereal flours can be added to cookie formulation to achieve better nutritional profile without causing severe technological problems.

Light buckwheat flour was used in gluten-free rice and buckwheat cookies. The substitution of rice flour in gluten-free cookie formulation by 10–30% light buckwheat flour increased mineral content, total phenolic content and rutin content, and antioxidant activity [83]. The shelf life study of gluten-free cookies based on a mixture (80:20) of rice flour and light buckwheat flour (chosen as optimal) suggested sensory properties as relevant parameters for predicting the endpoint of cookie shelf life rather than total aldehyde content.

Cookies were prepared from the composite flour containing various proportions of the whole amaranth flour. The physical properties of the amaranth-enriched cookies were affected in a positive way by demonstrating a decrease in bake loss, an increase in diameter, a higher spread ratio, and lesser hardness, leading to softer eating characteristics which are required in cookies. The composite cookies were darker in color at higher levels of substitution. So, the use of amaranth flour in cookie was effective for technological advantages of cookies [84].

Roasting of seeds is often done to enhance flavor and reduce antinutrients such as phytic acid and saponins. However, the effect of roasting on food quality has not yet been reported for quinoa. Such assumption led to gluten-free and allergen-free cake formulation maked up by different roasting times of quinoa. From the results, application of the resulting flours in an allergen-free, gluten-free cake formulation in particular may benefit from a blend of roasted and nonroasted quinoa flours when gelatinization is needed, but retrogradation must be limited for functionality and shelf life purposes [85].

3.2 Pasta

Pasta has a primary role in human nutrition, thanks to its complex carbohydrate profile, the large global distribution, and the extended shelf life. The World Health Organization (WHO) and Food and Drug Administration (FDA) consider pasta a good vehicle for the addition of different nutrients to diet [86]. Otherwise, there is an increasing interest of producers, consumers, and the scientific community toward the addition of high-protein vegetable ingredients deriving from pseudocereals and others to pasta formulations [87], even though on nutritional improvement, the replacement of semolina is still a challenge for the food industry, since the addition of alternative ingredients markedly affects technological and sensory properties [13].

In a recent study, fermented quinoa flour (with lactic and acid bacteria) was used for pasta fortification with the aim of enhancing its nutritional features. Addition of 20 g/100 g of quinoa flour to semolina was successful in improving the nutritional characteristics of pasta without compromising the technological and sensory quality.

Pasta containing fermented quinoa flour presented a higher nutritional profile compared to the other pasta, characterized by improved protein digestibility and quality, high nutritional scores, low predicted glycemic index, and high antioxidant potential. Fermentation as well other available technologies may be a successful way to produce pasta from quinoa (and other pseudocereals) with high nutritional potential.

Interest in amaranth flour has grown since studies have shown that it can contribute to the improvement of the structure and the cooking quality of pasta as well as enrichment of the essential mineral content in the case of gluten-free products [88, 89]. In line with that, amaranth leaves (DAL) and seed flour (AF) were used as ingredients for pasta production. Generally, amaranth pastas had lower cooking time than control pasta. Pasta with AF and DAL exhibited significantly higher content of protein, crude fiber, iron, zinc, magnesium, and potassium compared with the control pasta. Even though antioxidant capacity decreased due to the cooking process, AF and DAL have improved the pasta antioxidant capacity [90].

3.3 Extruded Products

Extrusion is a food process used to obtain a variety of foods from fundamental ingredients. This technology uses the extruder to produce foods as snacks, ready-to-eat breakfast cereals, and flours. Extrusion can be advantageous compared to other food processing techniques due to the short processing time [91].

Studies have shown that extrusion may have a definitive positive nutritional effect on food, if it is done appropriately. Specially, extruding of amaranth and quinoa has increased their nutritional value. Interestingly, extruded products from the pure grains of quinoa and amaranth (no cereal source), i.e., gluten-free, were produced in single-screw extruder [45].

A comprehensive study on impacts of extrusion processing conditions on quinoa flour showed moderate expansion characteristics as compared to widely used cereal grains, suggesting that this variety of quinoa is not well suited for making direct expanded products. However, this variety may be more useful in the products where direct expansion is not an important textural quality [91].

4 Sensory Aspects and Perceptions of Consumers

The food industries need to innovate their products constantly in order to satisfy consumer demand and maintain a leadership position in the market. The main factors impelling the preferences for food are appearance, taste, aroma, texture, cultural factors, cost, and the benefits that it promotes [92–94]. In this context, the sensorial analysis is a science used to analyze consumption products through human's sense as sight, smell, taste, touch, and hearing. The results obtained with the sensorial tests can help new food developer to evaluate the quality and consumers' satisfaction and give direction of a successful production in the market [95]. In addition, it is possible

to obtain information about different formulations, preferences of the products, or relationship between the variables, purchase intention, and consumers' perception [96]. An example of food innovation concerns to pseudocereals' use as ingredient in order to provide an attractive and functional food. Interestingly, in design of new food product, quinoa (20%) showed an acceptable sensory profile, so being an option for industries specialized on functional food development [97].

Currently, an expressive number of reports in the literature have indicated the benefits of ancient grains. Moreover, consumers are most concerned and consequently interested to buy products with health benefits. The pseudocereals present an acceptable flavor that consumers can easily adapt [51]. Therefore, quinoa, amaranth, and buckwheat present an increase sensory acceptability as the products are developed. However, the critical point is the difficulty of replacing 100% of wheat flour by pseudocereals [44, 98].

The use of quinoa and amaranth to develop extrudates (20, 30, 40% of pseudocereal flour) promotes a positive sensory perception characterized by crispness. However, when studying the temporal dominance profile of this snack cereals (sensorial technique used to study the profile of two or more sensations), during the mastication, crunchiness is the dominance sensation [99]. An acceptance test showed 70–78% of the preference of snacks being those produced with quinoa flour in the quantity 92–86% [8].

Also, the use of pseudocereals for gluten-free bread has been studied. The incorporation of 20% of quinoa or amaranth flour in gluten-free breads did not influence the texture perceptions of consumers and had a moderate acceptance. Additionally, the adhesiveness, amaranth aroma, and taste contributed positively for sensory acceptability. In contrast, quinoa showed negative drivers of linking as brown color of the crust and strong quinoa taste. In this context, amaranth flour seems to have better sensorial response than quinoa in substitution to starch in gluten-free bakery products [100].

Sensory evaluation of fresh spaghetti produced with amaranth flour showed a great sensory acceptance and purchase intention. Once again, the results indicate amaranth promising to gluten-free product [58]. In a recent finding, amaranth was used in porridge formulation, and from the consumers viewpoint, porridge showed a similar acceptability for maize-based porridge [101]. Thus, amaranth can be recognized as a potential ingredient to replace cereals or to be used in gluten-free products with sensorial acceptance of consumers.

Quinoa and buckwheat have been tested in a brewing process for the development of beverages. Fermented quinoa-based beverage was considered a potential drink, having good acceptability, since it is produced with bilberries (*Vaccinium myrtillus*) and has an added chocolate flavor [62]. Beer is another fermented beverage that has been investigated. Beer developed with quinoa had an interesting sensory profile characterized as black color, nutty aroma, grayish foam, and astringent. On the other hand, beer produced with buckwheat presented a quality characterized by bitterness and sparking aspect. Concluding, buckwheat beer is quite similar to traditional barley one.

Although pseudocereals are unpopular in some countries, the application of this grains in researches with focus in sensorial analyses should be become amaranth, quinoa and buckwheat, popular, and more applicable in the human food habits.

5 Conclusion

Despite all the issues discussed, pseudocereals are not widely used. High cost (imported grain as quinoa), little knowledge of their benefits by most consumers. Furthermore, pseudocereals present functional properties as excellent nutritional profile and bioactive compounds and are gluten-free. In this scenario, the Food Science and Technology has a crucial role to explore and disseminate knowledge about these grains. The pseudocereals present high commercial value and special functional and nutritional benefits next to popularize among people.

References

1. Schoenlechner R, Siebenhandl S, Berghofer E (2008) Pseudocereals. In: Arendt EK, Bello FD (eds) Gluten-free cereal products and beverages. Elsevier, Massachusetts, EUA, p 149–VI
2. Alvarez-Jubete L, Arendt K, Gallagher E (2010) Nutritive value of pseudocereals and their increasing use as functional gluten- free ingredients. Trends Food Sci Technol 21:106–113. https://doi.org/10.1016/j.tifs.2009.10.014
3. Ogrodowska D, Zadernowski R, Czaplick S et al (2014) Amaranth seeds and products – the source of bioactive compounds. Pol J Food Nutr Sci 64:165–170. https://doi.org/10.2478/v10222-012-0095-z
4. Srichuwong S, Curti D, Austin S et al (2017) Physicochemical properties and starch digestibility of whole grain sorghums, millet, quinoa and amaranth flours, as affected by starch and non-starch constituents. Food Chem 233:1–10. https://doi.org/10.1016/j.foodchem.2017.04.019
5. Nowak V, Du J, Charrondière U (2016) Assessment of the nutritional composition of quinoa (*Chenopodium quinoa* Willd). Food Chem 193:47–54. https://doi.org/10.1016/j.foodchem.2015.02.111
6. Pihlanto A, Mattila P, Mäkinen S, Pajari AM (2017) Function bioactivities of alternative protein sources and their potential health benefits. Food Funct 8:3443–3458. https://doi.org/10.1039/c7fo00302a
7. Matos ME, Rosell CM (2015) Understanding gluten-free dough for reaching breads with physical quality and nutritional balance. J Sci Food Agric Food Agric 95:653–661. https://doi.org/10.1002/jsfa.6732
8. Kahlon TS, Avena-Bustillos RJ, Chiu MM (2017) Sensory evaluation of gluten-free quinoa whole grain snacks. Heliyon 3:1–12. https://doi.org/10.1016/j.heliyon.2016.e00213
9. Peksa A, Kita A, Carbonell-Barrachina AA et al (2016) Sensory attributes and physicochemical features of corn snacks as affected by different flour types and extrusion conditions. LWT – Food Sci Technol 72:26–36. https://doi.org/10.1016/j.lwt.2016.04.034
10. Wang S, Opassathavorn A, Zhu F (2015) Influence of quinoa flour on quality characteristics of cookie, bread and Chinese steamed bread. J Texture Stud 46:281–292. https://doi.org/10.1111/jtxs.12128
11. Houben A, Hochstotter A, Becker T (2012) Possibilities to increase the quality in gluten-free bread production: an overview. Eur Food Res Technol 235:195–208. https://doi.org/10.1007/s00217-012-1720-0

12. Collar C, Jiménez T, Conte P, Fadda C (2014) Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads. Carbohydr Polym 113:149–158. https://doi.org/10.1016/j.carbpol.2014.07.020
13. Lorusso A, Verni M, Montemurro M et al (2017) Use of fermented quinoa flour for pasta making and evaluation of the technological and nutritional features. LWT – Food Sci Technol 78:215–221. https://doi.org/10.1016/j.lwt.2016.12.046
14. Kerpes R, Fischer S, Becker T (2017) The production of gluten-free beer: degradation of hordeins during malting and brewing and the application of modern process technology focusing on endogenous malt peptidases. Trends Food Sci Technol 67:129–138. https://doi.org/10.1016/j.tifs.2017.07.004
15. Matejčeková Z, Liptáková D, Valík L (2017) Functional probiotic products based on fermented buckwheat with *Lactobacillus rhamnosus*. LWT – Food Sci Technol 81:35–41. https://doi.org/10.1016/j.lwt.2017.03.018
16. Kocková M, Dilongová M, Hybenová E, Valík L (2013) Evaluation of cereals and pseudocereals suitability for the development of new probiotic foods. J Chem 2013:1–8
17. FAO (2017) The future of food and agriculture: trends and challenges. Food & Agriculture Organization of the United Nations, Rome
18. FAOSTAT (2014) Crops. In: Rome food and agriculture organization of the United Nations. http://www.fao.org/statistics/en/. Accessed 20 Oct 2017
19. de Morais EC, Alencar NMM (2015) Health benefits and food sources studies on gluten-free diets. In: Langdon RT (ed) Gluten-free diets. Nova Publishers, New York, pp 153–166
20. Ruiz KB, Biondi S, Oses R et al (2014) Quinoa biodiversity and sustainability for food security under climate change. A review. Agron Sustain Dev 34:349–359. https://doi.org/10.1007/s13593-013-0195-0
21. Drzewiecki J, Delgado-Licon E, Haruenkit R et al (2003) Identification and differences of total proteins and their soluble fractions in some pseudocereals based on electrophoretic patterns. J Agric Food Chem 51:7798–7804. https://doi.org/10.1021/jf030322x
22. Deutsch H, Poms R, Heeres H, Kamp J (2008) Labeling and regulatory issues. In: Arendt EK, Bello FD (eds) Gluten-free cereal products and beverages. Academic/Elsevier, Massachusetts, EUA, pp 149–190
23. Di Fabio A, Parraga G (2016) Origin, production and utilization of pseudocereals. In: Claudia Monika Haros RS (ed) Pseudocereals: chemistry and technology, 1st edn. Willey, Chichester, pp 1–27
24. Fletcher RJ (2016) Pseudocereals: overview, 2nd edn. Encyclopedia of food grains. Massachusetts, EUA. https://doi.org/10.1016/B978-0-08-100596-5.00039-1
25. Bressani R (2003) Amaranth. In: Caballero B (ed) Encyclopedia of food sciences and nutrition, 10th edn. Academic, Oxford, pp 166–173
26. Campbell CG (1997) Buckwheat: *Fagopyrum esculentum* Moench. Promoting the conservation and use of underutilized and neglected crops. International Plant Genetic Resorces Institute, Rome
27. Repo-Carrasco-Valencia RA, Serna LA (2011) Quinoa (*Chenopodium quinoa*, Willd.) as a source of dietary fiber and other functional components. Ciência e Tecnol Aliment 31:225–230. https://doi.org/10.1590/S0101-20612011000100035
28. Alvarez-Jubete L, Arendt EK, Gallagher E (2009) Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. Int J Food Sci Nutr 60:240–257. https://doi.org/10.1080/09637480902950597
29. Alencar NMM, Oliveira LC (2015) Technological functions of gluten and implications for celiac disease. In: Rivera H (ed) Gluten food sources, properties and health implications, 1st edn. Nova Publishers, New York, pp 1–345
30. Malalgoda M, Simsek S (2017) Celiac disease and cereal proteins. Food Hydrocoll 68:108–113. https://doi.org/10.1016/j.foodhyd.2016.09.024
31. Rastogi A, Shukla S (2013) Amaranth : a new millennium crop of nutraceutical values. Crit Rev Food Sci Nutr 52:109–125. https://doi.org/10.1080/10408398.2010.517876

32. Gorinstein S, Pawelzik E, Delgado-licon E et al (2002) Characterisation of pseudocereal and cereal proteins by protein and amino acid analyses. J Sci Food Agric 891:886–891. https://doi.org/10.1002/jsfa.1120
33. Mujica-Sanchez A, Jacobsen S, Izquierdo J (2001) Quinua (*Chenopodium quinoa* Willd.): ancestral cultivo andino, alimento del presente y del futuro. FAO/RLC, Santiago
34. Alves LF, Rocha MS, Gomes CCF (2008) Avaliação da qualidade protéica da Quinua Real (*Chenopodium quinoa* Willd) através de métodos biológicos. e-Scientia 1(1):1–16
35. Filho AMM, Pirozi MR, Borges JTDS et al (2017) Quinoa: nutritional, functional, and antinutritional aspects. Crit Rev Food Sci Nutr 57:1618–1630. https://doi.org/10.1080/10408398.2014.1001811
36. Wijngaard HH, Arendt EK (2006) Buckwheat. Cereal Chem 83:391–401
37. Janssen F, Pauly A, Rombouts I et al (2016) Proteins of Amaranth (*Amaranthus* spp.), buckwheat (*Fagopyrum* spp.), and Quinoa (*Chenopodium* spp.): a food science and technology perspective. Compr Rev Food Sci Food Saf 16:39–58. https://doi.org/10.1111/1541-4337.12240
38. Gewehr MF, Danelli D, de Melo LM et al (2012) Chemical analysis of quinoa flakes: characterization for use in food products. Braz J Food Technol 15:280–287
39. Steffolani ME, León AE, Pérez GT (2013) Study of the physicochemical and functional characterization of quinoa and kañiwa starches. Starch – Stärke 65:976–983. https://doi.org/10.1002/star.201200286
40. Li G, Wang S, Zhu F (2016) Physicochemical properties of quinoa starch. Carbohydr Polym 137:328–338. https://doi.org/10.1016/j.carbpol.2015.10.064
41. Zhu F (2016) Buckwheat starch: structures, properties, and applications. Trends Food Sci Technol 49:121–135. https://doi.org/10.1016/j.tifs.2015.12.002
42. Nascimento AC, Motaa C, Coelho I et al (2014) Characterisation of nutrient profile of quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus caudatus*), and purple corn (*Zea mays* L.) consumed in the North of Argentina: proximates, minerals and trace elements. Food Chem 148:420–426. https://doi.org/10.1016/j.foodchem.2013.09.155
43. Ogungbenle HN (2003) Nutritional evaluation and functional properties of quinoa (*Chenopodium quinoa*) flour. Int J Food Sci Nutr 54:153–158. https://doi.org/10.1080/09637480310000841 06
44. Valcárcel-Yamani B, Lannes SCDS (2012) Applications of *Quinoa* (*Chenopodium quinoa* Willd.) and Amaranth (*Amaranthus* Spp.) and their influence in the nutritional value of cereal based foods. Food Public Health 2:265–275. https://doi.org/10.5923/j.fph.20120206.12
45. Hemalatha P, Bomzan DP, Sathyendra Rao BV, Sreerama YN (2016) Distribution of phenolic antioxidants in whole and milled fractions of quinoa and their inhibitory effects on α-amylase and α-glucosidase activities. Food Chem 199:330–338. https://doi.org/10.1016/j.foodchem.2015.12.025
46. Alvarez-Jubete L, Wijngaard H, Arendt EK, Gallagher E (2010) Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chem 119:770–778. https://doi.org/10.1016/j.foodchem.2009.07.032
47. Gómez-Caravaca AM, Iafelice G, Verardo V et al (2014) Influence of pearling process on phenolic and saponin content in quinoa (*Chenopodium quinoa* Willd). Food Chem 157:174–178. https://doi.org/10.1016/j.foodchem.2014.02.023
48. Jung K, Richter J, Kabrodt K, Luecke IM, Schellenberg I, Herrling T (2006) The antioxidative power AP – a new quantitative time dependent (2D) parameter for the determination of the antioxidant capacity and reactivity of different plants. Spectrochim Acta Part A Mol Biomol Spectrosc 63:846–850. https://doi.org/10.1016/J.SAA.2005.10.014
49. Bunzel M, Ralph J, Steinhart H (2005) Association of non-starch polysaccharides and ferulic acid in grain amaranth (*Amaranthus caudatus* L.) dietary fiber. Mol Nutr Food Res 49:551–559. https://doi.org/10.1002/mnfr.200500030
50. Yawadio Nsimba R, Kikuzaki H, Konishi Y (2008) Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus* spp. seeds. Food Chem 106:760–766. https://doi.org/10.1016/j.foodchem.2007.06.004

51. Taylor JRN, Awika JM (2017) Future research needs for the ancient grains. In: Gluten-free ancient grains. Elsevier Ltd, p 0
52. Schoenlechner R, Siebenhandl S, Berghofer E (2008) 7 – Pseudocereals. In: Gluten-free cereal products and beverages. Massachusetts, EUA, p 149–VI
53. de Meo B, Freeman G, Marconi O, Booer C, Perretti G, Fantozzi, P (2011) Behaviour of malted cereals and pseudo-cereals for gluten-free beer production. J Inst Brew 117:541–546. https://doi.org/10.1002/j.2050-0416.2011.tb00502.x
54. Zhu F (2017) Structures, physicochemical properties, and applications of amaranth starch. Crit Rev Food Sci Nutr 57:313–325. https://doi.org/10.1080/10408398.2013.862784
55. Venskutonis PR, Kraujalis P (2013) Nutritional components of amaranth seeds and vegetables: a review on composition, properties, and uses. Compr Rev Food Sci Food Saf 12:381–412. https://doi.org/10.1111/1541-4337.12021
56. Konishi Y, Hirano S, Tsuboi H, Wada M (2004) Distribution of minerals in quinoa (*Chenopodium quinoa* Willd.) seeds. Biosci Biotechnol Biochem 68:231–234. https://doi.org/10.1271/bbb.68.231
57. Islas-rubio AR, De Calderon MA et al (2014) Effect of semolina replacement with a raw: popped amaranth flour blend on cooking quality and texture of pasta. LWT – Food Sci Technol 57:217–222. https://doi.org/10.1016/j.lwt.2014.01.014
58. Bastos GM, Júnior MSS, Caliari M et al (2016) Physical and sensory quality of gluten-free spaghetti processed from amaranth flour and potato pulp. LWT – Food Sci Technol 65:128–136. https://doi.org/10.1016/j.lwt.2015.07.067
59. Alencar NMM, Steel CJ, Alvim ID et al (2015) Addition of quinoa and amaranth flour in gluten-free breads: temporal profile and instrumental analysis. LWT – Food Sci Technol 62:1011–1018. https://doi.org/10.1016/j.lwt.2015.02.029
60. Naqash F, Gani A, Gani A, Masoodi FA (2017) Gluten-free baking: combating the challenges – a review. Trends Food Sci Technol 66:98–107. https://doi.org/10.1016/j.tifs.2017.06.004
61. Deželak M, Zarnkow M, Becker T, Košir IJ (2014) Processing of bottom-fermented gluten-free beer-like beverages based on buckwheat and quinoa malt with chemical and sensory characterization. J Inst Brew 120:360–370. https://doi.org/10.1002/jib.166
62. Urquizo FEL, Torres SMG, Tolonen T et al (2017) Development of a fermented quinoa-based beverage. Food Sci Nutr 5:602–608. https://doi.org/10.1002/fsn3.436
63. Chandla NK, Saxena DC, Singh S (2017) Processing and evaluation of heat moisture treated (HMT) amaranth starch noodles; an inclusive comparison with corn starch noodles. J Cereal Sci 75:306–313. https://doi.org/10.1016/j.jcs.2017.05.003
64. Li M, Zhu K, Sun Q et al (2016) Quality characteristics, structural changes, and storage stability of semi-dried noodles induced by moderate dehydration: understanding the quality changes in semi-dried noodles. Food Chem 194:797–804. https://doi.org/10.1016/j.foodchem. 2015.08.079
65. Suzuki T, Kim S, Mukasa Y et al (2010) Effects of lipase, lipoxygenase, peroxidase and free fatty acids on volatile compound found in boiled buckwheat noodles. J Sci Food Agric 90:1232–1237. https://doi.org/10.1002/jsfa.3958
66. Han L, Lu Z, Hao X et al (2012) Impact of calcium hydroxide on the textural properties of buckwheat noodles. J Texture Stud 43:227–234. https://doi.org/10.1111/j.1745-4603.2011.00331.x
67. Condes MC, Anon MC, Dufresne A, Mauri AN (2018) Food hydrocolloids composite and nanocomposite films based on amaranth biopolymers. Food Hydrocoll 74:159–167. https://doi.org/10.1016/j.foodhyd.2017.07.013
68. Tapia-Blacido D, Sobral PJ, Menegalli FC (2005) Development and characterization of biofilms based on amaranth flour (*Amaranthus caudatus*). J Food Eng 67:215–223. https://doi.org/10.1016/j.jfoodeng.2004.05.054
69. Martirosyan DM, Miroshnichenko LA, Kulakova SN et al (2007) Hypertension. Lipids Health Dis 12:1–12. https://doi.org/10.1186/1476-511X-6-1
70. Diogo A, Vieira S, Bedani R et al (2017) The impact of fruit and soybean by-products and amaranth on the growth of probiotic and starter microorganisms. Food Res Int 97:356–363. https://doi.org/10.1016/j.foodres.2017.04.026

71. FAO (2011) Quinoa: an ancient crop to contribute to word food security. Reg Off Lat Am Caribb. https://doi.org/10.1016/j.ecoser.2014.09.013
72. Ávila BP, Braganca GCM, Rockenbach R et al (2017) Physical and sensory characteristics of cake prepared with six whole-grain flours. J Food Meas Charact 11:1486–1492. https://doi.org/10.1007/s11694-017-9527-0
73. Rizzello CG, Lorusso A, Montemurro M, Gobbetti M (2016) Use of sourdough made with quinoa (*Chenopodium quinoa*) flour and autochthonous selected lactic acid bacteria for enhancing the nutritional, textural and sensory features of white bread. Food Microbiol 56:1–13. https://doi.org/10.1016/j.fm.2015.11.018
74. O'Shea N, Arendt E, Gallagher E (2014) State of the art in gluten-free research. J Food Sci 79:1067–1076. https://doi.org/10.1111/1750-3841.12479
75. Mišana A, Petelin A, Stubelj M et al (2017) Buckwheat – enriched instant porridge improves lipid profile and reduces inflammation in participants with mild to moderate hypercholesterolemia. J Funct Foods 36:186–194. https://doi.org/10.1016/j.jff.2017.06.056
76. Qin L, Sui X, Zeng H, Xu Z (2014) Fortification of the health benefit of buckwheat (*Fagopyrum tataricum*) tea. J Food Process Preserv 38:1882–1889. https://doi.org/10.1111/jfpp.12160
77. Pellegrini N, Agostoni C (2015) Nutritional aspects of gluten-free products. J Sci Food Agric 95:2380–2385. https://doi.org/10.1002/jsfa.7101
78. Jnawali P, Kumar V, Tanwar B (2016) Celiac disease: overview and considerations for development of gluten-free foods. Food Sci Hum Wellness 5:169–176. https://doi.org/10.1016/j.fshw.2016.09.003
79. Gao Y, Janes ME, Chaiya B et al (2017) Gluten-free bakery and pasta products: prevalence and quality improvement. Int J Food Sci Technol (in press): 1–14. https://doi.org/10.1111/ijfs.13505
80. Alencar NMM, de Carvalho Oliveira L (2017) Trends in bread consumption: non-wheat cereals, technological challenges and sensory quality. In: Lewis H (ed) Bread consumption, cultural significance and health effects. Nova Publishers, New York, p 276
81. Alvarez-Jubete L, Auty M, Arendt EK, Gallagher E (2010) Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. Eur Food Res Technol 230:437–445. https://doi.org/10.1007/s00217-009-1184-z
82. Marti A, Bottega G, Franzetti L et al (2015) From wheat sourdough to gluten-free sourdough: a non- conventional process for producing gluten-free bread. Int J Food Microbiol 50:1268–1274. https://doi.org/10.1111/ijfs.12757
83. Gallagher E, Gormley TR, Arendt EK (2004) Recent advances in the formulation of gluten-free cereal-based products. Trends Food Sci Technol 15:143–152. https://doi.org/10.1016/j.tifs.2003.09.012
84. Chauhan A, Saxena DC, Singh S (2016) Physical, textural, and sensory characteristics of wheat and amaranth flour blend cookies. Cogent Food Agric 2:1125773. https://doi.org/10.1080/23311932.2015.1125773
85. Rothschild J, Rosentrater KA, Onwulata C et al (2015) Influence of quinoa roasting on sensory and physicochemical properties of allergen-free, gluten-free cakes. Int J Food Sci Technol 50:1873–1881. https://doi.org/10.1111/ijfs.12837
86. Chillo S, Laverse J, Falcone PM, Del Nobile MA (2008) Quality of spaghetti in base amaranthus wholemeal flour added with quinoa, broad bean and chick pea. J Food Eng 84:101–107. https://doi.org/10.1016/J.JFOODENG.2007.04.022
87. Wang S, Zhu F (2016) Formulation and quality attributes of quinoa food products. Food Bioprocess Technol 9:49–68. https://doi.org/10.1007/s11947-015-1584-y
88. Fiorda FA, Soares MS, da Silva FA et al (2013) Microstructure, texture and colour of gluten-free pasta made with amaranth flour, cassava starch and cassava bagasse. LWT – Food Sci Technol 54:132–138. https://doi.org/10.1016/J.LWT.2013.04.020
89. Hidalgo MJ, Sgroppo SC, Camiña JM et al (2015) Trace element concentrations in commercial gluten-free amaranth bars. J Food Meas Charact 9:426–434. https://doi.org/10.1007/s11694-015-9250-7

90. Cárdenas-Hernández A, Beta T, Loarca-Piña G et al (2016) Improved functional properties of pasta: enrichment with amaranth seed flour and dried amaranth leaves. J Cereal Sci 72:84–90. https://doi.org/10.1016/J.JCS.2016.09.014
91. Kowalski RJ, Medina-Meza IG, Thapa BB et al (2016) Extrusion processing characteristics of quinoa (*Chenopodium quinoa* Willd.) var. cherry vanilla. J Cereal Sci 70:91–98. https://doi.org/10.1016/J.JCS.2016.05.024
92. Rozin P (2001) Food preference. In: International encyclopedia of social & behavioral sciences. pp 5719–5722
93. Raz C (2008) From sensory marketing to sensory design: how to drive formulation using consumers' input? Food Qual Prefer 19:719–726
94. Favalli S, Skov T, Byrne DV (2013) Sensory perception and understanding of food uniqueness: From the traditional to the novel. Food Res Int 50:176–188. https://doi.org/10.1016/j.foodres.2012.10.007
95. American Society for testing and materials standards sensory-evaluation. In: http://www.astm.org/Standards/sensoryevaluation-Stand
96. Stone H, Sidel JL (2004) Sensory evaluation practices, 3rd edn. Elsevier
97. Lavini A, Pulvento C, D'Andria R et al (2014) Quinoa's potential in the Mediterranean region. J Agron Crop Sci 200:344–360. https://doi.org/10.1111/jac.12069
98. Gimenez-Batista JA, Piskula M, Zielinski H (2015) Recent advances in development of gluten-free buckwheat products. Food Sci Technol 44:58–65. https://doi.org/10.1016/j.tifs.2015.02.013
99. Diaz JMR, Suuronen J-P, Deegan KC et al (2015) Physical and sensory characteristics of corn-based extruded snacks containing amaranth, quinoa and kaniwa flour. LWT – Food Sci Technol 64:1047–1056. https://doi.org/10.1016/j.lwt.2015.07.011
100. Alencar NMM, de Morais EC, Steel CJ et al (2017) Sensory characterisation of gluten-free bread with addition of quinoa, amaranth flour and sweeteners as an alternative for coeliac patients. Int J Food Sci Technol 52:872–879. https://doi.org/10.1111/ijfs.13349
101. Akande OA, Nakimbugwe D, Mukisa IM (2017) Optimization of extrusion conditions for the production of instant grain amaranth-based porridge flour. Food Sci Nutr 5:1–10. https://doi.org/10.1002/fsn3.513

Pesticide Residues in Fruits and Vegetables 58

Samira Mebdoua

Contents

S. Mebdoua (✉)
Laboratoire d'amélioration Intégrative des Productions Végétales, Ecole Nationale Supérieure Agronomique, Alger, Algeria

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_76

Abstract

Fruits and vegetables play an important role in human nutrition and health; they constitute an important part of our daily diet. They are important sources of carbohydrates, vitamins, trace minerals, and antioxidants. Therefore, they can be contaminated by pesticides used for the protection of their culture. The use of pesticides to control pests in fruits and vegetables can lead to the presence of pesticide residues. The level of these residues can be below the maximum residue limit (MRL) if good agricultural practices (GAP) were used. The presence of residues with level exceeding MRLs should be interpreted as violation of GAP. In many reports, pesticide residues are present in the majority of fruits and vegetables; they are more detected in fruits than in vegetables. The percentage of exceeding MRLs is less than 20% in most monitoring programs. The risk assessment for long-term and short-term exposure must be done for all pesticides detected to ensure consumer's health protection.

Keywords

Fruit · Vegetable · Pesticide residues · Risk assessment · MRL

1 Introduction

Pesticides are a subject of debate in today's society. In the past, these products were synonymous with the control of agricultural production, the guarantee of food resources, and the eradication of diseases transmitted by insects, but now, they became associated with a real public health problem. In few years, pesticides have gone from revolutionary products and benefactors to harmful products that must be eliminated [1].

In fact, the excessive and unreasonable use of pesticides since the middle of the twentieth century has led to a widespread contamination of the environment. Since the 1970s, these products and their metabolites have been detected in all compartments of the environment, including air, river water, and groundwater, and food [2, 3].

Pesticides, by their toxic properties, represent a real danger to man when they are not used under appropriate conditions. On the other hand, it seems complicated to remove their uses in the near future because our society depends on them (whether agricultural or nonagricultural use). The current global trend is to gradually decrease their use through the improvement of agricultural practices and the strengthening of the legislative measures related to their registration and use. To this end, the maximum residue limits (MRLs) in fruits and vegetables imposed by international legislation have become lower.

Fig. 1 Some vegetables and fruits

Fruit and vegetables are rich in vitamins and minerals. They also contain antioxidants, such as beta-carotene and vitamin C; they also contain fiber. This can help to control cholesterol levels and keep blood sugar levels steady. Many studies encourage eating at least five portions of fruit and vegetables each day (Fig. 1). Regular monitoring of residue levels in fruits and vegetables is so required to keep these products safe.

2 Definitions

According to FAO, the term "pesticide" means "any substance or combination of substances, or micro-organisms including viruses, intended to repel, destroy or control pests, including vectors of human or animal diseases, harmful pests, undesirable species of plants or animals causing damage or otherwise causing harm during the production, processing, storage, transport or marketing of food, agricultural products, timber and wood products, or food for animals, or that can be administered to animals to combat insects, arachnids and other endo- or ectoparasites. The term includes substances intended to be used as a growth regulator of insects or plants, as a defoliant, as a drying agent, as a fruit thinning agent or for preventing the premature fall of fruit, as well as substances applied to crops, before or after harvest, to protect products from deterioration during storage and transportation. This term also includes the synergistic and detoxifying products of pesticides when they are essential for the satisfactory performance of the pesticide" [4].

In this chapter, the word pesticide is synonymous with an active ingredient to differentiate it from the commercial pesticide. Indeed, pesticides are rarely used in the form of an active ingredient alone; they are sold in mixtures of one or more active ingredients and adjuvants and give rise to different formulations. Different types of adjuvants are used [5]:

- Emulsifiers stabilize an organic phase in water. They ensure the easy dilution in water of a concentrated liquid product. Once mixed, the pesticide is evenly dispersed in water in tiny droplets.
- Wetting agents or spreaders are added when the liquid to be sprayed remains in the form of a drop instead of spreading on the surface of the plant leaf. Wetting agents are surfactants; they allow the droplet of slurry to expand to wet a larger leaf area.
- Solvents are used for liquid formulations; they allow dilution of active ingredient. There are only a few active substances that can be dissolved in water; the most used solvents are organic solvents such as hydrocarbons, alcohols, and oil.
- The carrier substances are neutral and harmless substances that carry and dilute the active ingredient in dry formulations (powders or granules); the most used carriers are silica, aluminum or magnesium silicate, and talc.
- Dispersants are used to obtain stable and homogeneous suspensions of solid active substances; the dispersing agent plays the same role as the emulsifier in liquid formulations: it stabilizes the suspension of the powder in water. The dispersing agent makes it possible to distribute the powder uniformly in the water in small particles; a homogeneous liquid is thus obtained, ready for application.
- Agglutinating agents or adhesives are added to help the pesticide to attach to the leaf surface, thus decreasing the risk of leaching of the active ingredient during rain.
- Coloring agents are added to reduce the risk of accidents, for example, by clearly showing the difference between treated (and therefore toxic and nonedible) seeds and untreated seeds.
- Synergists are adjuvant that improves the treating action of active ingredient.

Pesticides can be classified according to the target pests in herbicides, fungicides, insecticides, and acaricides.

3 Herbicides

These are substances intended to destroy or limit the growth of plants that are herbaceous or woody. Desiccant herbicides are pesticides that destroy the above-ground parts of plants to facilitate harvesting.

Herbicides can be active preemergent, that is, on seed, or postemergent, when the plant is emerged from the soil. Their action is systemic if they cross the cuticle of the undesirable plant and are transported throughout the plant or local if they act only at the level of the organ on which they are applied [6].

3.1 Herbicides Acting on Plant Growth

3.1.1 Synthetic Phytohormones

Phenoxyalkanoic acids, or aryloxyacids, include 2,4-D (2,4-dichlorophenoxyacetic acid), MCPA (4-chloro-2-methylphenoxyacetic acid), mecoprop, and dichlorprop; they are associated with derivatives of benzoic acids (dicamba, tricamba) and pyridine derivatives (picolinic acid), of the same mechanism of action. These compounds indeed have auxinic activity; they are called synthetic phytohormones [6]. Used at low doses to increase the size of the fruits and prevent their fall, they have at high dose an herbicide activity. They cause an abnormal development of the undesirable plant. They are systemic herbicides, widely used in agriculture, for the maintenance of forests, and in products for domestic use.

3.1.2 Nonhormonal Compounds

Amides (especially dichloroacetanilides), dinitroanilines (pendimethalin, trifluralin), certain carbamates (phenmedipham, chlorpropham, asulam), and thiocarbamates (molinate, triallate) block the growth of the plant by a nonhormonal mechanism [6]. Amides act by inhibiting protein synthesis of the plant and root elongation. Alachlor and metolachlor, which are dichloroacetanilides, are the two main representatives of this family, the most widely used in the world, pre- and postemergence [7].

Dinitroanilines are derivatives of 2,6-dinitro-4-benzene-amine. They are also widely used worldwide, preplanted and preemergent on cereal crops and for many nonagricultural and ornamental uses. The mode of action of dinitroanilines is the inhibition of mitosis, therefore of cell growth, by blocking the polymerization of microtubules.

3.2 Photosynthesis Disruptors

3.2.1 Triazines

These substances block the transfer of electrons in the photosynthesis reactions. They are photosynthetic inhibitors at photosystem II, site A. The products of this family are used pre- and postemergence on crops and all sites requiring weeding; the penetration is both root and foliar, but migration is important by root [7]. Other chemical families act in the same way as benzothiadiazoles (bentazone), nitriles (bromoxynil), and pyridazinones (chloridazone or pyrazon).

3.2.2 Substituted Ureas

The substituted ureas (diuron, linuron, monuron) are photosynthetic inhibitors in photosystem II, site B. These herbicides migrate only in the xylem (upward only). The lesions appear after the exit of the cotyledons and the first true leaves.

3.2.3 Bipyridines

Bipyridines or quaternary ammoniums (paraquat, diquat) cause the formation of superoxides leading to lipoperoxidation and disturbances in oxidation-reduction

reactions [8, 9]. They act by contact; their effect is very fast. They are very toxic, very soluble in water, nonselective, and strongly fixed on the clay-humic complex so that their persistence of action is null.

3.3 Inhibitors of Protein Synthesis

3.3.1 Inhibitors of Glutamine Synthesis

This is glufosinate-ammonium, which has a leaf penetration and acts primarily by contact (little scattering in the phloem/xylem). It acts by inhibiting glutamine synthetase [8]; it is nonselective and broad spectrum.

3.3.2 Inhibitors of Aromatic Amino Acid Synthesis

These glycines (glyphosate) have a leaf penetration and a systemic action. It acts by inhibiting the enolpyruvylshikimate phosphate (EPSP) synthase. This is the group of herbicides that spread the most in the plant. Widely used worldwide in agriculture as for amateur gardens, they are totally nonselective and broad spectrum and can be very toxic to crops also. Glyphosate is quickly and firmly adsorbed on soil particles. It therefore has no action in the soil, which allows a culture to be implanted in a short time after treatment [9].

3.3.3 Inhibitors of Branched Amino Acid Synthesis

These are mainly imidazolinones (imazamox, etc.), sulfonylureas (amidosulfuron, chlorsulfuron, sulfosulfuron, tribenuron-methyl, etc.), and triazolopyrimidines (florasulam, metosulam, etc.). They act by inhibition of acetolactate synthase [9]. These compounds all have both foliar and root penetration and significant migration in weeds. They can cause atrophy of grasses and yellowing (chlorosis) or purple discoloration of inter-nerval regions.

3.4 Inhibitors of Lipid Synthesis

Most lipid synthesis inhibitors are inhibitors of the enzyme acetyl-coenzyme A carboxylase, also called “vegetative point destructors of grasses.” They act by blocking the level of the meristems of the initial stage of the synthesis of fatty acids; the chemical families concerned are aryloxyphenoxy propionates “fops” and cyclohexanediones “dimes.” They are widely used in combination with other herbicides for the weeding of cereal crops. Another type of lipid synthesis inhibitors that uses a mechanism other than the inhibition of the enzyme acetyl-coenzyme A carboxylase is the case of thiocarbamates.

Aryloxyphenoxypropionates act only against grasses. They all have a systemic mode of action, but diclofop and fenoxaprop spread little and do not control perennial grasses.

Cyclohexanedione “dimes” such as clethodim and cycloxydim are also systemic anti-grass compounds with a systemic mode of action.

4 Fungicides

Agricultural fungicides can be used to control phytopathogenic fungi that can cause damage to crop plants and crops. Potential losses caused by fungal diseases range from 10% to 30% depending on the crop [10]. Fungicides are used on seeds, soil, and crops, as well as on stored commodities, in feeds. The treatments are preventive most often, or applied at the first symptoms of infestation, more rarely curative; some, anti-sporulating, prevent the spread of the fungus.

The products are either systemic or penetrating, if they penetrate the cuticle but act only at the level of the organ treated or surface [6].

4.1 Fungicides Acting on Mitosis and Cell Division

The methyl benzimidazole carbamates act by blocking mitosis by interaction with tubulin [6]. Benzimidazoles were the first systemic polyvalent fungicides with healing properties, but resistance has developed in a very large number of pathogens, for example, benomyl, carbendazim, thiophanate-methyl, and thiabendazole. Benzamides also act with the same mode of action, for example, fluopicolide and zoxamide.

Iprovalicarb, cymoxanil, and dimetomorph belong to different chemical families but have a similar mode of action.

4.2 Fungicides Acting on Respiration

Strobilurins (azoxystrobin, kresoxim-methyl, pyraclostrobin, etc.) show a broad spectrum of activity, excellent preventive and curative action, long persistence of action, and low doses of use but were soon confronted with the development of resistance.

Other active substances of different chemical nature act with a similar mode of action of famoxadone, fenamidone, dinocap, meptyldinocap, fluazinam, and cyazofamid.

4.3 Fungicides Acting on the Sterol Biosynthesis in the Membrane

Triazoles are part of a group of fungicidal molecules called IDM (demethylation inhibitors). They act by disrupting the biosynthesis of the cell membranes of the fungus. They act on an enzyme called 14 alpha-demethylase or CYP51; thus, they prevent the synthesis of ergosterol, one of the main constituents of fungi cell membranes resulting in the increase of membrane permeability and cell disintegration, causing premature death of the pathogen [11]. Several active ingredients exist: bitertanol, bromuconazole, difenoconazole, epoxiconazole, flutriafol, hexaconazole,

myclobutanil, penconazole, propiconazole, prothioconazole, tebuconazole, tetraconazole, triadimenol, triticonazole, flusilazole, and cyproconazole.

Other fungicides belonging to other chemical families also act on the synthesis of sterols; these are triforine, prochloraz, fenhexamid, spiroxamine, and fenarimol.

4.4 Fungicides Affecting the Biosynthesis of Amino Acids or Proteins

It is essentially the family of anilinopyrimidines (cyprodinil, mepanipyrim, and pyrimethanil) whose primary target is not known.

4.5 Fungicides Acting by Contact: Multi-sites

Inorganic active substances: They account for a large part, in tonnage, of the fungicides used. They are active especially at the stage of germination, in prevention. These are sulfur, polysulfides, and copper derivatives (copper sulfate, copper oxychloride).

Dithiocarbamates: They are active mainly in prevention, by inhibiting the germination of the spores of many fungi, and are used in the treatment of aerial parts, seeds, or soils. They must be applied often because they are rapidly hydrolyzed and not systemic [12]. Examples are mancozeb, maneb, ziram, zineb, thiram, propineb, and metiram.

Phthalimides: They are not volatile or water-soluble. They act preventively by inhibiting the germination of fungal spores; they are systemic fungicides that appeared in the early 1950s. Their characteristic group S-CCl3 on nitrogen is responsible for their interaction with cell thiols. Their spectrum of action is quite broad; they are not persistent in the environment, for example, captan and folpel.

Chloronitriles: Chlorothalonil is a broad-spectrum anti-fungal, inhibiting enzymatic reactions in fungal spores. Non-water-soluble and nonvolatile, its persistence in soils is quite important (up to 3 months) and is very toxic to fish. It is used on the aerial parts of many crops and in some xyloprotective products [13].

5 Insecticides

The major chemical families of compounds with insecticide activity, sometimes also acaricide, anti-arachnid, and nematicide, are organochlorines, organophosphorus, carbamates, and pyrethroids. Other compounds are also widely used, such as rotenone, fipronil of the phenylpyrazole family, imidacloprid of the neonicotinoid family, and abamectin of the avermectin family. The products are active on the insect either by ingestion and digestive absorption or by contact and absorption at the cuticle or by inhalation.

At the level of the organism to be protected (plant or animal), there are the so-called "contact" products, which are present only at the level of the organ on which they are applied, and the "systemic" or endotherapeutic products, which migrate into the body and protect it completely. Insecticide targets are, depending on the compounds, the nervous system, the biosynthesis of chitin, the production of energy, or the hydration of insects. Hormones that control molts and chemical mediators that allow communication between insects or between insects and plants can also be used [6].

5.1 Insecticides Acting on the Nervous System

Some neurotoxic compounds are axon poisons: organochlorines such as DDT and pyrethroids block the closure of voltage-gated sodium channels, and endosulfan, phenylpyrazoles, and avermectins block the GABA-coupled chlorine channel. Others are synaptic poisons, such as acetylcholine esterase inhibitors (organophosphorus and carbamate), and neonicotinoids, which are irreversible cholinergic receptor agonists. These neurotoxins all cause neuronal hyper-excitation, and their specificity for insects compared to mammals or other animals is more or less strong, so organophosphorus and carbamate can be very neurotoxic for mammals, while avermectins and pyrethroids are less neurotoxic.

5.1.1 Organochlorines

Organochlorines were the first organic insecticides used, with DDT (dichlorodiphenyltrichloroethane), marketed in 1942. It played a very important role in the fight against the vectors of typhus, malaria, and cholera. Characterized by a very low toxicity for mammals, these compounds, very persistent, accumulate in fats and along the food chain (bioaccumulation). Compounds such as lindane (gamma-hexachlorocyclohexane) and methoxychlor, slightly less lipophilic, were then synthesized [6]. Most organochlorines act as modulators of sodium channel (DDT, aldrin, lindane, methoxychlor, etc.). The use of this type of organochlorines in agriculture has been banned in the world since the years 1960–1970.

5.1.2 Organophosphorus

Organophosphorus insecticides, obtained from the search for chemical weapons in the 1930s, were marketed from 1942 with the very toxic and quickly abandoned TEPP (tetraethyl pyrophosphate) and then the parathion in 1944 [13]. They have gradually replaced organochlorines, which are too persistent.

The toxic action of insecticides or acaricides of organophosphorus is due to the selective inhibition of acetylcholinesterase. The mites have developed a resistance to most organophosphorus compounds causing the withdrawal of their use.

They can be classified into three groups: aliphatics (e.g., malathion), aromatics (e.g., parathion), and heterocyclics (e.g., phosalone), or according to the presence of sulfur atoms into: organophosphorus compounds (e.g., dichlorvos), thio-organophosphorus compounds (e.g., diazinon), and dithio-organophosphorus compounds

(e.g., malathion) [13]. Aromatic and sulfurous derivatives are the most persistent; most compounds are however not persistent in the environment (they are hydrolyzed quickly). They do not accumulate in living organisms either. They are generally not very volatile, except chlorpyrifos, dichlorvos, and fenthion, and very lipophilic. They act by contact and by ingestion, sometimes also by inhalation, on a large variety of insects and worms. Some have a systemic spread in the plant or animal.

5.1.3 Carbamates

Carbamates, and more particularly *N*-methyl aryl carbamates, are potent insecticides; the first molecule, dimetan, was synthesized by Ciba-Geigy in 1945, and then carbaryl, still widely used, by Union Carbide in 1957 [13]. *N*-methyl carbamates of oxime, such as aldicarb, are also nematicides and miticides. Most are less volatile and fat-soluble (except pirimicarb, which is highly water-soluble). These products have a broad spectrum of action; some are systemic. They act by contact and by ingestion, sometimes also by inhalation, on a large variety of insects and worms. Carbofuran and aldicarb, used to treat soil, are among the most dangerous pesticides for mammals, while carbaryl and propoxur are very well tolerated and incorporated into many household products.

5.1.4 Pyrethroids

Pyrethroids, synthesized later, have the advantage of being nonpersistent and not very toxic for mammals; they are chemically similar to pyrethrum, derived from plants of the family Asteraceae. The latter is a mixture of esters of pyrethric and chrysanthemic acids, two derivatives of cyclopropane carboxylic acid. It is a diverse group, which includes allethrins, tetramethrin and resmethrins which are relatively photolabile compounds, and also more stable and persistent halogenated compounds: permethrin, deltamethrin, fenvalerate and cypermethrin. They are generally classified into two groups, type I (not containing cyano radical CN: permethrin, tetramethrin) and type II pyrethroids (comprising a cyano radical CN: deltamethrin, fenvalerate). Most of the compounds used are esters of 3-phenoxyphenyl alcohol. Very little volatile and very lipophilic, they are almost insoluble in water. They act by contact and ingestion and lead to immediate stupefaction (knockdown effect) and then the death of the unwanted insect; synergists, such as piperonyl butoxide, increase their insecticidal activity. Pyrethroids are widely used in all types of crops and nonagricultural uses but are toxic to bees and aquatic life [14].

5.1.5 Neonicotinoids

Neonicotinoids have become the best-selling insecticide class in the world, used to control biting-sucking insects, some beetles, and Lepidoptera [15]. The first neonicotinoid on the market, imidacloprid, has the distinction of being more selective for insects than for mammals [16]. Neonicotinoids are the only insecticides with three modes of application: treatment of the aerial parts of the plant, soil treatment, and coating of the seed. However, their uses are subject to controversy because of the harmful effects they generate on nontarget organisms such as bees and pollinating insects [17, 18].

5.2 Other Insecticides

The insecticides presented below have the advantage of being specific to insects and are very toxic to mammals. We will only present them very quickly.

5.2.1 Insecticides Inhibiting Molting

Benzoyl phenyl ureas, among the recent insecticides, were synthesized from the 1970s; they are contact insecticides and active after ingestion by the insect. Hexaflumuron, lufenuron, and diflubenzuron act by inhibiting chitin biosynthesis, the main component of the insect cuticle which disrupts molting. They also have ovicide action.

Juvenoids are compounds that mimic the action of juvenile hormones of insects: when the cerebral secretion of these hormones ceases, the larvae can be transformed into adults. Under the action of juvenoids, the insect remains immature and cannot reproduce. This group includes hydroprene, methoprene, pyriproxyfen, and fenoxycarb; they are also called growth regulators.

5.2.2 Insecticides and Acaricides Acting on Respiration

This group of pesticides contains mainly stannic derivatives which are specific acaricides; they act by contact and ingestion by inhibiting the oxidative phosphorylation at the level of the mitochondria; the most well-known acaricides in this group are azocyclotin, cyhexatin, and fenbutatin oxide.

6 Fungicides and Insecticides Authorized on Fruits and Vegetables

The production of fruits and vegetables is often confronted with biotic stresses exerted by different bio-aggressors (insect pests, fungal and bacterial diseases, weeds, etc.). These bio-aggressors cause considerable losses: yield decreases and deterioration of the quality of fruit or vegetables at harvest and storage. Crop losses may be quantitative or qualitative [19]. Quantitative losses result from reduced productivity, leading to a smaller yield. Qualitative losses from pests may result from reduced nutritional content, reduced market quality, reduced storage characteristics, or contamination of the harvested product with pests, parts of pests, or the toxic products of pests (e.g., mycotoxins from plant-parasitic fungi) [20].

In order to ensure sufficient production of fruits and vegetables for a growing population, agriculture often uses pest control through the use of pesticides; these products remain in most cases the most effective means and the most easy to handle and allow better optimization of yields.

6.1 Fungicides and Insecticides Most Authorized on Fruits

Fruit production can be attacked by several fungal and bacterial diseases, among which we can mention late blight, powdery mildew, peach leaf curl, scab, fire blight

(*Erwinia amylovora*), and monilia. Several fungicidal molecules are authorized all over the world to prevent or treat these diseases; these fungicides can be of the same chemical families with the same mode of action or of different chemical families with the same or different modes of action. The existence of several pesticides to treat the same disease or the same insect is very important in order to avoid the problems of resistance.

The most common fruit pests are aphids, psyllids, mites, cochineal, thrips, and leafhoppers.

The following table (Table 1) summarizes the list of fungicides and insecticides most authorized for each type of fruit [21, 22].

6.2 Fungicides and Insecticides Most Authorized on Vegetables

The most common diseases found on vegetables are mildew, seedling blight, *Alternaria*, *Rhizoctonia*, and fusarium wilt, while the most harmful pests are mites, codling moth, leaf miner, aphids, moths, and Colorado potato beetle. Table 2 gives the most common diseases and pests of vegetables as well as the fungicides and insecticides authorized to control them.

7 Toxicological Risks of Pesticides

The presence of pesticides in the various compartments of the environment results mainly from the intentional application of these products on crops and forests (spreading or spraying) as part of the fight against bio-aggressors or for other uses (domestic, public health, fight against vector-borne diseases, etc.) but also unintentionally following accidents related to manufacturing, transport, and storage. Pesticides in the environment cause different types of pollution that can have negative effects on living organisms.

For humans, apart from occupational exposure, exposure to pesticides is indirect from their environment and due to the presence of these toxic substances in the natural environment, food, and drinking water [3].

Multiple routes of exposure to pesticides can be encountered in humans; occupational exposure is mainly for people handling the products. Farmers are a particularly exposed population; in agriculture, the exposure is mainly cutaneous and rarely oral; the absorption of pesticides by the skin is revealed as the most significant route [23]. One study showed that wearing a protective suit did not completely prevent operator skin contamination with dithiocarbamate fungicides [24]. In several epidemiological studies, there has been a significant association of pesticide use among farmers and the occurrence of certain types of cancer such as cancers of the lips, prostate, stomach, kidneys, brain, but also most cancers of the hematopoietic system (leukemia, multiple myeloma and especially non-Hodgkin's lymphoma), cutaneous melanoma and soft tissue sarcomas [25–34].

Table 1 Fungicides and insecticides most authorized on fruits

Crop	Authorized pesticides	Diseases or pests
Grapes	**Acaricide insecticides**: cypermethrin, acrinathrin, bifenthrin, clofentezine, cyhexatin, fenbutatin oxide, diclofop, dimethoate, fenpyroximate, hexythiazox, propargite, pyridaben, lambda-cyhalothrin, pirimicarb, chlorpyrifos, acephate, beta-cypermethrin, diafenthiuron, esfenvalerate, fenvalerate, fenitrothion, imidacloprid, malathion, methomyl, spirodiclofen, bromopropylate, flufenoxuron, fenoxycarb, lufenuron, indoxacarb, diazinon, phosalone, trichlorfon, spinosad, tetradifon, tau-fluvalinate	Leafhopper, mite, flea beetle; borer, budworm, cochineal
	Fungicides dimethomorph, pyriproxyfen, captan, famoxadone, cymoxanil, metalaxyl, tebuconazole, copper, chlorothalonil, metiram zinc, mancozeb, maneb, azoxystrobin, difenoconazole, cyprodinil, procymidone, benomyl, thiophanate-methyl, iprodione, carbendazim, fludioxonil, thiram, fenhexamid, ziram, sulfur, zoxamide, tolylfluanid, fosetyl-aluminum, folpel, propineb, zineb, benalaxyl, iprovalicarb, fluopicolide, fenamidone, hexaconazole, penconazole, triadimenol, boscalid, pyraclostrobin, tetraconazole, trifloxystrobin, myclobutanil, spiroxamine, kresoxim-methyl, dinocap, difenoconazole, bromuconazole, pyrimethanil, triflumizole, mefenoxam, fenbuconazole, pyrimethanil, flusilazole, ofurace, fenarimol	Late blight, black rot, excoriose, powdery mildew, gray rot
Apples	**Acaricide insecticides**: azocyclotin, abamectin, amitraze, fenpyroximate, pyridaben, tebufenpyrad, endosulfan, alphamethrin, cypermethrin, acrinathrin, bifenthrin, clofentezine, cyhexatin, fenbutatin oxide, diclofop, dimethoate, hexythiazox, propargite, azinphos-methyl, carbaryl, chlorpyrifos, dimethoate, lambda-cyhalothrin, pirimicarb, deltamethrin, diflubenzuron, acephate, beta-cypermethrin, diafenthiuron, esfenvalerate, fenvalerate, fenitrothion,	Mites, codling moth, leaf miner, aphids, leaf roller

(*continued*)

Table 1 (continued)

Crop	Authorized pesticides	Diseases or pests
	imidacloprid, malathion, methomyl, spirodiclofen, bromopropylate, flufenoxuron, fenoxycarb, lufenuron, indoxacarb, diazinon, phosalone, trichlorfon, spinosad, tetradifon, tau-fluvalinate, fenthion, methidathion, phosmet, tebufenozide, teflubenzuron, diazinon, methamidophos, oxydemeton-methyl, beta-cyfluthrin, etoxazole, fenazaquin	
	Fungicides: captan, famoxadone, cymoxanil, tebuconazole, copper, sulfur, metiram zinc, mancozeb, maneb, azoxystrobin, difenoconazole, cyprodinil, benomyl, thiophanate-methyl, carbendazim, thiram, ziram, sulfur, tolylfluanid, fosetyl-aluminum, folpel, propineb, zineb, hexaconazole, penconazole, triadimenol, pyraclostrobin, tetraconazole, trifloxystrobin, myclobutanil, spiroxamine, kresoxim-methyl, dinocap, difenoconazole, bromuconazole, pyrimethanil, triflumizole, mefenoxam, fenbuconazole, flusilazole, ofurace, fenarimol, dithianon, thiabendazole, bupirimate, cyproconazole, fluquinconazole, triadimefon, triforine, dodine, meptyldinocap, bitertanol	Powdery mildew, scab, fire blight
Pears	**Acaricide insecticides**: azocyclotin, abamectin, amitraze, fenpyroximate, pyridaben, tebufenpyrad, endosulfan, alphamethrin, cypermethrin, acrinathrin, bifenthrin, clofentezine, cyhexatin, fenbutatin oxide, diclofop, dimethoate, hexythiazox, propargite, azinphos-methyl, carbaryl, chlorpyrifos, dimethoate, lambda-cyhalothrin, pirimicarb, deltamethrin, diflubenzuron, acephate, beta-cypermethrin, diafenthiuron, esfenvalerate, fenvalerate, fenitrothion, imidacloprid, malathion, methomyl, spirodiclofen, bromopropylate, flufenoxuron, fenoxycarb, lufenuron, indoxacarb, diazinon, phosalone, trichlorfon, spinosad, tetradifon, tau-fluvalinate, fenthion, methidathion, phosmet, tebufenozide, teflubenzuron,	Mites, codling moth, leaf miner, aphids, psyllid, leafhopper

(*continued*)

Table 1 (continued)

Crop	Authorized pesticides	Diseases or pests
	diazinon, methamidophos, oxydemeton-methyl, beta-cyfluthrin, etoxazole, fenazaquin	
	Fungicides: captan, famoxadone, cymoxanil, tebuconazole, copper, sulfur, metiram zinc, mancozeb, maneb, azoxystrobin, difenoconazole, cyprodinil, benomyl, thiophanate-methyl, carbendazim, thiram, ziram, sulfur, tolylfluanid, fosetyl-aluminum, folpel, propineb, zineb, hexaconazole, penconazole, triadimenol, pyraclostrobin, tetraconazole, trifloxystrobin, myclobutanil, spiroxamine, kresoxim-methyl, dinocap, bromuconazole, pyrimethanil, triflumizole, mefenoxam, fenbuconazole, flusilazole, ofurace, fenarimol, dithianon, thiabendazole, bupirimate, cyproconazole, fluquinconazole, triadimefon, triforine, dodine, meptyldinocap, bitertanol	Powdery mildew, leaf rust, septoria, scab, fire blight
Apricot	**Acaricide insecticides**: propargite, bifenthrin, etoxazole, pyridaben, lambda-cyhalothrin, phosalone, azinphos-methyl, deltamethrin, fenitrothion, fenthion, esfenvalerate, methomyl, methidathion, endosulfan, alphamethrin, imidacloprid, pirimicarb, methamidophos	Mites, codling moth, lyda, small leaf miner, aphids
	Fungicides: carbendazim, copper, cyprodinil, fludioxonil, iprodione, bitertanol, fenbuconazole, tebuconazole, cyproconazole, difenoconazole, flusilazole, myclobutanil, procymidone, thiophanate-methyl, triadimenol, vinclozolin, bupirimate, dinocap, fenarimol, penconazole, sulfur, captan, mancozeb, maneb	*Coryneum*, monilia, powdery mildew, rust, scab
Almonds	**Acaricide insecticides**: hexythiazox, lambda-cyhalothrin, azinphos-methyl, fenthion, cyfluthrin, deltamethrin, phosalone, pirimicarb, esfenvalerate, fenitrothion	Mite, codling moth, caterpillar, leaf miner, aphids
	Fungicides: copper, thiophanate-methyl, thiram, ziram, dithianon, bitertanol, carbendazim, iprodione, procymidone, maneb, probineb, tolylfluanid	Bacteriosis, leaf curl, monilia, rust, scab

(*continued*)

Table 1 (continued)

Crop	Authorized pesticides	Diseases or pests
Cherries	**Acaricide insecticides**: bifenthrin, lambda-cyhalothrin, phosalone, sulfur, deltamethrin, diazinon, dimethoate, fenthion, malathion, pirimicarb, methomyl, rotenone	Cheimatobia, leaf miner, fly, aphids, leaf roller
	Fungicides: copper, dithianon, mancozeb, maneb, cyprodinil, fludioxonil, flusilazole, hexaconazole, iprodione, myclobutanil, tebuconazole, thiophanate-methyl	Bacterial canker, cylindrospermopsis, monilia
Figs	**Acaricide insecticides**: deltamethrin, cyfluthrin, fenitrothion, esfenvalerate, phosalone, phosmet, azinphos-methyl, lambda-cyhalothrin	Fly, psyllid, ringworm
	Fungicides: copper	fig canker, rust
Kiwi	**Acaricide insecticides**: cyfluthrin, deltamethrin, lambda-cyhalothrin	Leafhopper
	Fungicides: copper, procymidone	Botrytis, bacteriosis
Peach	**Acaricide insecticides**: propargite, pyridaben, bifenthrin, cyhexatin, hexythiazox, amitraze, clofentezine, etoxazole, fenazaquin, fenpyroximate, tebufenpyrad, lambda-cyhalothrin, phosalone tau-fluvalinate, chlorpyrifos, methidathion, endosulfan, deltamethrin, azinphos-methyl, fenitrothion, esfenvalerate, fenthion, malathion, alphamethrin, imidacloprid, methamidophos, pirimicarb, cypermethrin, diazinon, methomyl, naled, pymetrozine, rotenone, acrinathrin, carbaryl, fenoxycarb	Thrips, moth, mites codling moth, cochineal, aphids
	Fungicides: copper, carbendazim, thiophanate-methyl, captan, dodine, thiram, ziram, dithianon, bitertanol, cyprodinil, fludioxonil, fenbuconazole, iprodione, tebuconazole, cyproconazole, flusilazole, difenoconazole, myclobutanil, procymidone, mancozeb, bupirimate, dinocap, fenarimol, penconazole, hexaconazole, maneb	Bacteriosis, peach leaf curl, monilia, powdery mildew, rust, scab
Plum	**Acaricide insecticides**: propargite, pyridaben, bifenthrin, cyhexatin, hexythiazox, clofentezine, fenazaquin, tebufenpyrad, azinphos-methyl, fenoxycarb, lambda-cyhalothrin,	Mite, codling moth, cheimatobia, aphids, budworm, zebra

(*continued*)

Table 1 (continued)

Crop	Authorized pesticides	Diseases or pests
	alphamethrin, deltamethrin, phosalone, methidathion, sulfur, fenthion, imidacloprid, methamidophos, methomyl, pyrimicab	
	Fungicides: copper, captan, vinclozolin, bitertanol, carbendazim, cyproconazole, cyprodinil, fludioxonil, difenoconazole, flusilazole, iprodione, myclobutanil, mancozeb, procymidone, thiophanate-methyl, triadimenol, fenbuconazole, tebuconazole, maneb, dithianon	Monilia, rust, scab
Oranges	**Acaricide insecticides**: hexythiazox, lambda-cyhalothrin, methidathion, tebufenozide, abamectin, acetamiprid, acephate, thiamethoxam, cypermethrin, chlorpyrifos, azadirachtin, azocyclotin, lufenuron, **b**uprofezin, tau-fluvalinate, flufenoxuron, imidacloprid, deltamethrin, dimethoate, beta-cyfluthrin, spirodiclofen, fenthion, phosmet, fenpyroximate, pirimicarb, acrinathrin, thiacloprid, spinosad, propargite, fenbutatin oxide, cyhexatin, pyriproxyfen	Mites, leafhoppers, whitefly, *Ceratitis*, leaf miners, thrips, aphids
	Fungicides: mancozeb, fosetyl-aluminum, thiabendazole, chlorothalonil, folpel, metalaxyl, imazalil	Gummosis
Banana	**Acaricide insecticides**: spinosad, fosthiazate, cadusafos, fenamiphos, ethoprophos	Soil treatment, weevils
	Fungicides: difenoconazole, propiconazole, trifloxystrobin, fenpropidin, imazalil, thiabendazole, azoxystrobin, mancozeb, chlorothalonil, copper, triadimenol, bitertanol	*Cercospora*, powdery mildew
Pineapple	**Acaricide insecticides**: spinosad	Cochineal
	Fungicides: fosetyl-aluminum	*Phytophthora*
	Fungicides: chlorothalonil, mancozeb, maneb, myclobutanil, propamocarb HCl, azoxystrobin, fosetyl-aluminum, bupirimate, dinocap, penconazole, sulfur, diethofencarb, carbendazim, iprodione, procymidone, vinclozolin	*Alternaria*, anthracnose, mildew, powdery mildew, rot
Strawberries	**Acaricide insecticides**: alphamethrin, bifenthrin, deltamethrin, abamectin, acrinathrin, clofentezine, cyhexatin,	Mites, whiteflies, leafhoppers, aphids, thrips

(*continued*)

Table 1 (continued)

Crop	Authorized pesticides	Diseases or pests
	fenazaquin, fenbutatin oxide, hexythiazox, pyriproxyfen, endosulfan, lambda-cyhalothrin, carbofuran, pirimicarb, rotenone, diclofop, formetanate	
	Fungicides: iprodione, mancozeb, maneb, thiophanate-methyl, myclobutanil, thiram, cuivre, fosetyl-aluminum, azoxystrobin, bupirimate, hexaconazole, dinocap, penconazole, mefenoxam, carbendazim, cyprodinil, fludioxonil, diethofencarb, fenhexamid, mepanipyrim, procymidone, pyrimethanil, vinclozolin, metalaxyl	*Alternaria*, anthracnose, late blight, powdery mildew, gray mold, verticillium wilt, *Zythia*
Melon	**Acaricide insecticides**: bifenthrin, abamectin, acrinathrin, clofentezine, cyhexatin, diclofop, fenazaquin, hexythiazox, buprofezin, deltamethrin, methomyl, pymetrozine, cyromazine, carbofuran, alphamethrin, esfenvalerate, lambda-cyhalothrin, pirimicarb, rotenone, tau-fluvalinate, acrinathrin, formetanate	Whitefly, leaf miner, aphids, borer, thrips
	Fungicides: chlorothalonil, mancozeb, maneb, thiophanate-methyl, myclobutanil, cuivre, carbendazim, azoxystrobin, benalaxyl, fosetyl-aluminum, mefenoxam, propamocarb HCl, bupirimate, dinocap, fenarimol, fenbuconazole, penconazole, sulfur, triadimefon, triadimenol, diethofencarb, procymidone, vinclozolin	Anthracnose, cladosporiosis, late blight, powdery mildew

Several studies have shown neurological damage; thus, an increased risk of Parkinson's disease associated with the exposure of insecticides or herbicides has been shown, and this risk would be higher if exposed to organochlorines, organophosphorus, or carbamates [35]. Baldi et al. [36] showed in a study in France that occupational exposure to a pesticide was associated with twice the risk of developing Alzheimer's disease. It has also been associated with mild cognitive dysfunction and risk of dementia in Parkinson's [37].

In the event of acute exposure, pesticides are responsible for nearly three million serious poisonings and 220,000 deaths each year worldwide [38].

The entire population may be exposed to pesticides for domestic use but especially to residues of these pesticides through its environment (water, air, suspended particles, dust) and its diet. WHO experts say pesticide contamination of food is by

Table 2 Fungicides and insecticides most authorized on vegetables

Crop	Authorized pesticides	Diseases or pests
Beets	**Acaricide insecticides**: bifenthrin, cyfluthrin, deltamethrin, lambda-cyhalothrin, diazinon, carbofuran, imidacloprid, aldicarb	Mites, flea beetle, leafhoppers, aphids, atomaria
	Fungicides: bitertanol, cyproconazole, tetraconazole, difenoconazole, flusilazole, flutriafol, mancozeb, maneb, sulfur, iprodione	*Cercospora*, powdery mildew, phoma, pythium, ramulariosis, rust
Potatoes	**Acaricide insecticides**: alphamethrin, carbosulfan, ethoprophos, beta-cyfluthrin, chlorpyrifos, cypermethrin, deltamethrin, endosulfan, esfenvalerate, lambda-cyhalothrin, phosalone, pyrimicab, azinphos-methyl, tefluthrin, malathion	Wireworms, Colorado potato beetle, aphids, moths
	Fungicides: mancozeb, imazalil, benalaxyl, chlorothalonil, copper, cymoxanil, probineb, fluazinam, folpel, thiophanate-methyl, iprodione, thiabendazole, flutolanil, maneb, captan, mandipropamid, famoxadone, fenamidone, propamocarb HCl, zoxamide, benalaxyl, metalaxyl, dimethomorph, difenoconazole, iprovalicarb, azoxystrobin, ofurace, mefenoxam, fentin hydroxide, fluopicolide	*Alternaria*, fusariosis, silvery scab, mildew, phoma, rhizoctonia
Olive	**Acaricide insecticides**: carbaryl, methidathion, fenoxycarb, deltamethrin, dimethoate, fenthion, diazinon, lambda-cyhalothrin, malathion, trichlorfon, phosmet, chlorpyrifos	Cochineal, olive fly, psyllid, ringworm
	Fungicides: copper, mancozeb, difenoconazole, dodine, probineb	Peacock's eye, bacteriosis
Carrot	**Acaricide insecticides**: benfuracarb, carbofuran, chlorfenvinphos, diazinon, diethion, endosulfan, lambda-cyhalothrin, pirimicarb, rotenone, taufluvalinat	Fly, aphids
	Fungicides: azoxystrobin, chlorothalonil, difenoconazole, hexaconazole, iprodione, carbendazim, mancozeb, myclobutanil, pyrimethanil, mefenoxam, copper, dinocap, sulfur	*Alternaria*, mildew, powdery mildew, sclerotinia
Celery	**Acaricide insecticides**: lambda-cyhalothrin, cyromazine, chlorfenvinphos, diazinon, diethion	Leaf miner, aphids, flies

(*continued*)

Table 2 (continued)

Crop	Authorized pesticides	Diseases or pests
	Fungicides: copper, azoxystrobin, difenoconazole, mancozeb, maneb, probineb, propamocarb HCl,	Septoria, bacteriosis
Garlic	**Acaricide insecticides**: carbofuran, diazinon, chlorfenvinphos, lambda-cyhalothrin, azinphos-methyl, cyfluthrin, deltamethrin	Fly, aphids, ringworm, thrips
	Fungicides: iprodione, maneb, thiophanate-methyl, procymidone, thiram, chlorothalonil, mefenoxam, azoxystrobin, mancozeb, diethofencarb, carbendazim, prochloraz, vinclozolin, hexaconazole, tebuconazole	Botrytis, mildew, rust
Onion	**Acaricide insecticides**: carbofuran, diazinon, chlorfenvinphos, lambda-cyhalothrin, azinphos-methyl, cyfluthrin, deltamethrin	Fly, ringworm, thrips
	Fungicides: iprodione, maneb, thiophanate-methyl, procymidone, thiram, chlorothalonil, benalaxyl, cymoxanil, probineb, metalaxyl, fosetyl-aluminum, hexaconazole, mefenoxam, cuivre, propamocarb HCl, azoxystrobin, mancozeb, carbendazim, prochloraz, thiram	Botrytis, mildew, fusarium, rot
Lettuce	**Acaricide insecticides**: bifenthrin, abamectin, deltamethrin, cyromazine, methomyl, alphamethrin, pirimicarb, lambda-cyhalothrin, azinphos-methyl, dichlorvos, endosulfan, fenitrothion, malathion, methomyl, naled, phosalone, pymetrozine, rotenone, tau-fluvalinate	Whiteflies, leaf miner, aphids, thrips
	Fungicides: pyrimethanil, cymoxanil, mancozeb, benalaxyl, fosetyl-aluminum, maneb, metiram, sodium, cyprodinil, fludioxonil, iprodione, procymidone, vinclozolin, pencycuron, sodium trithiocarbonate	*Botrytis*, mildew, rot, rhizoctonia
Eggplant	**Acaricide insecticides**: deltamethrin, abamectin, acrinathrin, hexythiazox, buprofezin, methomyl, pymetrozine, pyriproxyfen, alphamethrin, carbaryl, lambda-cyhalothrin, rotenone, cyromazine, pirimicarb	Mite, whiteflies, Colorado potato beetles, leaf miners, aphids, thrips
	Fungicides: azoxystrobin, mancozeb, maneb, thiophanate-methyl, myclobutanil, carbendazim, diethofencarb, iprodione, procymidone, vinclozolin	*Alternaria*, late blight, powdery mildew, verticillium wilt, rot

(*continued*)

Table 2 (continued)

Crop	Authorized pesticides	Diseases or pests
Cucumber	**Acaricide insecticides**: abamectin, acrinathrin, clofentezine, fenazaquin, fenbutatin oxide, hexythiazox, deltamethrin, methomyl, pymetrozine, pyriproxyfen, cyromazine, alphamethrin, esfenvalerate, lambda-cyhalothrin, pirimicarb, endosulfan, rotenone	Mites, whiteflies, miners, moths, aphids, thrips
	Fungicides: chlorothalonil, mancozeb, maneb, myclobutanil, propamocarb HCl, azoxystrobin, fosetyl-aluminum, bupirimate, dinocap, penconazole, sulfur, diethofencarb, carbendazim, iprodione, procymidone, vinclozolin	*Alternaria*, anthracnose, mildew, powdery mildew, rot
Zucchini	**Acaricide insecticides**: abamectin, fenbutatin oxide, hexythiazox, deltamethrin, pymetrozine, pyriproxyfen, cyromazine, chlorfenvinphos, deltamethrin, esfenvalerate, lambda-cyhalothrin, methomyl, rotenone, pirimicarb	Mite, whitefly, leaf miner, aphids, thrips
	Fungicides: chlorothalonil, mancozeb, myclobutanil, propamocarb HCl, azoxystrobin, fosetyl-aluminum, bupirimate, dinocap, fenbuconazole, hexaconazole, penconazole, sulfur, diethofencarb, carbendazim, iprodione, procymidone, vinclozolin,	Anthracnose, cladosporiosis, mildew, powdery mildew
	Fungicides: chlorothalonil, mancozeb, maneb, thiophanate-methyl, myclobutanil, copper, carbendazim, azoxystrobin, benalaxyl, fosetyl-aluminum, mefenoxam, propamocarb HCl, bupirimate, dinocap, fenarimol, fenbuconazole, penconazole, sulfur, triadimefon, triadimenol, diethofencarb, procymidone, vinclozolin	Anthracnose, cladosporiosis, mildew, powdery mildew
Peppers	**Acaricide insecticides**: abamectin, acrinathrin, diclofop, acetamiprid, hexythiazox, buprofezin, deltamethrin, methomyl, pymetrozine, cyromazine, carbofuran, alphamethrin, esfenvalerate, lambda-cyhalothrin, thiamethoxam, lufenuron, chlorpyrifos	Mites, whiteflies, caterpillars, leaf miner, moths, aphids
	Fungicides: hexaconazole, azoxystrobin, iprodione, procymidone, diethofencarb, vinclozolin, carbendazim	*Alternaria*, powdery mildew, mildew

(*continued*)

Table 2 (continued)

Crop	Authorized pesticides	Diseases or pests
Tomatoes	**Acaricide insecticides**: alphamethrin, bifenthrin, deltamethrin, abamectin, acrinathrin, cyhexatin, diclofop, fenazaquin, fenbutatin oxide, hexythiazox, sulfur, buprofezin, methomyl, pymetrozine, pyriproxyfen, carbaryl, lambda-cyhalothrin, rotenone, cyromazine, indoxacarb, pirimicarb, tau-fluvalinate, formetanate	Mites, flea beetle, whiteflies, leafhoppers, leaf miner, Colorado potato beetle, thrips aphids,
	Fungicides: azoxystrobin, chlorothalonil, difenoconazole, famoxadone, cymoxanil, iprodione, mancozeb, maneb, thiophanate-methyl, copper, sodium trithiocarbonate, propamocarb HCl, carbendazim, hymexazol, benalaxyl, cyazofamid, folpel, fenamidone, bupirimate, hexaconazole, myclobutanil, sulfur, procymidone, pyrimethanil, fenhexamid	*Alternaria*, anthracnose, mildew, cladosporiosis, powdery mildew rot, fusarium
Beans	**Acaricide insecticides**: bifenthrin, diclofop, fenbutatin oxide, hexythiazox, benfuracarb, chlorpyrifos, carbofuran, deltamethrin, pirimicarb, lambda-cyhalothrin, alphamethrin	Mites, fly, aphids, borer
	Fungicides: iprodione, maneb, thiophanate-methyl, azoxystrobin, carbendazim, thiram, cuivre, cyprodinil, fludioxonil, diethofencarb, procymidone, pyrimethanil, vinclozolin, mancozeb	*Alternaria*, anthracnose, rust, rot
Peas	**Acaricide insecticides**: beta-cyfluthrin, cyfluthrin, lambda-cyhalothrin, deltamethrin, alphamethrin, pirimicarb, naled, azinphos-methyl, bifenthrin, cypermethrin, fenitrothion, methidathion, methomyl, phosalone, rotenone, tau-fluvalinate, triazamate, dichlorvos, malathion, endosulfan, esfenvalerate, oxydemeton-methyl, beta-cyfluthrin, zeta-cypermethrin	Bruche, aphids, thrips, leaf rollers
	Fungicides: azoxystrobin, hexaconazole, captan, carbendazim, fosetyl-aluminum, chlorothalonil, folpel, thiram, carboxen, cymoxanil, mefenoxam, fludioxonil, difenoconazole, cyproconazole, flutriafol, iprodione, maneb, mancozeb, procymidone, carboxin, mefenoxam, diethofencarb, metconazole, pyrimethanil, vinclozolin, thiophanate-methyl	Anthracnose, mildew, powdery mildew, rust, sclerotinia

(*continued*)

Table 2 (continued)

Crop	Authorized pesticides	Diseases or pests
Artichoke	**Acaricide insecticides**: bifenthrin, deltamethrin, endosulfan, methomyl, pirimicarb, rotenone	Noctuidae, flea beetle, aphids
	Fungicides: fosetyl-aluminum, copper, mancozeb, myclobutanil	Ascochytosis, mildew, powdery mildew, ramulariosis

far the most important route of exposure. Risk assessments attribute 90% of exposure to food versus 10% to water [3].

The chronic exposure of the population to pesticides is related to various neurobehavioral [39] and neurodegenerative disorders [36] (such as Parkinson's disease and Alzheimer's disease) but also disorders of the peripheral nervous system (neuromotor and neurosensory disorders) [40]. Other studies have shown a link between environmental exposure to pesticides and the risk of hormone-dependent cancers such as breast cancer [41].

Some pesticides are identified as endocrine disruptors. They act by interfering with natural hormones because of their high binding potential with estrogenic and androgenic receptors [42]. They can also interfere in the synthesis, metabolism, and elimination of these hormones causing a decrease in their concentration; this is the case of cyhalothrin and fipronil that can inhibit the production of thyroid hormones [43]. They can also affect spermatogenesis reducing male fertility [44].

The exposure of the child to pesticides can take place very early, in utero via the placenta following the exposure of the mother [45], but also after birth, either directly by exposure to domestic contaminations (pesticides used in the home or garden or living in an agricultural area) or via breast milk and food [46] or indirectly for children of parents professionally exposed (e.g., farmers). It should be noted that diet has been shown to be a major source of exposure of children to organophosphorus pesticides [47].

In children, endocrine-disrupting pesticides are likely to cause developmental abnormalities: low birth weight, fetal death, childhood cancer, cryptorchidism, and hypospadias [48]. Another study by Merhi [3] showed that pesticide exposure is linked to a 10% increase in the risk of developing childhood cancers. The results show a very significant increase in leukemias, non-Hodgkin's lymphomas, and Ewing's sarcomas. For all cancers, the "prenatal" exposure period would appear to be the most critical in that it showed a 36% increase in all cancers [3].

8 Exposure to Pesticide Residues Through Food and Risk Assessment

Pesticide residues have become the permanent concern of the scientific community and public health organizations around the world. In order to guarantee consumer safety, very strict international, community, and national legislation has been put in

place. Monitoring of pesticide residues is a key tool to ensure compliance with regulations and to monitor compliance with good agricultural practices [49]. Toxic residue obviously means any residue that may be of toxicological significance in the margin of residual doses; there is no toxic compound but rather toxic doses. For this, many highly sophisticated methods have been developed for detecting, identifying, and measuring multi-residues contaminating matrices of different natures [50, 51].

8.1 Pesticide Residues

According to the Codex Alimentarius [52], a pesticide residue is any substance (derivative, metabolite, impurity, etc.) present in food, agricultural products, or animal feed as a result of the use of pesticides.

Indeed, after application, phytosanitary products evolve quantitatively and qualitatively over time. The amount of active substance or its transformation products present in the plant at harvest constitutes the residue. Its importance depends first and foremost on the nature of the product used but also on a number of external conditions such as climate, conditions of use, dose, and, in particular, the time to harvest [1].

8.2 Maximum Residue Limit (MRL)

The MRL is the maximum level of residues found in a crop product following the application of a pesticide in accordance with good agricultural practice (GAP) and therefore the maximum concentration of pesticide residue that is legally permitted or recognized as acceptable on food, agricultural commodity, or animal feed; it is expressed in milligrams of residues per kilogram of foodstuff [53].

Good agricultural practices for an active substance and a given crop correspond to the practice that leads to a residue concentration below the MRL. It usually reflects:

- The respect of the time before harvest
- Compliance with the dose per hectare indicated by the manufacturer
- Compliance with the number of application per season

As a result, the MRL appears as an agronomic standard, which reflects a use of the active substance in line with good agricultural practice and not a toxicological standard. An exceedance of the MRL must be interpreted as a non-compliance with an agricultural practice (generally no respect of the preharvest period).

Codex MRLs are used as national standards by many countries; however, some countries continue to establish their own MRLs or tolerances and impose zero tolerance to residues of pesticides on imported crops which do not have nationally/regionally agreed-upon MRLs. An example of the variation in MRLs for some pesticides in grapes is shown in Table 3 [54].

Table 3 Comparison of Codex and national/regional MRLs ($mgkg^{-1}$) for grapes

Pesticide	Codex	EU	Japan	USA
Captan	None	3	5	50
Chlorpyrifos	0.5	0.5	1	0.5
Dimethoate	1	0.02	1	2
Endosulfan	1	0.5	1	2
Myclobutanil	1	1	2	1
Spinosad	0.5	None	0.5	0.5

8.3 Acceptable Daily Intake (ADI)

It is the amount of a chemical present in the diet, which can be ingested daily by the consumer throughout his life without adverse effects on his health. It is calculated on the basis of toxicological studies from NOAEL (no-observed-adverse-effect level) observed in animals, divided by a safety factor taking into account intraindividual variability, interspecies variability, uncertainty associated with experimental protocols, and, if necessary, the nature of the effects of the substance. If available, data from epidemiological studies in humans can also be used [55]. ADIs are set by scientific assessment organizations at the international (JMPR: joint meeting of pesticide residues FAO/WHO Expert Committee), community (EFSA: European Food Safety Authority), or even national level. The ADI is used for the evaluation of food additives, pesticide residues, and veterinary antiparasitics. It is expressed in milligrams of substance per kilogram of body weight and per day (mg kg^{-1} bw day^{-1}).

8.4 Acute Reference Dose (ARfD)

It refers to the maximum amount of active substance (expressed as mg of active substance per kg of body weight) that can be ingested by the consumer for a day or less, in food or drinking water, with no adverse health effects. It is calculated from a no-observed-adverse-effect level (NOAEL) and a safety factor. The NOAEL chosen for the calculation comes from the most appropriate study on a sensitive and representative animal species. The safety factor takes into account intra- and interspecies variability and the nature of the effects of the substance.

9 Analytical Methods Applied to the Research of Pesticide Residues

Pesticide residues in foods have received a great deal of attention as an important element in food safety, and as a result, national and international laws have become stricter in respecting MRLs. Monitoring residue levels in foods requires reliable methods of analysis.

Nevertheless, pesticides are generally present at very low concentrations in the environment and in food. Their number is high, and they are of different physicochemical natures. Research and quantification of their residues are a permanent challenge and require the implementation of highly sensitive and reliable multiresidue analysis methods [56]. Several methods of analysis exist, and their choice depends on the nature of the pesticides studied and the targeted matrices.

9.1 Sample Preparation

The analysis of pesticide residues in fruits and vegetables consists of sample preparation and instrumental determination. Although analytical instruments have long been developed, detector background noise, detection limits, and final quantification are often influenced by matrix interference [57]. Thus sample preparation is the critical step for an efficient and accurate analysis of traces of pesticide residues [58]. The purpose of sample preparation is to isolate minute quantities of the analyte from a large quantity of complex matrices and to eliminate interference from the food matrix as much as possible.

The process of sample preparation typically includes sampling/homogenization, extraction, and purification. Traditional extraction methods, especially liquid extraction, have been widely used in the analysis of pesticide residues; however, these methods are laborious and consume a lot of time and solvents and are subject to analyte losses. Thus, new extraction and purification methods are introduced in the field of analyses of pesticide residues in foods [59]. These are supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), solid-phase extraction (SPE), solid-phase microextraction (SPME), liquid-phase microextraction (LPME), QuEChERS, etc.

The QuEChERS method (acronym: quick, easy, cheap, effective, rugged, and safe) has become the most widely used technique in pesticide residue extraction in vegetables and fruits. Table 4 illustrates some examples of the use of this method for residue analysis in fruits and vegetables. This method was developed in 2003 by Anastassiades et al. [60], and its simple implementation is done in two short stages of extraction and purification. Its main advantages are to significantly simplify the work of sample preparation in the laboratory and to reduce the use of solvents and glassware. Its cost is low, and it generates small amounts of waste unlike other conventional techniques. This method has a few steps:

– Solid/liquid extraction using acetonitrile (ACN) and manual stirring for 1 min. Acetonitrile can extract a wide range of pesticides and is miscible with water (the moisture content of most fruits is in the range of 80–95%) which gives good penetration into the aqueous fraction samples, also allowing for relatively easy separation in subsequent steps.

Table 4 Examples of application of the QuEChERS method to the analysis of pesticide residues in fruits and vegetables

Pesticides	Matrices	Extraction	Purification	Pesticide determination	Recovery	References
229 pesticides	Lettuce and orange	15 mL ACN	1.8 g $MgSO_4$, 300 mg PSA	HPLC-MS/MS	70–120	[62]
		6 g $MgSO_4$, 1.5 g NaCl		GC-MS		
43 pesticides	Apple, lemon, and lettuce	10 mL ACN 5%	150 mg $MgSO_4$, 50 mg PSA, 50 mg C18,	LPGC-MS/MS	90–110	[63]
		HOAc (v/v)	7.5 mg GCB			
		7.5 g ammonium formate				
27 pesticides	Grapes	10 mL MeCN, 4 g $MgSO_4$ + 1 g NaCl	$MgSO_4$:150 mg	LP-GC/MS	70–120%	[64]
			PSA:50 mg			
			C18: 50 mg			
104 pesticides	Olives	10 mL MeCN, 4 g $MgSO_4$ + 1 g NaCl;	$MgSO_4$:750 mg	LC-MS/MS	70–120%	[65]
			PSA:250 mg			
			C18:250 mg			
			GCB: 250 mg			
212 pesticides	04 plant matrices	10 mL MeCN, 4 g MgSO4 + 1 g NaCl	/	UHPLC –TOF/MS	70–120%	[66]
32 pesticides	Fruits and vegetables	10 mL MeCN, 4 g $MgSO_4$ + 1 g NaCl	MgSO4:150 mg	LC–MS/MS	88–102%	[67]
			PSA:50 mg	GC-MS		
			C18: 50 mg			
150 pesticides	Fruits and vegetables	15 mL MeCN + 0.15 mL HAc (1%), 6 g $MgSO_4$ + 1.5 g NaAc	$MgSO_4$:150 mg	LP-GC/TOFMS	70–120%	[68]
			PSA:50 mg			
			C18:50 mg			
			GCB: 7.5 mg			
14 organochlorine pesticides	Fruits	10 mL MeCN 1 g Na_3Citrate dihydrate + 0.5 g Na_2HCitrate sesquihydrate + 1.0 g NaCl + 4.0 g $MgSO_4$	$MgSO_4$:900 mg	GC–MS	70–120%	[69]
			PSA:150 mg			
11 pesticides	Banana	10 mL MeCN, 1 g Na3Citrate dihydrate + 0.5 g Na_2HCitrate sesquihydrate	$MgSO_4$:750 mg	GC-NPD	67–118%	[70]
			PSA:125 mg			

(*continued*)

Table 4 (continued)

Pesticides	Matrices	Extraction	Purification	Pesticide determination	Recovery	References
		+ 1 gNaCl + 4 g $MgSO_4$				
215 pesticides	Vegetables	10 mL MeCN, 4 g $MgSO_4$ + 1 g NaCl	$MgSO_4$:150 mg	LC-MS/MS	70–120%	[71]
			PSA:50 mg	GC-MS/MS		
			C18: 50 mg			

- Addition of salts to promote the separation of phases and the transfer of pesticides to the organic phase; the salts used in the original version of QuEChERS method are NaCl and anhydrous MgSO4. Other salts are used in the modified versions; it is mainly trisodium citrate dihydrate and disodium hydrogen citrate sesquihydrate in the buffered citrate version and sodium acetate in the buffered acetate version.
- Purification on dispersed solid phase. In the case of pesticides in plants, it is composed of a mixture of several phases: grafted silica support octadecyl (C18) and a mixture of primary and secondary amines (PSA).

It should be noted that the QuEChERS method was first developed for pesticide residue research in fruits and vegetables [60] where water content is high; however, several authors have used this method on another matrix with a low water content such as cereals [61].

10 Determination of Pesticides

Once the extraction process is complete, the next step is the analysis of separating, identifying, and assaying isolated substances. Depending on the nature of the pesticides studied, two analytical separation techniques are generally used for their identification and quantification: gas chromatography (GC) and liquid chromatography. These techniques can be coupled to specific or universal detectors.

Mass spectrometry (MS) was coupled with GC in commercial instruments during the 1980s, and they were initially used for qualitative confirmation purposes in pesticide analyzes. In the 1990s, the performance characteristics of the instruments improved to the point that the detection limits were low enough that GC-MS could be used to replace the GC selective detectors for quantitative and qualitative analysis and reduce the need of multiple injections in the GC. By the end of the 1990s, GC-MS had become commonplace in surveillance laboratories, particularly in developed countries [72].

The operating principle of the mass spectrometer is based on the action of an electromagnetic field on the molecules leaving the GC column; the mass spectrometer breaks each molecule into ionized fragments and separates them according to their specific mass to charge (m/z).

11 Monitoring Pesticide Residues in Fruits and Vegetables

Descriptive data on pesticide contamination of fruit and vegetables are derived mainly from official country monitoring programs or from published research.

The majority of the fruits and vegetables samples were positive for at least one pesticide (Table 5). In Poland, a total of 144 samples (of black currants, red currants, raspberries, cherries, strawberries, blackberries, cauliflowers, and broccoli) were analyzed for the determination of 60 pesticides. Residues of 15 fungicides and insecticides were detected in 32% of samples. The percentage of samples with residues above the maximum residue limits (MRL) was 15%. Samples with multi-residues represented 9%. In the report on pesticide residue monitoring elaborated in the UK, residues were found in 79% of sample of fruits and vegetables; the most found residues are fungicides: boscalid and azoxystrobin.

In the USA, no residues were detected in 48% of 3905 samples of fruit and vegetable and 87.3% of samples had violative residues. In Algeria and Kuwait, samples with pesticide residues represent more than 57%. Exceeding of LMRs was observed in 12.5% of samples from Algeria and 21% of samples from Kuwait. In Turkey, detectable residues were found in 62.2% of 1432 samples of fresh fruits and vegetables collected from 2010 to 2012 [73].

Table 6 shows the result of pesticide residue analysis for some crop. A higher number of pesticide residues were found in apple (from 5 to 15) and strawberry (from 6 to 19). In tomatoes, two to nine pesticide residues were found. The number and the nature of residues detected in the same crop vary considerably and depend on the pesticide regulations of each country and also on the method of analysis (the number of pesticides sought).

The percentage of fruit with pesticide residues is higher than that of vegetables in the majority of monitoring programs carried out by the different countries (Fig. 2). Brazilian monitoring programs for pesticide residues in fruits and vegetables showed that 59% of fruit samples contained residues and only 36% of vegetable samples contained those [79]. Similar found was reported in US and EU monitoring programs [77, 80].

12 Risk Assessment Method for Dietary Exposure

The consumer is exposed to pesticides through residues that are found in fruit and vegetable; these residues constitute the liabilities of crop production treatments by pesticides. Alimentation is the bulk of consumer exposure; non-food exposure represents only a small part of total exposure.

The risks for the consumer are very difficult to evaluate because they are – except accidents – very small doses ingested almost daily for a very long time and the disorders they could cause are sometimes minimal and not taken in count, so it is very difficult if not impossible to make the link of cause and effect; it is essentially for this reason and for others already mentioned about the exposure of operators that epidemiological surveys are very difficult to implement and are therefore extremely rare.

Table 5 Results of monitoring residues in fruits and vegetables found in published works

Country	Sample number	Sample identity	Number of analyzed pesticides	Samples with residues (%)	Samples with multi-residues (%)	Sample with residues above LMR (%)	Number of detected residues (most found)	References
Poland	144	Black currants, red currants, raspberries, cherries, strawberries, blackberries, cauliflowers, and broccoli	60	32	9	15	15 (carbendazim, acetamiprid)	[74]
UK	62	Apple, banana, carrot,, raisin, strawberry, sugar snap pea, sweet pepper, and tomato	More than 300	79	66	0	42 (boscalid, azoxystrobin)	[75]
Kuwait	150	Tomato, bell pepper, eggplant, cucumber, zucchini, cabbage, carrot, potato, strawberry, watermelon, apple, and grapes	34	58	42	21	16 (imidacloprid, deltamethrin)	[76]
USA	3905	Fruits and vegetables	≈400	52.11	/	8.73	207 (imidacloprid, chlorpyrifos)	[77]
Algeria	160	Apples, pears, plums, peaches, nectarines, grapes, figs, apricots, strawberries, tomatoes, lettuce, potatoes, zucchini	10	57.5	32.5	12.5	08 (lambda-cyhalothrin, metalaxyl, and chlorpyrifos)	[78]

Table 6 Result of pesticide residues in some kind of fruits and vegetables found in published works

Country [reference]	Crop	Pesticides analyzed	Sample with residues	Sample with multi-residues	Residues detected	Sample with residue above LMR
UK [75]	Apples (11)	360	11	11	Boscalid, captan, chlorantraniliprole, cyprodinil, dodine, dithiocarbamates, dithianon, flonicamid, fludioxonil, indoxacarb, methoxyfenozide, myclobutanil, pyraclostrobin, thiacloprid, trifloxystrobin	0
	Strawberries (06)	358	6	6	Boscalid, bupirimate, cyprodinil, dimethomorph, ethirimol, fludioxonil, fenamidone, fenhexamid, fenpyrazamine, fluopyram, mepanipyrim, myclobutanil, penconazole, pyraclostrobin, pyrimethanil, quinoxyfen, tebufenpyrad, thiacloprid, trifloxystrobin	0
	Banana (14)	368	11	11	Azoxystrobin, imazalil, myclobutanil, thiabendazole	0
Kuwait [76]	Apple (10)	34	9	/	Deltamethrin, malathion, diazinon, imidacloprid, aldrin	8
	Strawberries (10)	34	7	/	Deltamethrin, fenpropathrin, malathion, diazinon,	4

(*continued*)

Table 6 (continued)

Country [reference]	Crop	Pesticides analyzed	Sample with residues	Sample with multi-residues	Residues detected	Sample with residue above LMR
					imidacloprid, thiophanate-methyl	
	Zucchini (12)	34	02	0	Imidacloprid	0
	Potato (10)	34	0	0	None	0
	Tomatoes (16)	34	14	/	Oxamyl, cypermethrin, profenofos, monocrotophos, pirimiphos-methyl, chlorpyrifos-methyl, imidacloprid, thiophanate-methyl, metalaxyl	3
Algeria [78]	Apple (32)	10	19	16	Benalaxyl, chlorpyrifos, cypermethrin, deltamethrin, lambda-cyhalothrin, metalaxyl	04
	Peach (20)	10	15	07	Benalaxyl, chlorpyrifos, deltamethrin, lambda-cyhalothrin, metalaxyl	07
	Tomato (10)	10	02	02	Benalaxyl, metalaxyl	00
	Potato (08)	10	07	01	Chlorpyrifos, lambda-cyhalothrin	02

12.1 Evaluation of Long-Term Exposure to Residues (Chronic Risk)

For an active substance, it is important to verify that the quantities of residues that an individual is likely to find daily in his diet do not exceed the toxicological reference standards of the acceptable daily intake (ADI) and the dose of acute reference (ARfD).

The theoretical long-term exposure of a consumer to a pesticide is evaluated by calculating theoretical maximum daily intake (TMDI) which makes it possible to verify that the quantity potentially ingested each day by a consumer is lower than the ADI.

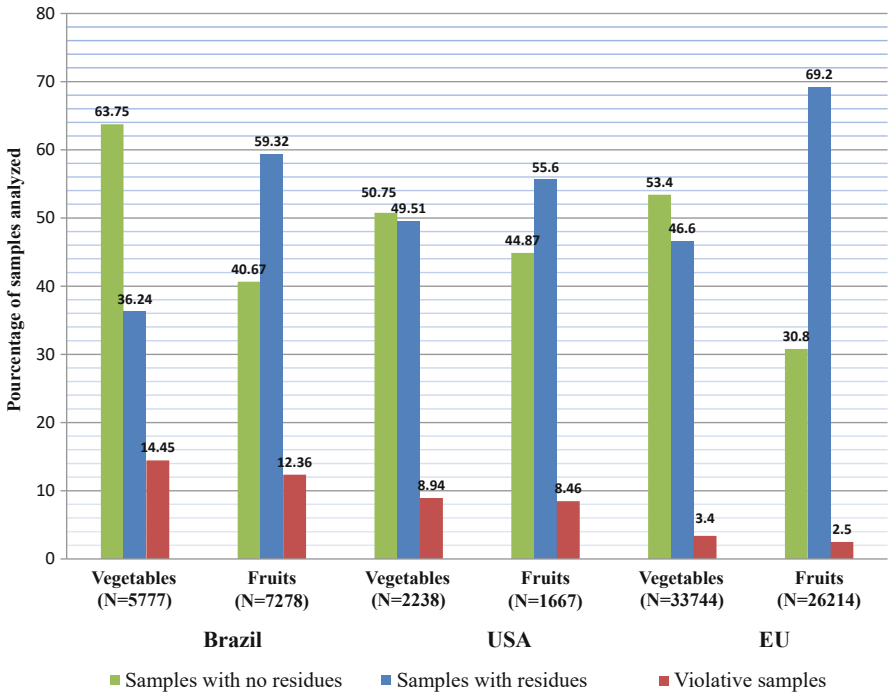


Fig. 2 Graphic presentation of pesticide residue monitoring results in Brazil, the USA, and EU

The TMDI is calculated as the sum of the daily average individual consumptions multiplied by the MRLs; it can be summarized by the following formula:

$$\text{TMDI} = \sum \text{MRLi} \times \text{Fi}$$

MRLi is the maximum residue limit for a given food commodity, and Fi is the average consumption of that food commodity per person [81].

This calculation is clearly maximalist because it is based on the assumption that all foods have residue levels equivalent to the MRLs. In reality, it must be considered that only a small percentage of food displays such levels. In 2008, according to the European Food Safety Authority (EFSA), 3.5% of the 70,000 samples analyzed for about 200 types of food exceeded the MRLs, compared to 4.2% in 2007 [82].

This calculation also recognizes that the entire crop is treated and that there is no reduction factors related to household or industrial processes during food preparation. If these studies lead to results far removed from reality, they still provide clues to identify priorities for food security and public health [55].

Recently, surveys that take into account actual data from pesticide concentration in commodities are being put in place and provide an image closer to reality. In this case, the long-term exposure is evaluated by calculating the estimated long-term dietary intake (ELTDI).

The ELTDI is determined by considering that all the food consumed daily is contaminated at the average level observed after the results of the quantification of pesticide residues; it is calculated according to this formula:

$$\text{ELTDI} = \Sigma\ (\text{RLi} \times \text{Fi})$$

RLi = the average residue concentration in a given commodity (mg kg^{-1})
Fi = the average daily consumption of this commodity per person (kg day^{-1})

12.2 Evaluation of Short-Term Exposure to Residues (Acute Risk)

The assessment of acute (short-term) exposure is based both on extreme food consumption and the highest residue level detected. In many studies, acute exposure was calculated only for pesticides exceeding the MRLs [83].

International estimated short-term intake (IESTI) values were calculated according to the WHO guidelines [84]. Depending on the food commodity, two groups were identified:

Group 1: It is the case where the residue in a composite sample reflects the residue level in a meal-sized portion of the commodity. This case shall apply to the commodity with a unit weight <25 g (e.g., strawberry, cherry).

$$\text{IESTI} = (\text{LP} \times \text{HR})\ \text{bw}^{-1}$$

Group 2: Meal-sized portion might have a higher residue level than the composite sample, when unit weight of a commodity is ≥25 g.

Case 2a: Unit weight U of a raw commodity is less than a large portion weight (e.g., apple, pear, peach).

$$\text{IESTI} = (\text{U} \times \text{HR} \times \text{v} + (\text{LP} - \text{U}) \times \text{HR})\ \text{bw}^{-1}$$

Case 2b: U is higher than a large portion weight (e.g., watermelon)

$$\text{IESTI} = (\text{U} \times \text{HR} \times \text{v})\ \text{bw}^{-1}$$

where LP represents highest large portion reported at the 97.5th percentile of eaters (kg of food day^{-1}), HR highest residue (mg kg^{-1}), bw body weight (adult = 60 kg, children = 15 kg), U unit weight of the edible portion (in kg), and v variability factor which is applied to the composite residue ($v = 7$ for a unit weight between 25 and 250 g, $v = 5$ for unit weight >250 g)

An example of assessment acute (short-term) exposure is given on Table 7.

Potential consumer short-term risk was identified for two pesticide/commodity combinations for children (chlorpyrifos/apple: 558.5% of ARfD; chlorpyrifos/

Table 7 Result of short-term exposure to chlorpyrifos residues

Pesticide residue	Crop	ARfD (mg kg^{-1} bw^{-1})	HR (mg kg^{-1})	IESTI children (mg kg^{-1} bw^{-1})	IESTI adult (mg kg^{-1} bw^{-1})	IESTI children/ ARfD (%)	IESTI adult/ ARfD (%)
Chlorpyrifos	Peach	0.1	0.747	0.0472	0.0138	47.2	13.8
	Apple		9.830	0.5585	0.2378	558.5	237.8
	Grape		1.978	0.1477	0.0394	147.75	39.4
	Potato		0.215	0.0194	0.0065	19.4	6.5
	Nectarine		0.785	0.0501	0.0147	50.1	14.7

grapes, 147.75% of ARfD) and for one pesticide/commodity combination (chlorpyrifos/apple: 237.8%) for the adult population [78].

The exceedances of the ARfD are due mainly to the exceeding of the MRLs; these identified pesticide-commodity combinations should lead to an intensification of the controls of pesticides use and to the identification of the origin nonconformities and the implementation of corrective measures.

13 Conclusions

The use of pesticides in fruit and vegetable culture has become a common practice, and it is indispensable for intensive production. Unfortunately, this practice is not without risks; in fact, these pesticides generate residues that accumulate in fruits and vegetables, and they can constitute a danger to the consumer health. The pesticides used in fruit and legumes are numerous and are from different chemical families with also different modes of action. Among them, there are neurotoxin insecticides, endocrine disruptors, and others suspected to cause cancer. Residue analysis was performed using multi-residue research ensuring a rapid determination and sufficient sensibility. Many national and regional monitoring programs found that pesticide residues and multi-residues were detected in the majority of vegetables and fruits. Therefore, it is indispensable to know the degree of contamination of fruits and vegetables through a multi-residue research and to assess the risk of chronic and acute exposure for the general population and for the most vulnerable populations such as children and pregnant women.

References

1. Fillatre Y (2011) Produits phytosanitaires: Développement d'une méthode d'analyse multi-résidus dans les huiles essentielles par couplage de la chromatographie liquide avec la spectrométrie de masse en mode tandem. Dissertation, University of Angers
2. Schiavon M, Jacquin F (1972) Contribution to the technical study of the migration of some organic compounds in soils. Bull de l'Ecole Natl Supérieure d'Agronomie et des Ind Aliment:221–225

3. Merhi M (2008) Etude de l'impact de l'exposition à des mélanges de pesticides à faibles doses: caractérisation des effets sur des lignées cellulaires humaines et sur le système hématopoïétique murin. Dissertation, University of Toulouse
4. [FAO] Food and Agriculture Organization (2003) Code international de conduite pour la distribution et l'utilisation des pesticides (version révisée). FAO, Rome
5. Boland J, Koomen I, van Lidth de Jeude J, Oudejans J (2004) Agrodok 29: Les pesticides: composition, utilisation et risques. Fondation Agromisa, Wageningen
6. Fournier J (1988) Chimie des Pesticides. Cultures et techniques, Nantes
7. Stevens JT, Sumner DD (1991) Herbicides. In: Hayes WJ, Laws ER (eds) Handbook of pesticide toxicology. Academic Press, San Diego
8. Hess FD (2000) Light –dependent herbicides: an overview. Weed Sci 48:160–170
9. Gauvrit C (2005) Modes d'action des herbicides. In: Regnault-Roger C (ed) Enjeux phytosanitaire pour l'agriculture et l'environnement. Lavoisier, Paris
10. Oerke EC (1996) The impact of disease and disease control on crop production. In: Lyr H, Russell PE, Sisler H (eds) Modern fungicides and antifungal compounds I. Intercept, Andover
11. Maumené C (2008) Un fongicide à la loupe: le mode d'action des triazoles. Perspect Agric 345:60–61
12. Testud F, Marcotullio E (2001) Les dithiocarbamates. In: Testud F, Garnier R, Delemotte B (eds) Toxicologie humaine des produits phytosanitaires. Eska – Lacassagne, Paris
13. Testud F (2001) Les Carbamates. In: Testud F, Garnier R, Delemotte B (eds) Toxicologie humaine des produits phytosanitaires. Eska – Lacassagne, Paris
14. Ray DE (1991) Pesticides derived from plants and other organisms. In: Hayes WJ, Laws ER (eds) Handbook of pesticide toxicology. Academic, San Diego
15. Elbert A, Haas M, Springer B, Thielert W, Nauen R (2008) Applied aspects of neonicotinoid uses in crop protection. Pest Manag Sci 64:1099–1105
16. Tomizawa M, Casida JE (2003) Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu Rev Entomol 48:339–364
17. Laurent FM, Rathahao E (2003) Distribution of [(14)C] imidacloprid in sunflowers (*Helianthus annuus* L.) following seed treatment. J Agric Food Chem 51:8005–8010
18. Maxim L, Van Der Sluijs JP (2007) Uncertainty: cause or effect of stakeholders' debates? Analysis of a case study: the risk for honeybees of the insecticide Gaucho. Sci Total Environ 376:1–17
19. Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43
20. Culliney TW (2014) Crop losses to arthropods. In: Pimentel D, Peshin R (eds) Integrated pest management pesticide problems, vol 3. Springer, New York
21. Couteaux A, Le Jeune V (2011) Index phytosanitaire ACTA 2011, 47th edn. ACTA, Paris
22. [DPVCT] Direction de la Protection des Végétaux et Contrôles Techniques (2015) Index des produits phytosanitaires à usage agricole. http://www.inpv.edu.dz/institut/wp-content/uploads/2016/03/INDEX_PRODUITS _PHYTO_2015.pdf. Accessed 20 Jan 2016
23. Jakubowski M, Trzcinka-Ochocka M (2005) Biological monitoring of exposure: trends and key developments. J Occup Health 47:22–48
24. Baldi I, Lebailly P, Jean S, Rougetet L, Dulaurent S, Marquet P (2006) Pesticide contamination of workers in vineyards in France. J Expo Sci Environ Epidemiol 16:115–124
25. Van Leeuwen JA, Waltner-Toews D, Abernathy T, Smit B, Shoukri M (1999) Associations between stomach cancer incidence and drinking water contamination with atrazine and nitrate in Ontario (Canada) agroecosystems, 1987–1991. Int J Epidemiol 28:836–840
26. Blair A, Zheng T, Linos A, Stewart PA, Zhang YW, Cantor KP (2001) Occupation and leukemia: a population-based case-control study in Iowa and Minnesota. Am J Ind Med 40:3–14
27. Buzio L, Tondel M, De Palma G, Buzio C, Franchini I, Mutti A, Axelson O (2002) Occupational risk factors for renal cell cancer: an Italian case-control study. La Medecina del Lavaro 93:303–309
28. Hu J, Mao Y, White K (2002) Renal cell carcinoma and occupational exposure to chemicals in Canada. Occup Med 52:157–164

29. Mills PK, Yang R (2003) Prostate cancer risk in California farm workers. J Occup Environ Med 45:249–258
30. Alavanja MC, Hoppin JA, Kamel F (2004) Health effects of chronic pesticide exposure: cancer and neurotoxicity. Annu Rev Public Health 25:155–197
31. McCauley LA, Anger WK, Keifer M, Langley R, Robson MG, Rohlman D (2006) Studying health outcomes in farmworker populations exposed to pesticides. Environ Health Perspect 114:953–960
32. Van Maele-Fabry G, Libotte V, Willems J, Lison D (2006) Review and metaanalysis of risk estimates for prostate cancer in pesticide manufacturing workers. Cancer Causes Control 17:353–373
33. Provost D, Cantagrel A, Lebailly P, Jaffre A, Loyant V, Loiseau H, Vital A, Brochard P, Baldi I (2007) Brain tumours and exposure to pesticides: a case control study in southwestern France. Occup Environ Med 64:509–514
34. Van Maele-Fabry G, Duhayon S, Mertens C, Lison D (2008) Risk of leukaemia among pesticide manufacturing workers: a review and meta-analysis of cohort studies. Environ Res 106:121–137
35. McCormack AL, Thiruchelvam M, Manning-Bog AB, Thiffault C, Langston JW, Cory-Slechta DA, Di Monte DA (2002) Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. Neurobiol Dis 10:119–127
36. Baldi I, Lebailly P, Mohammed-Brahim B, Letenneur L, Dartigues JF, Brochard P (2003) Neurodegenerative diseases and exposure to pesticides in the elderly. Am J Epidemiol 157:409–414
37. Bosma H, vanBoxtel MP, Ponds RW, Houx PJ, Jolles J (2000) Pesticide exposure and risk of mild cognitive dysfunction. Lancet 356:912–913
38. [WHO] World Health Organization (1992) Our planet, our health: report of the WHO Commission on Health and Environment. WHO, Geneva
39. Baldi I, Filleul L, Mohammed-Brahim B, Fabrigoule C, Dartigues JF, Schwall S, Drevet JP, Salamon R, Brochard P (2001) Neuropsychologic effects of long term exposure to pesticides: results from the French Phytoner study. Environ Health Perspect 109:839–844
40. Cole DC, Carpio F, Julian J, Leon N (1998) Assessment of peripheral nerve function in an Ecuadorian rural population exposed to pesticides. J Toxicol Environ Health 55:77–91
41. Cohn BA (2011) Developmental and environmental origins of breast cancer: DDT as a case study. Reprod Toxicol 31:302–311
42. Tabb MM, Blumberg B (2006) New modes of action for endocrine-disrupting chemicals. Mol Endocrinol 20:475–482
43. Sugiyama S, Shimada N, Miyoshi H, Yamauchi K (2005) Detection of thyroid system disrupting chemicals using in vitro and in vivo screening assays in *Xenopus laevis*. Toxicol Sci 88:367–374
44. Roeleveld N, Bretveld R (2008) The impact of pesticides on male fertility. Curr Opin Obstet Gynecol 20:229–233
45. Saunders M, Fox D, Salisbury C, Strokes V, Palmer A, Preece A (2004) Placental transfer and foetal uptake of pesticides. Toxicol Appl Pharmacol 197(3):341
46. Jurewicz J, Hanke W, Johansson C, Lundqvist C, Ceccatelli S, van den Hazel P, Saunders M, Zetterstrom R (2006) Adverse health effects of children's exposure to pesticides: what do we really know and what can be done about it. Acta Paediatr Suppl 95:71–80
47. Lu C, Barr DB, Pearson MA, Waller LA (2008) Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. Environ Health Perspect 116:537–542
48. Carbone P, Giordano F, Nori F, Mantovani A, Taruscio D, Lauria L, Figa-Talamanca I (2006) Cryptorchidism and hypospadias in the Sicilian district of Ragusa and the use of pesticides. Reprod Toxicol 22:8–12
49. Nasreddine L, Parent-Massin D (2002) Food contamination by metals and pesticides in the European Union. Should we worry? Toxicol Lett 127:29–41

50. Fussell RJ, Jackson AK, Reynolds SL, Wilson MF (2002) Assessment of the stability of pesticides during cryogenic sample processing: 1. Apples J Agric Food Chem 50:441–448
51. Baril A, Whiteside M, Boutin C (2005) Analysis of a database of pesticide residues on plants for wildlife risk assessment. Environ Toxicol Chem 24:360–371
52. Commission du Codex Alimentarius (1994) Commission du Codex Alimentarius: Résidus de pesticides dans les denrées alimentaires. FAO/OMS, Rome
53. Couteaux A, Le Jeune V (2004) Index phytosanitaire ACTA 2004, 40e edn. ACTA, Paris
54. Racke KD (2007) Pesticide residues in food and international trade: regulation and safety considerations. In: Ohkawa H, Miyagawa H, Lee PW (eds) Pesticide chemistry: crop protection, Public Health, Environmental Safety. Wiley-vch, Verlag, Weinheim
55. Nougadère A (2015) Surveillance des expositions alimentaires aux résidus de pesticides: développement d'une méthode globale d'appréciation quantitative du risque pour optimiser l'évaluation et la gestion du risque sanitaire. Dissertation, University of Toulouse
56. LeDoux M (2011) Analytical methods applied to the determination of pesticide residues in foods of animal origin: a review of the past two decades. J Chromatogr A 1218:1021–1036
57. Zollner P, Leitner A, Berner D, Kleinova M, Jodlbauer J, Mayer BX, Lindner W (2003) Improving LC–MS/MS analyses in complex food matrices, part I: sample preparation and chromatography. LC GC Eur 16:163–168
58. Ferrer C, Jose Gómez M, García-Reyes JF, Ferrer I, Thurman EM, Fernández-Alba AR (2005) Determination of pesticide residues in olives and olive oil by matrix solid-phase dispersion followed by gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry. J Chromatogr A 1069:183–194
59. Zhang L, Liu S, Cui X, Pan C, Zhang A, Chen F (2012) A review of sample preparation methods for the pesticide residue analysis in foods. Cent Eur J Chem 10:900–925
60. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid- phase extraction" for the determination of pesticide residues in produce. J AOAC Int 86:412–431
61. Lehotay SJ (2011) QuEChERS sample preparation approach for mass spectrometric analysis of pesticide residues in foods. In: Zweigenbaum J (ed) Mass spectrometry in food safety: methods and protocols. Springer, Heidelberg
62. Lehotay SJ, De Kok A, Hiemstra M, Van Bodegraven P (2005) Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. J AOAC Int 88:595–614
63. Gonzalez-Curbelo MA, Lehotay SJ, Hernandez-Borges J, Rodriguez-Delgado MA (2014) Use of ammonium formate in QuEChERS for high throughput analysis of pesticides in food by fast, low-pressure gas chromatography and liquid chromatography tandem mass spectrometry. J Chromatogr A 1358:75–84
64. Cunha SC, Fernandes JO, Alves A, Oliveira MBPP (2009) Fast low-pressure gas chromatography–mass spectrometry method for the determination of multiple pesticides in grapes, musts and wines. J Chromatogr A 1216:119–126
65. Gilbert-López B, García-Reyes JF, Lozano A, Fernández-Alba AR, Molina-Díaz A (2010) Large-scale pesticide testing in olives by liquid chromatography-electrospray tandem mass spectrometry using two sample preparation methods based on matrix solid-phase dispersion and QuEChERS. J Chromatogr A 1217:6022–6035
66. Lacina O, Urbanova J, Poustka J, Hajslova J (2010) Identification/quantification of multiple pesticide residues in food plants by ultra-high-performance liquid chromatography-time-of-flight mass spectrometr. J Chromatogr A 1217:648–659
67. Lehotay SJ, Son KA, Kwon H, Koesukwiwat U, Fu W, Mastovska K, Hoh E, Leepipatpiboon N (2010) Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. J Chromatogr A 1217:2548–2560
68. Koesukwiwat U, Lehotay SJ, Miao S, Leepipatpiboon N (2010) High throughput analysis of 150 pesticides in fruits and vegetables using QuEChERS and low-pressure gas chromatography-time-of-flight mass spectrometry. J Chromatogr A 121:6692–6703

69. Cieślik E, Sadowska-Rociek A, Ruiz JMM, Surma-Zadora M (2011) Evaluation of QuEChERS method for the determination of organochlorine pesticide residues in selected groups of fruits. Food Chem 125:773–778
70. Hernández-Borges J, Cabrera JC, Rodríguez-Delgado M, Hernandez-Suarez EM, Sauco VG (2009) Analysis of pesticide residues in bananas harvested in the Canary Islands (Spain). Food Chem 113:313–319
71. Alla SAG, Loutfy NM, Shendy AH, Ahmed MT (2015) Hazard index, a tool for a long term risk assessment of pesticide residues in some commodities, a pilot study. Regul Toxicol Pharmacol 73:985–991
72. Latif YS, Sherazi TH, Bhanger MI (2011) Assessment of pesticide residues in commonly used vegetables in Hyderabad, Pakistan. Ecotoxicol Environ Saf 74:2299–2303
73. Bakırcı GT, Acay DBY, Bakırcı F, Otles S (2014) Pesticide residues in fruits and vegetables from the Aegean region, Turkey. Food Chem 160:379–392
74. Stachniuk A, Szmagara A, Czeczko R, Fornal E (2017) LC-MS/MS determination of pesticide residues in fruits and vegetables. J Environ Sci Health B 52:446–457
75. PRiF [Pesticide Residues in Food] (2017) School fruit and vegetable scheme report on pesticide residues monitoring: summer term 2017 https://www.gov.uk/government/collections/pesticide-residues-in-food-results-of-monitoring-programm. Accessed 20 Dec 2017
76. Jallow MFA, Awadh DG, Albaho MS, Devi VY, Ahmad N (2017) Monitoring of pesticide residues in commonly used fruits and vegetables in Kuwait. Int J Environ Res Public Health 2017(14):833. https://doi.org/10.3390/ijerph14080833
77. U.S. Food and Drug Administration (2017) Pesticide residue monitoring program fiscal year 2015. http://www.fda.gov/food/foodborneillnesscontaminants/pesticides/default.htm. Accessed 12 Nov 2017
78. Mebdoua S, Lazali M, Ounane SM, Tellah S, Nabi F, Ounane G (2017) Evaluation of pesticide residues in fruits and vegetables from Algeria. Food Addit Contam Part B Surveill 10:91–98
79. Jardim ANO, Caldas ED (2012) Brazilian monitoring programs for pesticide residues in food-results from 2001 to 2010. Food Control 25:607–616
80. [EFSA] European Food Safety Authority] (2017) The 2015 European Union report on pesticide residues in food. EFSA J 15:4791. https://doi.org/10.2903/j.efsa.2017.4791, 134 pp
81. [WHO] World Health Organization (1997) Guidelines for predicting dietary intake of pesticide residues (revised). http://www.who.int/foodsafety/publications/chem/ en/pesticide _en.pdf. Accessed 25 Apr 2016
82. [EFSA] European Food Safety Authority (2010) Scientific report of EFSA 2008 annual report on pesticide residues according to Article 32 of Regulation (EC) No 396/20051. European Food Safety Authority; EFSA, Parme
83. [FAO] Food and Agriculture Organization (2016) Evaluation of pesticide residues: for estimation of maximum residue limits and calculation of dietary intake, training manual. FAO, Rome
84. [WHO] World Health Organization (2014) International estimated short-term intake (IESTI) [Internet]. http://www.who.int/foodsafety/chem/guidance_for_IESTI_calculation.pdf. Accessed 3 June 2017

Banana and Plantains: Improvement, Nutrition, and Health

59

Siddhesh B. Ghag and Thumballi R. Ganapathi

Contents

Abstract

Banana is one of the oldest cultivated plant known for its dietary and medicinal properties. Banana is grown in the tropical and subtropical regions of the world and constitutes the staple food of the people. They are classified as dessert or sweet bananas and cooking bananas or plantains depending on whether they can be eaten raw or not. Banana plant parts such as the roots, pseudostem, fruits, and inflorescence is used in some or the other way and therefore it is rightly called as the "Kalpatharu" in India. Banana and plantains contain important bioactive compounds such as phenolics, flavonoids, carotenoids, biogenic amines, sterols,

S. B. Ghag
UM-DAE Centre for Excellence in Basic Sciences, Mumbai, Maharashtra, India
e-mail: siddhesh.ghag@cbs.ac.in

T. R. Ganapathi (✉)
Plant Cell Culture Technology Section, Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, India
e-mail: trgana@barc.gov.in

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_73

and antimicrobial compounds which make bananas a perfect functional food for health improvement. Presently, research is focused on exploring and identifying compounds, refining the techniques of isolation and purification, and using it in modern medicines. Moreover, bananas are also being used as a platform to produce and accumulate important nutrients like vitamins and minerals by biofortification strategies.

Keywords
Bananas · Bioactive compounds · Carotenoids · Dopamine · Antimicrobials · Biofortification

1 Introduction

Banana and plantains belong to the order Zingiberales and genus *Musa* [1]. These are large, perennial, monocotyledonous herbs that arise from the underground rhizomes. The fruits are usually elongate, cylindrical, and curved at the ends enclosed in thin or thick skin with color ranging from yellow to green. The fruits of dessert banana are sweet whereas those of plantains are starchy. Banana and plantains are listed as the fourth most important crop grown all across the globe along the tropical and subtropical regions [2]. The annual world production of banana was about 113.28 million tons in the year 2016 [3]. India is the largest producer with a production of about 29.124 million tones that accounts approximately one-third of total world production [3]. Banana and plantains are the staple food of the developing regions of Africa and South East Asia. This plant has numerable uses other than being a source of food. Different parts of the plant are used in medicines, preparing beverages, flavorings, clothing, and in various religious and ceremonial practices [2, 4]. Several varieties are grown across the world but only a few of these are categorized as superior ones for international markets. The ones belonging to this category are the Cavendish cultivars namely, Grand Naine, Dwarf and Giant Cavendish, Robusta, etc. Banana is the first solid food recommended for infants and so it is the most practical crop for biofortification programs to eliminate nutrient deficiency that is prevalent in most developing regions [5]. Inherently, hundred grams of raw banana contains 22.84 g carbohydrates, 2.6 g fiber, 64 IU vitamin A, 8.7 mg vitamin C, and 358 mg potassium [6]. Moreover, banana plant parts such as fruit, peel, flower, and stem have been used orally or topically as remedies in folk medicine in Africa, India, Asia, and America for the treatment of ulcers, hyperglycemia, and stomach disorders [7]. Presence of some specific bioactive compounds such as apigenin glycosides, myricetin glycoside, myricetin-3-*O*-rutinoside, naringenin glycosides, kaempferol-3-*O*-rutinoside, dopamine, *N*-acetyl serotonin, and rutin has been reported in different species of *Musa* [8]. Presently, the trend is to identify and characterize unique bioactive compounds demonstrating therapeutic potential. Moreover, deficiency of important nutrients in the diet results in chronic ailments which increase the mortality rate. Supplementation of these nutrients through natural foods is the most viable approach to alleviate nutrient deficiencies. Great progress

has been made in the recent past to identify wild cultivars or biofortify domesticated varieties through biotechnology and generate nutritionally improved food crops [9, 10]. Few banana cultivars which have high market value are cultivated worldwide and grown as monocultures in the major banana growing regions. However, each region has its own set of varieties which are mostly cultivated for domestic consumption. These exotic varieties are source of a few vital nutrients. Such varieties can be identified as supplement food to curb nutrient deficiency. Moreover, the genes responsible for these nutrients in these varieties can be identified and engineered into the Cavendish varieties. This chapter will comprehensively describe the natural bioactive compounds present in bananas and its biofortification by genetic modification imparting health benefits.

2 Bioactive Compounds in Banana and their Health Benefits

2.1 Phenolics

Phenolic compounds such as flavonoids (including flavones, flavonols, and flavanones), anthocyanins, and condensed tannins are present in banana tissues [11–13]. Basically these are involved in defense mechanisms and are known to have health promoting effects. Phenolic compounds act as antioxidants and provide protection against microbial infections thereby contributing towards mitigation of chronic and infectious diseases. The antioxidant activity is attributed to the ability of phenolic compounds to scavenge free radicals or chelate metal ions [14–16]. Thus, addition of natural phenolic compounds to dietary supplements, food components, and drugs has gained importance. Particular phenolic compounds and their derivatives are present at different concentrations in different banana tissues at different points of their growth cycle [17–19]. Anthocyanin pigments such as petunidin, malvidin, pelargonidin, delphinidin, cyanidin, and peonidin have been observed in banana bracts and fruits [20–22]. Condensed tannins are more prominently present in the pulps of unripe fruits of diverse dessert and plantain banana cultivars. Flavonoids, such as apigenin, naringenin, myricetin, kaempferol, and quercetin were found in the sap of dessert banana species [8]. The extract of banana peel has around 29 mg/g as gallic acid equivalents of total phenolic, gallocatechin around 158 mg/100 g dry weight, prodelphinidins of around 3952 mg/kg, and a few hundred mg of flavonol glycosides per kg [23, 24]. There is considerable level of total free phenolics in the pulp ranging from 11.8 to 90.4 mg of gallic acid equivalents per 100 g of fresh weight [25]. There are significant levels of tannins in unripe and ripe banana fruits; however, the levels are lower in the latter than the former. Banana pulp is a rich source of catecholamines such as dopamine which is a strong water soluble antioxidant. Dopamine levels ranged from 80 to 560 mg per 100 g in peel and 2.5 to 10 mg in pulp [26]. Soluble condensed tannins such as epicatechin, epigallocatechin, and gallocatechin-catechin dimer were reported in banana pulp [27, 28]. Banana inflorescence is also a rich source of phenolic compounds such as anthocyanidins, tannins, and other glycosides. The total phenolic content was around 1690 mg

equivalent of gallic acid per100 g of the dry sample [18]. The banana flower extract have a strong antioxidant activity, iron chelation, and antihemolytic activity [29].

2.2 Carotenoids

Carotenoids are noteworthy for their wide distribution and structural diversity. Carotenoids are often described as provitamin A and are 40-carbon terpenoids having isoprene as their basic structural unit. These includes α and β carotenes, lycopenes, lutein, and xanthin; among which α and β carotenes predominate and are easily converted to vitamin A [30, 31]. However, out of the 600 carotenoids identified in nature 50 possess provitamin activity and they exist as red, orange, and yellow pigments in plants and animal tissues, fungi, and algae [31, 32]. Dietary supplements of provitamin A are recommended in the treatment of cancer and eye diseases. Vitamin deficiency is a serious issue among children and women especially in the developing regions of the world. Lack of vitamin A in the diet leads to increased mortality associated with vision, anemia, and normal growth and development. Supplementing vitamin A through natural food sources is still the sustainable approach to alleviate vitamin A deficiency. Banana being the staple food in the developing countries of Asia and Africa, the content of provitamin A was determined in several known cultivars of banana. However, the famous Cavendish cultivar has very low levels of provitamin A content of about 21–70 μg of β-carotene per 100 g of edible portion as compared to other nonpopular varieties such as Fe'i bananas (Asupina) and karat [5, 33–37]. Bananas found in the Micronesia and eastern Africa have higher levels of carotenoids which can be included in the diet as vitamin A supplement [38, 39]. Bananas found in some regions of Australia with yellow or yellow-orange flesh have shown to contain high levels of carotenoids such as Asupina, Kirkirnan, Pisang Raja, Horn Plantain, Pacific Plantain, Kluai Khai Bonng, Wain, Red Dacca, Lakatan, and Sucrier [36]. Edible banana genotypes from Papua New Guinea have the highest levels of β-carotene with values as high as 2594 μg/100 g of the pulp [40]. Indian cultivars such as Red banana and Karpooravalli showed maximum accumulation of carotenoid content in the pulp and peels followed by Rasthali and Hill banana [41]. Another Indian cultivar, Nendran which has orange-colored flesh, has high β-carotene content (1362 μg/100 g) in the edible pulp [42]. There are two isoforms of phytoene synthase gene observed in banana fruits; however, only one isoform is expressed in the edible pulp. Interestingly, a recent report proved that both the isoforms were expressed in the Nendran pulp and thus the increased β-carotene content [42]. The carotenoids content in banana fruit increases during ripening. The mean total provitamin A carotenoids ranged from 560 to 4680 mg/100 g fresh weight when estimated in unripe fruit and was from 1680 to 10,630 mg/100 g fresh weight in ripe fruit [43]. The change of banana peel from green to yellow during ripening is due to change in the contents of chlorophylls and carotenoids. Therefore carotenoids content of banana peel was estimated and was found to be 3–4 μg per gram as lutein equivalent [44]. Yellow banana (*Musa paradisiaca sapientum*) contains around

6.2 mg/g of carotenoid in the peels and was shown to increase the serum retinol levels in mice fed with purified carotenoids from the peels [45]. Xanthophyll compounds such as neoxanthin, violaxanthin, zeaxanthin, and lutein were also detected in banana tissues [46]. Intake of dietary carotenoids is associated with protection against chronic diseases such as certain cancers, cardiovascular diseases, diabetes, inflammatory diseases, arteriosclerosis, and age-related degeneration [38, 47].

2.3 Fatty Acids and Phytosterols

The major lipophilic compounds present in banana include the fatty acids, sterols, and steryl esters. Bananas are very low in saturated fat content and have almost no cholesterol. However, the total omega-6 fatty acids content is doubled than the omega-3 fatty acids content. A total of 0.2–0.4% of fatty acids is present in banana which consists of a mixture of palmitic, oleic, linoleic, and linolenic acids together with lower amount of phytosterol [48–50]. Oliviera et al. [51] identified compounds such as campesterol, stigmasterol, sitosterol and fatty acids, such as palmitic, stearic, linoleic, linolenic, 22-hydroxydocosanoic, 24-hydroxytetracosanoic, and 26-hydroxyhexacosanoic acids in the lipophilic extract (in dichloromethane) of Dwarf Cavendish. Fatty acids are abundantly present in the banana pulp as compared to the peels; however, the phytosterol content is high in peels than in the pulp tissues [52]. β-sitosterol followed by campesterol and stigmasterol are the major sterols found in banana pulp, whereas cycloeucalenone and 31-norcyclolaudenone are abundantly present in the banana peel tissues [52, 53]. Villaverde et al. [54] analyzed the lipophilic extractives of the unripe peel of ten banana cultivars namely "Giant Cavendish," "Chinese Cavendish," "Grand Nain," "Gruesa," "Williams," "Ricasa," "Eilon," "Zelig," "Dwarf Red," and "Silver" and found that the content ranged from 2% to 3%. Cycloeucalenone was the chief sterol identified in peels of "Williams" and "Dwarf Red" varieties. Vilela et al. [49] determined the lipophilic extract from the ripe pulp of banana fruit of the same ten cultivars by gas chromatography-mass spectrometry and showed that fatty acids constitute 68.6–84.3%, whereas sterols are 11.1–28.0% of the total amount of lipophilic components.

Phytosterols have numerous health benefits such as lowering the cholesterol level in the blood, lowering cardiovascular disease, and they also act as immune system modulators and possess anticancer properties [55–58]. A daily intake of 1.6–2 g/day of phytosterols is known to reduce cholesterol absorption from the gut by about 30% and plasma LDL cholesterol levels by 8–10% [56, 59]. Lowering the low-density lipoprotein cholesterol uptake and thereby decreasing its level in the blood lowers the risk of cardiovascular diseases [58]. β-sitosterol and its glycoside complex appears to target specific T-helper lymphocytes, increases T_H1-related cytokines and decreases T_H2-related cytokines, increases lymphocyte proliferation, and improves natural killer cell activity [60]. Moreover, phytosterols have shown to subside overactive antibody responses, and this property can be exploited in numerous disease conditions such as chronic viral infections, tuberculosis, rheumatoid

arthritis, allergies, cancer, and auto-immune diseases to ameliorate clinical manifestations [60, 61]. Phytosterols are described as one of the anticancer compounds in plants known to target cancers of breast, prostate, lung, liver, colon, stomach, and ovary [62]. The precise mechanism of action of these phytosterols on cancer cells is poorly understood; however, it seems to target cancerous cells through multiple ways such as inhibition of carcinogen production, cancer cell growth and multiplication, invasion and metastasis and induction of cell cycle arrest and apoptosis, boosting of immune recognition of cancer, influencing hormonal dependent growth of endocrine tumors, and altering sterol biosynthesis [62–64]. Three phytosterols purified from banana flowers, β-sitosterol, 31-norcyclolaudenone, and (24R)-4α,14α,24-trimethyl-5α-cholesta-8,25(27)-dien-3β-ol, had the inhibitory activity against α-glucosidase and α-amylase and thus are capable in prevention of the diseases associated with abnormal blood sugar such as diabetes [65].

2.4 Amines

Biologically active amines or biogenic amines are nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones and may act as either psychoactive or vasoactive. They possess a 3,4-dihydroxyphenyl (catechol) nucleus and are generally called catecholamines. Bananas are a rich source of catecholamines such as dopamine, epinephrine, and norepinephrine which are formed from L-tyrosine [66–68]. Eating bananas has shown to significantly increase catecholamines in the urinary excretion and norepinephrine and dopamine levels in blood and urine [69, 70]. Banana pulp and peel both contains substantial amount of dopamine which has a strong antioxidant activity [26]. Dopamine levels ranged from 80 to 560 mg per 100 g in peel and 2.5–10 mg in ripe pulp of Cavendish banana. Dopamine levels decrease with increased ripening [71]. DOPA was also produced in the in vitro banana cultures supplemented with L-phenylalanine [72]. The catecholamines, epinephrine, and norepinephrine are involved predominantly in the sympathetic control of blood pressure and metabolic processes especially fat and glucose turnover [73, 74]. Dopamine is an important neurotransmitter for controlling behavior and motor functions [75, 76]. Insufficient dopamine can lead to depression, loss of motor control, and lack of motivation to carry out routine tasks. Increasing dopamine directly through dietary sources can help in alleviating these symptoms. Banana being the rich dietary source of dopamine and L-tyrosine, it can be used as a natural food supplement to restore low dopamine levels. Serotonin, another neurotransmitter which is formed from L-tryptophan, is present in appreciable amounts in both pulp and peel of ripe and unripe bananas [77, 78]. Moreover, the serotonin levels in pulp reduced during ripening in Cavendish and "Prata" banana varieties [79, 80]. Few reports claim that increasing dietary serotonin alleviates mood and reduces anxiety; however, dietary serotonin cannot cross the blood brain barrier [81]. Furthermore, banana in addition to serotonin also contains ample L-tryptophan which can be assimilated and converted to serotonin. However, more research is necessary in this

aspect to understand the role of such dietary supplements on brain activity. Bananas contain tyramine (1667 ng/g) which is known to increase blood pressure [71, 82, 83]. Bananas also contain diamines and polyamines such as putrescine, cadaverine, spermine, spermidine which are known to play key role as cytoprotectants in various cellular functions [80, 84, 85].

2.5 Antimicrobial Compounds

Banana plant extract derived from roots, leaves, fruit pulp and peels, inflorescence, and pseudostem have shown to possess antibacterial, antifungal, and antiviral activity [19, 86, 87]. The acetone and methanolic extract obtained from pulp of three banana species Pisang Berangan (*Musa acuminate* AA/AAA), Pisang Mas (*Musa acuminate* AA), and Pisang Nipah (*Musa balbisiana* BBB) demonstrated antibacterial activity against thre Gram-negative bacteria namely, *Pseudomonas aeruginosa* and *Escherichia coli* but not against gram positive bacteria *Staphylococcus aureus* and *Streptococcus mutans.*[88]. The ethanolic extracts of *Musa paradisiaca* L. cv. puttabale leaves showed broad spectrum antibacterial activity against *Bacillus subtilis*, *E. coli*, *P. aeruginosa,* and *S. aureus* with high inhibitory potency against *E. coli* and *S. aureus* [89]. However, [90] showed that the aqueous extracts of *Musa paradisiaca* leaf possess better antibacterial activity than the ethanolic one. The methanolic extracts of *Musa sapientum* L. subsp. *sylvestris* pulp, peel, and seeds were investigated for its antibacterial property against five Gram-positive and eight Gram-negative bacteria wherein the pulp extract showed best antibacterial activity among the three [91]. Ethyl acetate and ethanolic extract of dried pulp and peel of *Musa sapientum* L. subsp. *sylvestris* exhibited antibacterial activity against four Gram-positive and four Gram-negative bacteria whereas only ethyl acetate extract of seeds showed antibacterial activity against the same group of bacteria [92]. In yet another study, ethanolic extracts of *Musa sapientum* peels were effective in inhibiting *Salmonella typhi*, *E. coli*, *Klebsiella pneumoniae*, and *S. aureus* [93]. An alcoholic extract of banana peels was also effective against periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* [94]. Moreover the decoction of banana peels were used along with chitosan in wound healing management [95]. Tin et al. [96] tested the antibacterial activity of the methanolic extracts obtained from *Musa balbisiana* cv. Saba inflorescence against Gram-positive bacteria (*S. aureus*, *B. cereus*, *Listeria monocytogenes*, and *Brochothrix thermosphacta*) and Gram-negative bacteria (*S. typhimurium*, *S. enteritidis*, *E. coli* O157:H7, *Enterobacter sakazakii*, *Yersinia enterocolitica*, and *Vibrio parahaemolyticus*). The buds and bracts of the inflorescence of Mysore banana (*Musa paradisiaca* cv. *Mysore*) showed a wide spectrum of inhibition against foodborne pathogenic bacteria such as *S. aureus*, *B. cereus*, *L. monocytogenes*, and *Vibrio parahaemolyticus* [97]. The methanolic extract of *Musa acuminata* flower showed antimicrobial activity against bacteria like *S. aureus*, *Proteus mirabilis*, *Bacillus subtilis*, *Micrococcus* sp. and *Salmonella* sp. and fungi like *Aspergillus niger* and *Candida albicans* [98]. The ethanolic extracts derived

from corm tissues of *Musa acuminate* cv. *Grand naine* and *Musa paradisiaca* cv. Puttabale showed significant inhibition against *P. vulgaris*, *P. aeruginosa*, and *S. aureus*; moderate activity against *S. typhi*, *S. paratyphi*, *K. pneumoniae*, and *B. subtilis*; and very less against *E. coli* [99]. Ethyl acetate extract of leaf of *Musa acuminata* Colla, *Musa troglodytarum* L., *Musa sapientum* L., and *Musa paradisiaca* L. displayed antibacterial activity against multidrug resistant clinical pathogens causing nosocomial infection such as *E. coli*, *P. aeruginosa*, *Enterobacter aerogenes*, *K. pneumoneae*, *P. mirabilis*, *Shigella flexneri*, *Citrobacter* sp., *S. aureus* MRSA, and *Enterococcus faecalis* [100]. Methanolic extracts of banana (*Musa sapientum* and *Musa acuminata* colla) peels were shown to contain oils having stearic, palmitic, oleic, and linoleic acids and their methyl esters as well as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 5-(hydroxymethyl)-2-furancarboxyaldehyde, cyclododecane, dibutyl phthalate, b-sitosterol, sesamin, and episesamin as fatty acids which were speculated to be responsible for the antibacterial activity against *Bacillus* spp., *Pseudomonas* spp., *E. coli*, *S. aureus*, *Streptococcus* spp., *Klebsiella* spp., *Proteus* spp., and *Salmonella* spp. [101]. Aqueous extract of banana pseudostem showed 21 mm diameter zone of inhibition to *Streptococcus aureus* and 17 mm to *P. aerugenosa* [102]. The methanolic extract of pseudostem of *Musa acuminata* showed inhibitory activity against the drug-sensitive and drug-resistant variants of *Mycobacterium tuberculosis* H37Rv at a concentration of 200 μg/mL [103].

Banana tissue extracts show significant antibacterial and antifungal activity owing to the high content of tannins and other phenolic compounds [104, 105]. The ethanol, acetone, and petroleum ether extracts of banana leaves showed inhibition of *Aspergillus terreus* and *Penicillium solitum* growth [106]. The phenolic compounds extracted in acetone from *Musa acuminata* leaves were prepared as a hydrogel formulation which showed strong antifungal activity against *C. albicans* [107].

Banana plant extracts have also demonstrated antiviral activity against important human viruses. The ethanolic extract of *Musa acuminata* inflorescence showed antiviral activity against simple human herpesvirus type 1 and simple human herpesvirus type 2, both resistant to Acyclovir [108]. Many naturally occurring lectins show antiviral activity wherein they specifically bind to the oligosaccharides present on the viral envelope thereby preventing viral entry [109]. BanLec is a jacalin-related lectin isolated from the fruits of *Musa acuminata* that recognizes the high mannose structures and binds to gp120 of human immunodeficiency virus type-1 (HIV-1). Binding of BanLec to gp120 prevents the entry of the HIV-1 into the cells [86]. Besides, BanLec is known to stimulate T-cell proliferation and act as T-cell mitogen which induces the formation of IgG4 antibodies [110–112]. BanLec was also shown to be interacting with insulin receptors on M210B4 cells and induced a mitogen-activated protein kinase (MEK)-dependent ERK signaling in these cells [113]. Another homodimeric fructose-binding lectin was isolated from *Musa acuminata* (Del Monte banana) which was capable of eliciting a mitogenic response in murine splenocytes and inducing the expression of the cytokines interferon-gamma, tumor necrosis factor-alpha, and interleukin-2 in splenocytes. Further,

it also inhibited proliferation of leukemia cells (L1210) and hepatoma cells (HepG2) and the activity of HIV-1 reverse transcriptase [114]. Thus, banana plant waste which includes the roots, pseudostem, fruit peels, flower buds, and leaves are the potential source of bioactive compounds and nutraceuticals which can be extracted, purified, and valorized into value-added products.

2.6 Antioxidant Compounds

It is a well-established concept that plant-based diet reduces oxidative stress related diseases such as cancer, diabetes, obesity, cardiac diseases, and Parkinson's disease [115, 116]. Antioxidants are the compounds which can efficiently scavenge and eliminate free radicals and reactive oxygen or nitrogen species which contribute to such chronic diseases [117]. Banana is a rich source of antioxidants and is known to have a stronger antioxidant activity even in the simulated gastrointestinal extracts in vitro [118]. Banana pulp and peel contains phenolics, flavonols, dopamine, carotenoids, and vitamin C which are the potent antioxidants and known to scavenge free radicals [26, 119–122]. The pulp tissues from *Musa sapientum* (Latundan banana) and *Musa acuminata* (Red Dacca) showed higher radical scavenging activity than *Musa acuminata* (Cavendish banana) [123]. Fifteen banana varieties were evaluated for their antioxidant potential and found that the ripe pulp is more efficient in removal of free radicals as compared to the unripe pulp. Moreover, the antioxidant property was also found to be genotype specific as "Ouro" (AA) cultivar seemed to be superior to the other cultivars analyzed, presenting 95.36% of free radical removal percentage; while "Marmelo" (ABB) was the least efficient in free radical removal [124]. However, the antioxidant potential was found to be higher in ripe peel than in unripe peel in these 15 varieties. Herein the antioxidant property in these varieties was also attributed to the phenolic compounds, carotenoids, vitamin C, and minerals present in the extracts [124]. The total antioxidant activity, radical scavenging activity, and CUPRAC was tested with the methanolic extract of peel, pulp, and seed of *Musa sapientum* L. ssp. *sylvestris* fruits wherein the seed extract showed better free radical scavenging activity compared to the other two extracts [125]. Vijaykumar et al. [126] showed that when normal and high fat diet-fed rats when fed with flavonoids from raw banana have high glutathione and increased catalase and superoxide dismutase activity. This increase was characteristically correlated with the decreased levels of peroxidation products such as malondialdehyde, hydroperoxides, and conjugated dienes. Ethanolic extract of peels from Monthan (AAB), Karpooravalli (ABB) Nendran (AAB), Kadali (AB), Pachainadan (AABS), Poovan (AAB), Rasthali (AAB), Robusta (AAB), and Sevvazhai (AAA) were tested for their free radical scavenging activity by DPPH, ABTS, and lipid peroxidation inhibition assay [127]. Authors speculated that the high ABTS scavenging activity in Rasthali was due to its high total phenol content whereas the high lipid peroxidation inhibition activity of Poovan was because of its high flavonoid content. Another antioxidant, gallocatechin was identified in banana fruits. Abundance of gallocatechin in the peel (158 mg/100 g dry wt) than in pulp (29.6 mg/100 g dry wt) described the

strong antioxidant activity of the banana peel extract, against lipid auto oxidation, than that of the banana pulp extract [23]. Banana inflorescence which is another by-product of banana industry is loaded with bioactive compounds with potent antioxidant activity [128–130]. Ethanolic extracts of banana flowers have maximum antioxidant activity because of the presence of flavonoid and polyphenols [129, 131]. Ethanolic extract of inflorescence of two Indian varieties Poovan and Monthan was tested for their antioxidant potential and correlated with the phenolic, flavonoid, and vitamin E content. The flower sample of Poovan showed a higher phenolic content (13.45 ± 0.35 mg/g) than samples extracted from the Monthan (9.3 ± 0.36 mg/g) variety. Moreover, the flavonoid content was 6.4 ± 0.2 mg/100 g, 5.53 ± 0.42 mg/100 g and vitamin E content was 1.42 ± 0.11 mg/kg, 1.04 ± 0.07 mg/kg for Poovan and Monthan, respectively [130]. Schmidt et al. [18] optimized the extraction method for obtaining high levels of phenolic compounds and flavonoids from inflorescence of *Musa cavendishii*. A temperature of 60 °C, ethanol concentration of 50%, time of 30 min, and stirring extraction without the use of ultrasound gave highest amounts of phenolic compounds and flavonoids and thus best antioxidant activity. Carotenoids and tocopherols present in banana also impart antioxidant properties [35, 36, 132]. All these studies designate banana as an important source for bioactive molecules having strong antioxidant property.

Other health benefits of banana have been summarized in Table 1.

3 Banana and Plantains: Improvement Through Genetic Engineering for Biofortification

Numerous plant foods or bioactive ingredients derived from plants have been investigated for their role in disease prevention and health. Bananas have long been known as a medicinal plant in the Indian medicine and is effective and advantageous in the treatment of several diseases [4]. Banana is also a suitable crop to achieve biofortification of vitamins and minerals. Biofortification can be achieved by conventional breeding or through transgenic approaches. Edible cultivars of banana being sterile, transgenic strategy to achieve biofortification is primarily exercised in this crop. Queensland University of Technology and the National Agricultural Research Organization of Uganda developed transgenic bananas with elevated levels of provitamin A by expressing the phytoene synthase 1 gene [5]. These provitamin A biofortified transgenic Cavendish bananas lines were field tested in Australia to achieve a level of 55 μg/g dw β-carotene equivalent in the fruit. Moreover, biofortification of banana to alleviate minerals deficiency (such as iron and zinc) is also underway in some research laboratories. Transgenic banana cv. Rasthali plants were generated by expressing soybean ferritin gene. These lines showed a 6.32-fold increase in iron accumulation and a 4.58-fold increase in the zinc levels in the leaves [154]. In yet another study, banana ferritin gene *MusaFer1* when overexpressed in transgenic banana plants showed increased levels of iron in the leaf and root tissues [155]. However, the increased content of iron or other minerals in the edible fruit tissues has not been estimated till now. These genes can be driven by fruit

Table 1 Other health benefits of banana

Health benefit	Source material	Activity	Reference
Anticancer	*Musa acuminata* bract	Antiproliferative activity against breast cancer cell line	[133]
	Musa sapientum fruits	Inhibit the growth of colon cancer cell line HCT-116	[134]
	Musa paradisiaca flower	Antiproliferative effect on the cervical cancer cell line HeLa	[135]
Antidiabetic	*Musa nana* Lour peel	Antihyperglycemic activity in alloxan-induced diabetic mice	[136]
	Musa sapientum infructescence stalks	Reduced blood glucose levels in diabetic rats and glucose transport across rat jejuna and Caco-2 monolayers; induced a 50% decrease in their Na^+/K^+ ATPase activity	[137]
	Musa sapientum, Musa acuminata, and Musa acuminate pulp	α-amylase and α-glucosidase inhibitory activities	[123]
	Musa x *paradisiaca* L.	Regulation of glucose homeostasis and the presence of rutin as the major compound having antidiabetic properties	[138]
	"Prata" variety fruits	Antihyperglycemic effects; protection against oxidative damage, the protein oxidation in both the liver and kidneys were prevented in diabetic rats, lipid peroxidation was prevented in the liver	[139]
	Musa sp. var. Nanjangud rasa bale flower	Antihyperglycemic activity via inhibition of α-glucosidase and in antidiabetogenic effect by inhibition of polyol pathway and protein glycation; increasing the hepatic glucose utilization in diabetic rats by stimulating insulin secretion from the remnant β-cells	[140, 141]
	Musa paradisiaca flowers	Reversed the permanent hyperglycemia within a week in alloxan-induced diabetic rats	[142]
	Musa paradisiaca root	Recovery of the serum insulin level in streptozotocin-induced diabetic rat	[143]
Anti-inflammatory	*Musa sapientum* peel	Exhibited the most potent NO inhibitory activity	[144]
	Green dwarf banana flour (*Musa* sp. AAA)	Prevented the glutathione depletion and inhibited myeloperoxidase activity and lipid peroxidation; inhibition of alkaline phosphatase activity	[145]

(*continued*)

Table 1 (continued)

Health benefit	Source material	Activity	Reference
Antiulcer	*Musa sapientum* var. Paradisiaca	Antiulcerogenic and mucosal protective actions	[146]
	Musa sapientum fruit	Antiulcerogenic activity	[147]
	Musa sapientum peel	Antiulcer and ulcer healing activity	[148]
	Musa paradisiaca tepal and peel	Strengthening the gastric mucosa and decreasing the gastric juice acidity	[149]
	Musa sp. Palo and Horn varieties	Gastroprotective effect	[150]
	Musa paradisiaca leaves	Antiulcer activity	[151]
	Musa sapientum Linn.	Reduction in the number of ulcer and ulcer index	[152]
	Musa sapientum var. Paradisiaca	Antiulcer activity and anti-*Helicobacter pylori* activity	[153]

pulp specific promoter to accumulate vital minerals in the banana fruit tissues [156, 157]. Attempts have also been made to increase the anthocyanidin content in transgenic banana plants and decrease proanthocyanidin content by targeting the branch point enzyme anthocyanidin reductase (MusaANR1) by RNA interference [158]. Downregulation of *MusaANR1* gene did not increase the anthocyanin levels in the transgenic plants. Better understanding of the complex network of regulation in pigment production and accumulation in plant tissues would help to achieve the desired goal. Biofortification has a long way to go and is yet to be fully exploited to reap its benefits in near future. However, few proof of principle studies have provided evidences and support its effectiveness [5, 159–161].

4 Conclusion

Bananas and plantains are largely consumed all over the world as a staple food and for medicinal purposes. There are more than 1000 banana cultivars identified around the globe each having a peculiar phytochemical characteristic. The medicinal properties of banana have always been acknowledged in the ancient texts against diseases and ailments. Current research is focused on the identification and characterization of pharmacological active compounds in banana. Banana contains considerable amount of bioactive compounds for health promotion and thus the phytochemical and pharmacological studies of bananas and plantains have received much interest. Many studies have demonstrated and proved the beneficial properties of banana as an antioxidant, anticancer, antidiabetic, and antimicrobial. Although different cultivars and different tissues of banana have demonstrated difference in the production and activity of bioactive molecules, it is necessary to further identify, characterize, and use them for generating biofortified varieties. Moreover, bananas are rich source

of flavonoids, carotenoids, and biogenic amines which can be explored as functional food source against serious ailments. Utilizing banana tissue extracts for medical application needs rigorous understanding of efficacy, safety, and toxicity. However, considering the seminal work published in the last decade on the use of banana plant extracts for series of ailments, one could expect the use of banana bioactive compounds in modern medicine in near future.

References

1. Martin G, Baurens FC, Cardi C, Aury JM, D'Hont A (2013) The complete chloroplast genome of banana (*Musa acuminata*, Zingiberales): insight into plastid monocotyledon evolution. PLoS One 8:e67350
2. Bakry F, Carreel F, Jenny C, Horry JP (2009) Genetic improvement of banana. In: Breeding plantation tree crops: tropical species. Springer, New York, pp 3–50
3. FAOSTAT (2016) http://www.fao.org/faostat. Accessed on 22 Dec 2017
4. Kumar KPS, Bhowmik D, Duraivel S, Umadevi M (2012) Traditional and medicinal uses of banana. J Pharmacogn Phytochem 1:51–63
5. Paul JY, Khanna H, Kleidon J, Hoang P, Geijskes J, Daniells J, Zaplin E, Rosenberg Y, James A, Mlalazi B, Deo P (2017) Golden bananas in the field: elevated fruit pro-vitamin A from the expression of a single banana transgene. Plant Biotechnol J 15:520–532
6. National Nutrient Database (2016) https://ndb.nal.usda.gov/ndb/foods/show/2159. Accessed on 30 May 2017
7. Imam MZ, Akter S (2011) *Musa paradisiaca* L. and *Musa sapientum* L.: a phytochemical and pharmacological review. J Appl Pharm Sci 1:14–20
8. Pothavorn P, Kitdamrongsont K, Swangpol S, Wongniam S, Atawongsa K, Svasti J et al (2010) Sap phytochemical compositions of some bananas in Thailand. J Agric Food Chem 58:8782–8787
9. Hefferon KL (2015) Nutritionally enhanced food crops; progress and perspectives. Int J Mol Sci 16:3895–3914
10. Singh SP, Gruissem W, Bhullar NK (2017) Single genetic locus improvement of iron, zinc and β-carotene content in rice grains. Sci Rep 7:6883
11. Horry J, Jay M (1988) Distribution of anthocyanins in wild and cultivated banana varieties. Phytochemistry 27:2667–2672
12. Schieber A, Stintzing FC, Carle R (2001) By-products of plant food processing as a source of functional compounds – recent developments. Trends Food Sci Technol 12:401–413
13. Kitdamrongsont K, Pothavorn P, Swangpol S, Wongniam S, Atawongsa K, Svasti J, Somana J (2008) Anthocyanin composition of wild bananas in Thailand. J Agric Food Chem 56:10853–10857
14. Afanas'ev IB, Dcrozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI (1989) Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem Pharmacol 38:1763–1769
15. Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20:933–956
16. Atala E, Fuentes J, Wehrhahn MJ, Speisky H (2017) Quercetin and related flavonoids conserve their antioxidant properties despite undergoing chemical or enzymatic oxidation. Food Chem 234:479–485
17. De Ascensao ARFDC, Dubery IA (2003) Soluble and wall-bound phenolics and phenolic polymers in *Musa acuminata* roots exposed to elicitors from *Fusarium oxysporum* f. sp. *cubense*. Phytochemistry 63:679–686

18. Schmidt MM, Prestes RC, Kubota EH, Scapin G, Mazutti MA (2015) Evaluation of antioxidant activity of extracts of banana inflorescences (*Musa cavendishii*). CyTA-J Food 13:498–505
19. Mathew NS, Negi PS (2017) Traditional uses, phytochemistry and pharmacology of wild banana (*Musa acuminata* Colla): a review. J Ethnopharmacol 196:124–140
20. Lewis DA, Fields WN, Shaw GP (1999) A natural flavonoid present in unripe plantain banana pulp (*Musa sapientum* L. var. *paradisiaca*) protects the gastric mucosa from aspirin-induced erosions. J Ethnopharmacol 65:283–288
21. Pazmiño-Durána EA, Giusti MM, Wrolstad RE, Glória MBA (2001) Anthocyanins from banana bracts (*Musa paradisiaca*) as potential food colorants. Food Chem 73:327–332
22. Bennett RN, Shiga TM, Hassimotto NM, Rosa EA, Lajolo FM, Cordenunsi BR (2010) Phenolics and antioxidant properties of fruit pulp and cell wall fractions of postharvest banana (*Musa acuminata* Juss.) cultivars. J Agric Food Chem 58:7991–8003
23. Someya S, Yoshiki Y, Okubo K (2002) Antioxidant compounds from bananas (*Musa Cavendish*). Food Chem 79:351–354
24. Rebello LP, Ramos AM, Pertuzatti PB, Barcia MT, Castillo-Muñoz N, Hermosín-Gutiérrez I (2014) Flour of banana (*Musa* AAA) peel as a source of antioxidant phenolic compounds. Food Res Int 55:397–403
25. Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 99:191–203
26. Kanazawa K, Sakakibara H (2000) High content of dopamine, a strong antioxidant, in Cavendish banana. J Agric Food Chem 48:844–848
27. de Pascual-Teresa S, Santos-Buelga C, Rivas-Gonzalo JC (2000) Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. J Agric Food Chem 48:5331–5337
28. Santos JR, Bakry F, Brillouet JM (2010) A preliminary chemotaxonomic study on the condensed tannins of green banana flesh in the Musa genus. Biochem Syst Ecol 38:1010–1017
29. Loganayaki N, Rajendrakumaran D, Manian S (2010) Antioxidant capacity and phenolic content of different solvent extracts from banana (*Musa paradisiaca*) and mustai (*Rivea hypocrateriformis*). Food Sci Biotechnol 19:1251–1258
30. Johnson EJ (2002) The role of carotenoids in human health. Nutr Clin Care 5:56–65
31. Kiokias S, Proestos C, Varzakas T (2016) A review of the structure, biosynthesis, absorption of carotenoids-analysis and properties of their common natural extracts. Curr Res Nutr Food Sci J 4:25–37
32. Akoh CC, Min BD (1997) Food lipid chemistry, nutrition and biotechnology. Marcel Dekker, New York
33. Wills RBH, Lim JSK, Greenfield H (1986) Composition of Australian foods. 31. Tropical and sub-tropical fruit. Food Technol Aust 38:118–123
34. Holden JM, Eldridge AL, Beecher GR, Buzzard IM, Bhagwat S, Davis CS, Douglas LW, Gebhardt S, Haytowitz D, Schakel S (1999) Carotenoid content of U.S. foods: an update of the database. J Food Compos Anal 12:169–196
35. Englberger L, Schierle J, Aalbersberg W, Hofmann P, Humphries J, Huang A, Lorens A, Levendusky AM, Daniells J, Marks GC, Fitzgerald MH (2006) Carotenoid and vitamin content of Karat and other Micronesian banana cultivars. Int J Food Sci Nutr 57:399–418
36. Englberger L, Wills RB, Blades B, Dufficy L, Daniells JW, Coyne T (2006) Carotenoid content and flesh color of selected banana cultivars growing in Australia. Food Nutr Bull 27:281–291
37. Buah S, Mlalazi B, Khanna H, Dale JL, Mortimer CL (2016) The quest for golden bananas: investigating carotenoid regulation in a Fe'i group *Musa* cultivar. J Agric Food Chem 64:3176–3185
38. Englberger L, Darnton-Hill I, Coyne T, Fitzgerald MH, Marks GC (2003) Carotenoid-rich bananas: a potential food source for alleviating vitamin A deficiency. Food Nutr Bull 24:303–318

39. Ekesa BN, Poulaert M, Davey MW, Kimiywe J, Van den Bergh I, Blomme G, Dhuique-Mayer C (2012) Bioaccessibility of provitamin A carotenoids in bananas (*Musa* spp.) and derived dishes in African countries. Food Chem 133:1471–1477
40. Fungo R, Pillay M (2011) β-Carotene content of selected banana genotypes from Uganda. Afr J Biotechnol 10:5423–5430
41. Arora A, Choudhary D, Agarwal G, Singh VP (2008) Compositional variation in β-carotene content, carbohydrate and antioxidant enzymes in selected banana cultivars. Int J Food Sci Technol 43:1913–1921
42. Dhandapani R, Singh VP, Arora A, Bhattacharya RC, Rajendran A (2017) Differential accumulation of β-carotene and tissue specific expression of phytoene synthase (MaPsy) gene in banana (*Musa* sp) cultivars. J Food Sci Technol 54:4416–4426
43. Ekesa B, Nabuuma D, Blomme G, Van den Bergh I (2015) Provitamin a carotenoid content of unripe and ripe banana cultivars for potential adoption in eastern Africa. J Food Compos Anal 43:1–6
44. Subagio A, Morita N, Sawada S (1996) Carotenoids and their fatty-acid esters in banana peel. J Nutr Sci Vitaminol 42:553–566
45. Suparmi S, Prasetya H, Martosupono M, Sunaryanto LT (2014) Effect of beta-Carotene from yellow ambon banana peel on rat serum retinol level. J Pure Appl Chem Res 3:83–87
46. Kumar SR, Baskaran V (2012) Assay of carotenoid composition and retinol equivalents in plants. In: Preedy VR (ed) Vitamin A and carotenoids: chemistry, analysis, function and effects. RSC Publishing, London, pp 221–248
47. Voutilainen S, Nurmi T, Mursu J, Rissanen TH (2006) Carotenoids and cardiovascular health. Am J Clin Nutr 83:1265–1271
48. Lehrman L, Kabat EA (1937) The fatty acids associated with banana starch. J Am Chem Soc 59:1050–1051
49. Vilela C, Santos SA, Villaverde JJ, Oliveira L, Nunes A, Cordeiro N, Freire CS, Silvestre AJ (2014) Lipophilic phytochemicals from banana fruits of several *Musa* species. Food Chem 162:247–252
50. Morais DR, Rotta EM, Sargi SC, Bonafe EG, Suzuki RM, Souza NE, Matsushita M, Visentainer JV (2017) Proximate composition, mineral contents and fatty acid composition of the different parts and dried peels of tropical fruits cultivated in Brazil. J Braz Chem Soc 28:308–318
51. Oliveira L, Freire CS, Silvestre AJ, Cordeiro N, Torres IC, Evtuguin D (2006) Lipophilic extractives from different morphological parts of banana plant "Dwarf Cavendish". Ind Crop Prod 23:201–211
52. Oliveira L, Freire CS, Silvestre AJ, Cordeiro N (2008) Lipophilic extracts from banana fruit residues: a source of valuable phytosterols. J Agric Food Chem 56:9520–9524
53. Knapp FF, Nicholas HJ (1969) The sterols and triterpenes of banana pulp. J Food Sci 34:584–586
54. Villaverde JJ, Oliveira L, Vilela C, Domingues RM, Freitas N, Cordeiro N, Freire CS, Silvestre AJ (2013) High valuable compounds from the unripe peel of several *Musa* species cultivated in Madeira Island (Portugal). Ind Crop Prod 42:507–512
55. Ortega RM, Palencia A, López-Sobaler AM (2006) Improvement of cholesterol levels and reduction of cardiovascular risk via the consumption of phytosterols. Br J Nutr 96:S89–S93
56. Marangoni F, Poli A (2010) Phytosterols and cardiovascular health. Pharmacol Res 61:193–199
57. Genser B, Silbernagel G, De Backer G, Bruckert E, Carmena R, Chapman MJ, Deanfield J, Descamps OS, Rietzschel ER, Dias KC, März W (2012) Plant sterols and cardiovascular disease: a systematic review and meta-analysis. Eur Heart J 33:444–451
58. Ras RT, van der Schouw YT, Trautwein EA, Sioen I, Dalmeijer GW, Zock PL, Beulens JW (2015) Intake of phytosterols from natural sources and risk of cardiovascular disease in the European prospective investigation into cancer and nutrition-the Netherlands (EPIC-NL) population. Eur J Prev Cardiol 22:1067–1075

59. Quilez J, Garcia-Lorda P, Salas-Salvado J (2003) Potential uses and benefits of phytosterols in diet: present situation and future directions. Clin Nutr 22:343–351
60. Bouic PJ, Lamprecht JH (1999) Plant sterols and sterolins: a review of their immune-modulating properties. Altern Med Rev 4:170–177
61. Valerio M, Liu HB, Heffner R, Zivadinov R, Ramanathan M, Weinstock-Guttman B, Awad AB (2011) Phytosterols ameliorate clinical manifestations and inflammation in experimental autoimmune encephalomyelitis. Inflamm Res 60:457–465
62. Ramprasath VR, Awad AB (2015) Role of phytosterols in cancer prevention and treatment. J AOAC Int 98:735–738
63. Bradford PG, Awad AB (2007) Phytosterols as anticancer compounds. Mol Nutr Food Res 51:161–170
64. Woyengo TA, Ramprasath VR, Jones PJ (2009) Anticancer effects of phytosterols. Eur J Clin Nutr 63:813–820
65. Sheng Z, Dai H, Pan S, Ai B, Zheng L, Zheng X, Prinyawiwatkul W, Xu Z (2017) Phytosterols in banana (*Musa* spp.) flower inhibit α-glucosidase and α-amylase hydrolysations and glycation reaction. Int J Food Sci Technol 52:171–179
66. Crout JR, Sjoerdsma A (1959) The clinical and laboratory significance of serotonin and catechol amines in bananas. N Engl J Med 261:23–26
67. Davidson L, Vandongen R, Beilin LJ (1981) Effect of eating bananas on plasma free and sulfate-conjugated catecholamines. Life Sci 29:1773–1778
68. Lassois L, De CC, Frettinger P, De LL, Lepoivre P, Haïssam MJ (2011) Catecholamine biosynthesis pathway potentially involved in banana defense mechanisms to crown rot disease. Commun Agric Appl Biol Sci 76:591–601
69. Kuchel OT, Buu NT, Serri O (1982) Sulfoconjugation of catecholamines, nutrition, and hypertension. Hypertension 4:III93
70. de Jong WH, Post WJ, Kerstens MN, de Vries EG, Kema IP (2010) Elevated urinary free and deconjugated catecholamines after consumption of a catecholamine-rich diet. J Clin Endocrinol Metab 95:2851–2855
71. Romphophak T, Siriphanich J, Ueda Y, Abe K, Chachin K (2005) Changes in concentrations of phenolic compounds and polyphenol oxidase activity in banana peel during storage. Food Preserv Sci 31:111–115
72. Bapat VA, Suprasanna P, Ganapathi TR, Rao PS (2000) In vitro production of L-DOPA in tissue cultures of banana. Pharm Biol 38:271–273
73. Laverty R (1978) Catecholamines: role in health and disease. Drugs 16:418–440
74. Nagatsu T (2006) The catecholamine system in health and disease. Proc Jpn Acad Ser B Phys Biol Sci 82:388–415
75. Goerendt IK, Messa C, Lawrence AD, Grasby PM, Piccini P, Brooks DJ (2003) Dopamine release during sequential finger movements in health and Parkinson's disease: a PET study. Brain 126:312–325
76. Narvaes R, Martins de Almeida RM (2014) Aggressive behavior and three neurotransmitters: dopamine, GABA, and serotonin-A review of the last 10 years. Psychol Neurosci 7:601
77. Waalkes TP, Sjoerdsma A, Creveling CR, Weissbach H, Udenfriend S (1958) Serotonin, norepinephrine, and related compounds in bananas. Science 127:648–650
78. Badria FA (2002) Melatonin, serotonin, and tryptamine in some Egyptian food and medicinal plants. J Med Food 5:153–157
79. Vettorazzi (1974) 5-Hydroxytryptamine content of bananas and banana products. Food Cosmet Toxicol 12:107–113
80. Adão RC, Glória MB (2005) Bioactive amines and carbohydrate changes during ripening of Prata'banana (*Musa acuminata* × *M. balbisiana*). Food Chem 90:705–711
81. Young SN (2007) How to increase serotonin in the human brain without drugs. J Psychiatry Neurosci 32:394
82. Rice SL, Eitenmiller RR, Koehler PE (1976) Biologically active amines in food: a review. J Milk Food Technol 39:353–358

83. Czajkowska-Mysłek A, Leszczyńska J (2017) Risk assessment related to biogenic amines occurrence in ready-to-eat baby foods. Food Chem Toxicol 105:82–92
84. Baston O, Moise D, Barna O, Pricop E (2009) Bioactive amines content in "Dwarf Cavendish banana" stored at different temperatures. Lucr ŞtiinŃ seria Agron 52:603–606
85. Hunter DC, Burritt DJ (2012) Polyamines of plant origin - An important dietary consideration for human health. In: Venketeshwer R (ed). Phytochemicals as nutraceuticals - Global approaches to their role in nutrition and health. InTech: Rijeka, Croatia. pp. 225–244.
86. Swanson MD, Winter HC, Goldstein IJ, Markovitz DM (2010) A lectin isolated from bananas is a potent inhibitor of HIV replication. J Biol Chem 285:8646–8655
87. Martinez JA (2012) Natural Fungicides obtained from plants. In: D. Dhanasekaran, N. Thajuddin, and A. Panneerselvam (eds). In Fungicides for Plant and Animal Diseases. InTech: Rijeka, Croatia pp. 1–28.
88. Jalani FF, Mohamad S, Shahidan WN (2014) Antibacterial effects of banana pulp extracts based on different extraction methods against selected microorganisms. Asian J Biomed Pharm Sci 4:14
89. Naikwade PV, Gaurav S, Sharayu D, Kailas J (2014) Evaluation of antibacterial properties of *Musa paradisiaca* L. Leaves. In: Proceedings of the national conference on conservation of natural resources & biodiversity for sustainable development
90. Asuquo EG, Udobi CE (2016) Antibacterial and toxicity studies of the ethanol extract of *Musa paradisiaca* leaf. Cogent Biol 2:1219248
91. Zafar IM, Saleha A, Hoque MM, Sohel RM (2011) Antimicrobial and cytotoxic properties of different extracts of *Musa sapientum* L. subsp. *sylvestris*. Int Res J Pharm 2:62–65
92. Jain P, Bhuiyan MH, Hossain KR, Bachar SC (2011) Antibacterial and antioxidant activities of local seeded banana fruits. Afr J Pharm Pharmacol 5:1398–1403
93. Ehiowemwenguan G, Emoghene AO, Inetianbor JE (2014) Antibacterial and phytochemical analysis of banana fruit peel. IOSR J Pharm 4:18–25
94. Kapadia SP, Pudakalkatti PS, Shivanaikar S (2015) Detection of antimicrobial activity of banana peel (*Musa paradisiaca* L.) on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*: an in vitro study. Contemp Clin Dent 6:496
95. Franco PB, Almeida LA, Marques RF, da Silva MA, Campos MG (2017) Chitosan associated with the extract of unripe banana peel for potential wound dressing application. Int J Polym Sci 2017:9761047
96. Tin HS, Padam BS, Vairappan CS, Abdullah MI, Chye FY (2015) Effect of preparation and extraction parameters of banana (*Musa balbisiana* cv. Saba) inflorescence on their antibacterial activities. Sains Malays 44:1301–1307
97. Padam BS, Tin HS, Chye FY, Abdullah MI (2012) Antibacterial and antioxidative activities of the various solvent extracts of banana (*Musa paradisiaca* cv. *Mysore*) inflorescences. J Biol Sci 12:62–73
98. Sumathy V, Lachumy SJ, Zakaria Z, Sasidharan S (2011) In vitro bioactivity and phytochemical screening of *Musa acuminata* flower. Pharmacologyonline 2:118–127
99. Venkatesh KV, Girish Kumar K, Pradeepa K, Santhosh Kumar SR (2013) Antibacterial activity of ethanol extract of *Musa paradisiaca* cv. Puttabale and *Musa acuminate* cv. Grand Naine. Asian J Pharm Clin Res 6:169–172
100. Karuppiah P, Mustaffa M (2013) Antibacterial and antioxidant activities of *Musa* sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed 3:737–742
101. Mordi RC, Fadiaro AE, Owoeye TF, Olanrewaju IO, Uzoamaka GC, Olorunshola SJ (2016) Identification by GC-MS of the components of oils of banana peels extract, phytochemical and antimicrobial analyses. Res J Phytochem 10:39–44
102. Onyema CT, Ofor CE, Okudo VC, Ogbuagu AS (2016) Phytochemical and antimicrobial analysis of banana pseudo stem (*Musa acuminata*). Br J Pharm Res 10:1–9
103. Camacho-Corona MD, Ramírez-Cabrera MA, Santiago OG, Garza-González E, Palacios ID, Luna-Herrera J (2008) Activity against drug resistant-tuberculosis strains of plants used in

Mexican traditional medicine to treat tuberculosis and other respiratory diseases. Phytother Res 22:82–85
104. Scalbert A (1991) Antimicrobial properties of tannins. Phytochemistry 30:3875–3883
105. Min BR, Pinchak WE, Merkel R, Walker S, Tomita G, Anderson RC (2008) Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. Sci Res Essay 3:066–073
106. Meenashree B, Vasanthi VJ, Mary RN (2014) Evaluation of total phenolic content and antimicrobial activities exhibited by the leaf extracts of *Musa acuminata* (banana). Int J Curr Microbiol Appl Sci 3:136–141
107. Bankar AM, Dole MN (2016) Formulation and evaluation of herbal antimicrobial gel containing *Musa acuminata* leaves extract. J Pharmacog Phytochem 5:1
108. Martins FO, Fingolo CE, Kuster RM, Kaplan MA, Romanos MT (2009) Antiviral activity of *Musa acuminata* Colla, Musaceae. Rev Bras Farmacogn 19:781–784
109. Mitchell CA, Ramessar K, O'keefe BR (2017) Antiviral lectins: selective inhibitors of viral entry. Antivir Res 142:37–54
110. Koshte VL, Van Dijk W, Van Der Stelt ME, Aalberse RC (1990) Isolation and characterization of BanLec-I, a mannoside-binding lectin from *Musa paradisiac* (banana). Biochem J 272:721–726
111. Mo H, Winter HC, Van Damme EJ, Peumans WJ, Misaki A, Goldstein IJ (2001) Carbohydrate binding properties of banana (*Musa acuminata*) lectin. FEBS J 268:2609–2615
112. Winter HC, Oscarson S, Slättegård R, Tian M, Goldstein IJ (2005) Banana lectin is unique in its recognition of the reducing unit of 3-*O*-β-glucosyl/mannosyl disaccharides: a calorimetric study. Glycobiology 15:1043–1050
113. Bajaj M, Hinge A, Limaye LS, Gupta RK, Surolia A, Kale VP (2010) Mannose-binding dietary lectins induce adipogenic differentiation of the marrow-derived mesenchymal cells via an active insulin-like signaling mechanism. Glycobiology 21:521–529
114. Cheung AH, Wong JH, Ng TB (2009) *Musa acuminata* (Del Monte banana) lectin is a fructose-binding lectin with cytokine-inducing activity. Phytomedicine 16:594–600
115. Carlsen MH, Halvorsen BL, Holte K, Bøhn SK, Dragland S, Sampson L, Willey C, Senoo H, Umezono Y, Sanada C, Barikmo I (2010) The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutr J 9:3
116. Rahman T, Hosen I, Islam MT, Shekhar HU (2012) Oxidative stress and human health. Adv Biosci Biotechnol 3:997
117. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5:9
118. Bhatt A, Patel V (2015) Antioxidant potential of banana: study using simulated gastrointestinal model and conventional extraction. Indian J Exp Biol 53:457–461
119. Harris PL, Poland GL (1939) Variations in ascorbic acid content of bananas. J Food Sci 4:317–327
120. Fatemeh SR, Saifullah R, Abbas FM, Azhar ME (2012) Total phenolics, flavonoids and antioxidant activity of banana pulp and peel flours: influence of variety and stage of ripeness. Int Food Res J 19:1041–1046
121. Padilla-Camberos E, Flores-Fernández JM, Canales-Aguirre AA, Barragán-Álvarez CP, Gutiérrez-Mercado Y, Lugo-Cervantes E (2016) Wound healing and antioxidant capacity of *Musa paradisiaca* Linn. peel extracts. J Pharm Pharmacog Res 4:165–173
122. Heng Z, Sheng O, Yan S, Lu H, Motorykin I, Gao H, Li C, Yang Q, Hu C, Kuang R, Bi F (2017) Carotenoid profiling in the peel and pulp of 36 selected Musa varieties. Food Sci Technol Res 23:603–611
123. Adedayo BC, Oboh G, Oyeleye SI, Olasehinde TA (2016) Antioxidant and antihyperglycemic properties of three banana cultivars (*Musa* spp.) Scientifica 2016:8391398
124. Aquino CF, Salomão LC, Ribeiro S, Rocha M, Siqueira DL, Cecon PR (2016) Carbohydrates, phenolic compounds and antioxidant activity in pulp and peel of 15 banana cultivars. Rev Bras Frutic 38(4):e-090

125. Imam MZ, Akter S, Mazumder ME, Rana MS (2011) Antioxidant activities of different parts of *Musa sapientum* L. ssp. sylvestris fruit. J Appl Pharm Sci 1:68–72
126. Vijayakumar S, Presannakumar G, Vijayalakshmi NR (2008) Antioxidant activity of banana flavonoids. Fitoterapia 79:279–282
127. Baskar R, Shrisakthi S, Sathyapriya B, Shyampriya R, Nithya R, Poongodi P (2011) Antioxidant potential of peel extracts of banana varieties (*Musa sapientum*). Food Nutr Sci 2:1128
128. Bhaskar JJ, Chilkunda ND, Salimath PV (2011) Banana (*Musa* sp. var. elakki bale) flower and pseudostem: dietary fiber and associated antioxidant capacity. J Agric Food Chem 60:427–432
129. Sheng ZW, Ma WH, Gao JH, Bi Y, Zhang WM, Dou HT, Jin ZQ (2011) Antioxidant properties of banana flower of two cultivars in China using 2, 2-diphenyl-1-picrylhydrazyl (DPPH,) reducing power, 2, 2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate (ABTS) and inhibition of lipid peroxidation assays. Afr J Biotechnol 10:4470–4477
130. Arya Krishnan S, Sinija VR (2016) Proximate composition and antioxidant activity of banana blossom of two cultivars in India. Int J Agric Food Sci Technol 7:13–22
131. Joseph J, Paul D, Kavitha MP, Dineshkumar B, Menon JS, Bhat AR, Krishnakumar K (2014) Preliminary phytochemical screening and in vitro antioxidant activity of banana flower (*Musa paradisiaca* AAB Nendran variety). J Pharm Res 8:144–147
132. Waghmare JS, Kurhade AH (2014) GC-MS analysis of bioactive components from banana peel (*Musa sapientum* peel). Eur J Exp Biol 4:10–15
133. Roobha JJ, Saravanakumar M, Aravindhan KM, Suganyadevi P (2011) In vitro evaluation of anticancer property of anthocyanin extract from *Musa acuminata* bract. Res Pharm 1:17–21
134. Dahham SS, Mohamad TA, Tabana YM, Majid AM (2015) Antioxidant activities and anticancer screening of extracts from banana fruit (*Musa sapientum*). Acad J Cancer Res 8:28–34
135. Nadumane VK, Timsina B (2014) Anti-cancer potential of banana flower extract: an in vitro study. Bangladesh J Pharmacol 9:628–635
136. Wu HM, Xu FH, Hao J, Yang Y, Wang X (2015) Antihyperglycemic activity of banana (*Musa nana* Lour.) peel and its active ingredients in alloxan-induced diabetic mice. In 3rd international conference on material, mechanical and manufacturing engineering, pp 231–238
137. Jaber H, Baydoun E, Ola EZ, Kreydiyyeh SI (2013) Anti-hyperglycemic effect of the aqueous extract of banana infructescence stalks in streptozotocin-induced diabetic rats. Plant Foods Hum Nutr 68:83–89
138. Kappel VD, Cazarolli LH, Pereira DF, Postal BG, Madoglio FA, Buss ZD, Reginatto FH, Silva FR (2013) Beneficial effects of banana leaves (*Musa* x *paradisiaca*) on glucose homeostasis: multiple sites of action. Rev Bras Farmacogn 23:706–715
139. Silva AR, Cerdeira CD, Brito AR, Salles BC, Ravazi GF, Moraes GD, Rufino LR, Oliveira RB, Santos GB (2016) Green banana pasta diet prevents oxidative damage in liver and kidney and improves biochemical parameters in type 1 diabetic rats. Arch Endocrinol Metab 60:355–366
140. Ramu R, Shirahatti PS, Zameer F, Dhananjaya BL, Prasad N (2016) Assessment of in vivo antidiabetic properties of umbelliferone and lupeol constituents of banana (*Musa* sp. var. Nanjangud Rasa Bale) flower in hyperglycaemic rodent model. PLoS One 11:e0151135
141. Ramu R, Shirahatti PS, Zameer F, Ranganatha LV, Prasad MN (2014) Inhibitory effect of banana (*Musa* sp. var. Nanjangud rasa bale) flower extract and its constituents Umbelliferone and Lupeol on α-glucosidase, aldose reductase and glycation at multiple stages. S Afr J Bot 95:54–63
142. Jawla S, Kumar Y, Khan MS (2012) Antimicrobial and antihyperglycemic activities of *Musa paradisiaca* flowers. Asian Pac J Trop Biomed 2:S914–S918
143. Mallick C, Maiti R, Ghosh D (2006) Comparative study on antihyperglycemic and antihyperlipidemic effects of separate and composite extract of seed of *Eugenia jambolana* and root of *Musa paradisiaca* in streptozotocin-induced diabetic male albino rat. Iran J Pharmacol Ther 5:27–33

144. Phuaklee P, Ruangnoo S, Itharat A (2012) Anti-inflammatory and antioxidant activities of extracts from *Musa sapientum* peel. J Med Assoc Thail 95:S142–S146
145. Scarminio V, Fruet AC, Witaicenis A, Rall VL, Di Stasi LC (2012) Dietary intervention with green dwarf banana flour (*Musa* sp AAA) prevents intestinal inflammation in a trinitrobenzenesulfonic acid model of rat colitis. Nutr Res 32:202–209
146. Tandel KR, Shah BK (2012) Evaluation of gastric antiulcerogenic action of vegetable plantain banana (*Musa sapientum* var. Paradisiaca) in aspirin plus pylorus ligated albino rats. Int J Pharm Sci Res 3:4387
147. Prabha P, Karpagam T, Varalakshmi B, Packiavathy AS (2011) Indigenous anti-ulcer activity of *Musa sapientum* on peptic ulcer. Pharm Res 3:232
148. Onasanwo SA, Emikpe BO, Ajah AA, Elufioye TO (2013) Anti-ulcer and ulcer healing potentials of *Musa sapientum* peel extract in the laboratory rodents. Pharm Res 5:173
149. Ulser T (2016) Antiulcer activity of *Musa paradisiaca* (banana) tepal and skin extracts in ulcer induced albino mice. Malays J Anal Sci 20:1203–1216
150. Pannangpetch P, Vuttivirojana A, Kularbkaew C, Tesana S, Kongyingyoes B, Kukongviriyapan V (2001) The antiulcerative effect of Thai Musa species in rats. Phytother Res 15:407–410
151. Bhatnagar S, Garg VK, Sharma PK, Jain S (2011) Pelagia Research Library. Der Pharmacia Sinica 2:40–43
152. Gangwar AK, Ghosh AK (2014) To estimate the antiulcer activity of leaves of *Musa sapientum* Linn. by ethanol induced method in rats. Int J Pharmacog Phytochem Res 6:53–55
153. Goel RK, Sairam K, Rao CV, Raman A (2001) Role of gastric antioxidant and anti-*Helicobactor pylori* activities in anti ulcerogenic activity of plantain banana (*Musa sapientum* var. paradisiaca). Indian J Exp Biol 39:719–722
154. Sunil Kumar GB, Srinivas L, Ganapathi TR (2011) Iron fortification of banana by the expression of soybean ferritin. Biol Trace Elem Res 142:232–241
155. Yadav K, Patel P, Srivastava AK, Ganapathi TR (2017) Overexpression of native ferritin gene *MusaFer1* enhances iron content and oxidative stress tolerance in transgenic banana plants. PLoS One 12:e0188933
156. Clendennen SK, López-Gómez R, Gómez-Lim M, Arntzen CJ, May GD (1998) The abundant 31-kilodalton banana pulp protein is homologous to class-III acidic chitinases. Phytochemistry 47:613–619
157. Itou N, Kuroda H, Takane KI, Aoki K, Shibata D, Ezura H, Tanase K, inventors (2011) In planta innovations inc., University of Tsukuba, Kazusa Dna Research Institute, assignee. Fruit-specific promoter. United States patent application US 14/007,813
158. Ghag SB, Shekhawat UK, Ganapathi TR (2015) Silencing of *MusaANR1* gene reduces proanthocyanidin content in transgenic banana plants. Plant Cell Tissue Organ Cult (PCTOC) 121:693–702
159. Haas JD, Beard JL, Murray-Kolb LE, del Mundo AM, Felix A, Gregorio GB (2005) Iron-biofortified rice improves the iron stores of nonanemic Filipino women. J Nutr 135:2823–2830
160. Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH (2011) Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr Bull 32:S31–S40
161. Gurmu F, Hussein S, Laing M (2014) The potential of orange-fleshed sweet potato to prevent vitamin A deficiency in Africa. Int J Vitam Nutr Res 84:65–78

Veterinary Antibiotics in Animal Diet: Effects on Waste/Environment

60

Lizbeth E. Robles Jimenez, Juan C. Angeles Hernandez, Jorge Osorio Avalos, Xunde Li, Edward Rob Atwill, Octavio Castelan Ortega, and Manuel Gonzalez Ronquillo

Contents

Abstract

Residues of veterinary antibiotics in the environment pose a risk for aquatic and terrestrial ecosystems. The degradation of antibiotics in the environment has not been fully characterized in spite of the long periods of persistence of residues in the environment. This chapter reviews the main antibiotics found in water bodies and on land as well as its effects on microbial communities, along with some strategies that have been proposed by global organizations to control the use of antibiotics in the livestock production industry.

Keywords

Veterinary antibiotic · Pollution · Environmental · Residues

L. E. Robles Jimenez · J. C. Angeles Hernandez · J. Osorio Avalos · O. Castelan Ortega · M. Gonzalez Ronquillo (✉)
Departamento de Nutricion Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma del Estado de Mexico, Toluca, Estado de Mexico, Mexico
e-mail: lizroblez@hotmail.com; crucifixio_8@hotmail.com; josorioa@uaemex.mx; oaco2002@yahoo.com.mx; mrg@uaemex.mx

X. Li · E. R. Atwill
Western Institute for Food Safety and Security, School of Veterinary Medicine, University of California Davis, Davis, CA, USA
e-mail: xdli@ucdavis.edu; ratwill@ucdavis.edu

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_41

1 Introduction

Antibiotics play an important role in the treatment of diseases of food-producing animals (livestock, poultry, and aquaculture), as well as being used as growth promoters [1]. There are approximately 250 types of antibiotics registered for veterinary use [2, 3], including more than 2000 different products that contain over 400 active ingredients [4]. Tetracyclines appear to be the most widely used veterinary antibiotics, followed by sulfonamides and macrolides [5].

In 2010, approximately 63,151 tons of antibiotics were consumed by livestock industry worldwide. This number is expected to increase by 67% as 105,596 tons per year in 2030 [6]. China is one of the main consumers and producers of antibiotics, with the consumption that was 21,000 tons of antibiotics in 2015 [7] (Fig. 1). The majority of veterinary antibiotics are destined for pig production worldwide [8].

The presence of pharmaceutical antibiotics in terrestrial and aquatic systems has increased concerns [9, 10] of the potential risks of promoting bacterial resistance [11, 12] and contamination [13]. In addition, these antibiotic residues may have toxic effects on marine and terrestrial ecosystems due to the potential physiological effects on organisms. These negative effects were not given enough attention in the past; leading to concerns of antibiotics has been primarily on farms use antibiotics [14]. The antibiotic residues are released into the environment through manure and urine [15, 16] either as its original form or as metabolites that have similar biological activities as its original form [17, 18]. Figure 2 shows the typical route of the antibiotics once they enter a farm. Antibiotics are poorly absorbed in the animal's intestine, and approximately 40–90% are excreted as parenteral compounds or metabolites into water or soil [8, 19].

According to different studies, antibiotics can accumulate in the soil for indeterminate periods of time when their entry rate exceeds the dispersion rate [20]. For example, accumulated antibiotics were detected for a period of up to 20 years in Mexican soil [21]. Similarly, antibiotics have been found in the mangrove areas located in the provinces of Thai Binh, Nam Dinh, and Ca Mau and in the forests of Can Gio in Vietnam. It has been reported that 0.73 g kg^{-1} of trimethoprim, 0.82 g kg^{-1} of sulfamethoxazole, and 2.62 g kg^{-1} of norfloxacin were detected in mud samples in these locations [22] as well as in other parts of the world (Table 1).

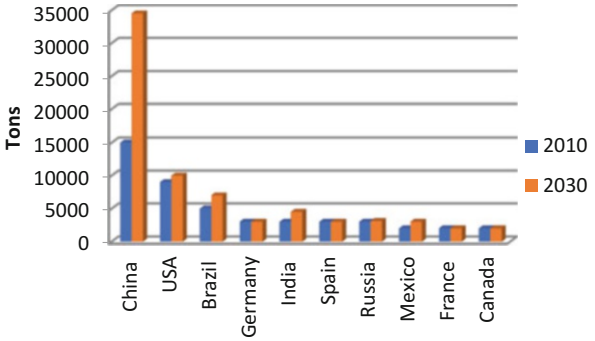


Fig. 1 Consumption of antibiotics in the livestock industry by the top ten countries in 2010–2030 (projected for 2030) [6]

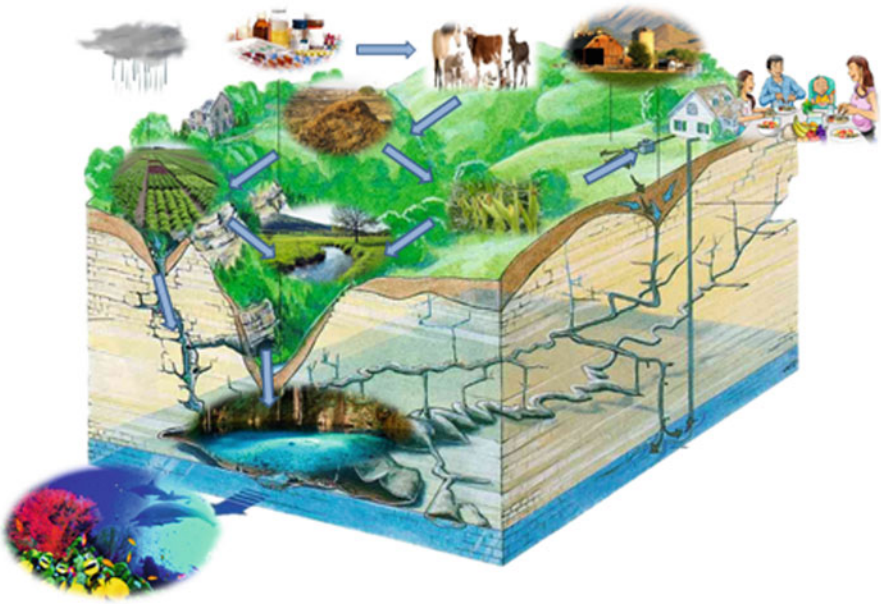


Fig. 2 Route of the antibiotics, from their entrance to a farm to their excretion to the environment through manure system. Antibiotic residues could be incorporated into the fields and plants, as well as enter into the aquifer

Therefore, the aim of the present review is to provide an overview of the fate of antibiotics in the environment, the time of degradation, and the potential damage to ecosystems.

2 Destination of Antibiotics in the Soil

Antibiotics have been globally implemented in animal feed to treat disease, improve resistance to disease, and promote growth since 1950 [39–41]. In 2013, 48,400 tons of antibiotics were used in China, mainly for swine production, of which 49.5% was discharged to the environment after being metabolized by the animal [42]. It is estimated that the Chinese swine industry generated 3190 million tons of manure every year [43].

The residual levels of veterinary pharmaceutical products are generally higher in the soil than in the water. For instance, it has been reported that the concentration of tetracycline was 900 μg/kg in soil but 0.4 μg/L in water, and the levels of ciprofloxacin were 52 μg/kg in soil but 0.4 μg/L in water [15].

Not all antibiotics found in environmental samples have detrimental effects on the ecosystems and agroecosystems [44]. It is important to identify which ones are harmful. Antibiotics' persistence in the environment can vary due to its half-life, which is affected by several factors such as water pH, temperature, geographical

Table 1 Antibiotics found in samples of soil, mud, and manure

Family	Name	Concentration	Localization	Country	Reference
Quinolones	Norfloxacin	0.43 g/ kg	Mud	Vietnam	Tuan and Munekage [22]
	Norfloxacin	225.45 mg/kg	Manure	China	Zhao et al. [23]
	Ofloxacin	1.6 μg/kg	Soil	China	Hu et al. [24]
Fluoroquinolones		0.13–0.75 mg/kg	Manure	Austria	Martínez Cerballo et al. [25]
		33. 56 μg kg	Soil	China	Shi et al. [26]
	Ciprofloxacin	808.3 μg/kg	Manure	China	Hou et al. [27]
		75.6 μg/kg	Manure	China	Ma and Chen, [28]
		0.1 mg kg	Soil	China	Hu et al. [24]
	Enrofloxacin	1420.76 mg/kg	Manure	China	Zhao et al. [23]
		140.71 μg/kg	Soil	China	Ma and Chen [28]
Sulfonamides	Sulfamethoxazole	2.62 g/ kg	Mud	Vietnam	Tuan and Munekage [22]
		18.9 μg/kg	Manure	China	Hou et al. [27]
		0.9 μg/kg	Soil	China	Hu et al. [24]
	Sulfachloropyridazine	34.9 μg/kg	Manure	China	Hou et al. [27]
	Sulfadimidine	1–2 mg/kg	Manure	Germany	Chistian et al. [29]
	Sulfadoxine	9.1 mg/kg	Soil	China	Hu et al. [24]

Trimethoprim		0.82 g/ kg	Mud	Vietnam	Tuan and Munekage [22]
Tetracyclines	Chlortetracycline	2668.9 μg/kg	Manure	China	Hou et al. [27]
		754.4 mg/kg	Manure	China	Pan et al. [30]
		0.039 mg/kg	Soil	Germany	Hamschert et al. [31]
		0.88 μg/kg	Soil	Korea	Awad et al. [32]
		1079 μg/kg	Soil	China	Hu et al. [24]
		0.19–208.9 mg kg	Soil	China	Lin et al. [33]
		119–307 9 mg kg	Soil	China	Xie et al. [34]
		11,440 μg/kg	Soil	China	Tang et al. [35]
	Oxytetracycline	0.3 mg/kg	Soil	UK	Boxall et al. [36]
		183.5 mg kg	Soil	China	Hu et al. [24]
		2690 μg/kg	Soil	China	Tang et al. [35]
		397.6 μg kg	Soil	China	Zhang et al. [37]
Macrolides	Tylosin	1.4 μg/kg	Manure	China	Hou et al. [27]
		0.0–37.1 μg/kg	Manure	Italy	Liguoro et al. [38]
Nitrofurans		85.1 μg/kg	Manure	China	Hou et al. [27]

location, etc. [45]. Persistence is also influenced by the animal species from which they come, their respective nutrition and intestinal microbiota [21, 23]. Some antibiotics, such as penicillin, are easily degraded in a matter of hours or few days. Other antibiotics, such as macrolides (i.e., tylosin), fluoroquinolones (i.e., ciprofloxacin), and tetracyclines, may persist for several months or even years. Those antibiotics will be more harmful to the environment due to the greater accumulation [46]; some antibiotics can even be transformed from the metabolites back into the original compound [47].

Veterinary antibiotics have been monitored in manure or sludge that is routinely applied to crops, which is the first stage of degradation or transformation when interacting with microorganisms [48, 49]. A process known as "sequestration" can be presented, which is given by kinetic absorption and diffusion in micro- and nanopores that are too small for microorganisms' enzymes to remove antibiotics temporarily from contact and biological absorption; due to this process the antibiotics decrease over the time, but there is a transient storage for time not yet defined [21, 50].

Antibiotics are currently considered as emerging contaminants [51], which can affect the structure and function of indigenous bacterial communities and increase the abundance of antibiotic-resistant bacteria by stimulating the expression of resistant genes [52]. An experiment investigating the interaction of antibiotics and microorganisms found that the soil microorganisms could utilize some antibiotics as their organic substrates; however, the enzymatic activities of dehydrogenase and urease were obviously inhibited by the antibiotic over the time [53].

In addition, insufficient attentions has been presented to the effect caused by antibiotics in the soil on the bacterial communities responsible for anoxic denitrification [54], which is due to the very short half-life (hours to months) of the drug in anoxic soils [8, 55]. One study showed that the presence of antibiotics could inhibit denitrification and the activity of denitrifying genes in soils [56]. Photo degradation of antibiotics or metabolites is unlikely to occur in soils, because light penetration is limited [57]. However, runoff and transport facilitated by particles can dissipate all antibiotics in the environment [58, 59].

Vertical leaching or percolation in groundwater occurs mainly in preferential flow paths; however, it is restricted to a few antibiotics such as sulfonamides; therefore most of the antibiotics applied with manure to soil is retained in the surface soil [60, 61].

Plants absorb small amounts of antibiotics (Fig. 3). Kumar et al. [62] observed an accumulation in plant tissue (2–17 ng/g fresh weight) of chlortetracycline, and Grote et al. [63] found the same antibiotic in wheat grain (~44 ng/g). Therefore, the elimination of antibiotics from the soil by means of plants absorption does not represent an effective alternative since the amount of absorption is very small. However, the antibiotics may cause risks to plants, because they may have toxic effects on the vegetal organism [64, 65].

These toxic effects vary from organism to organism. Migliore et al. [66] observed that the leaves and internodes of Lythrum salicaria absorb and accumulate antibiotics at low concentrations and present toxicity at high concentrations, while roots, cotyledons, and cotyledon petioles always exhibit toxic effects regardless of concentration. Migliore et al. [67] found that sulfadimethoxine at

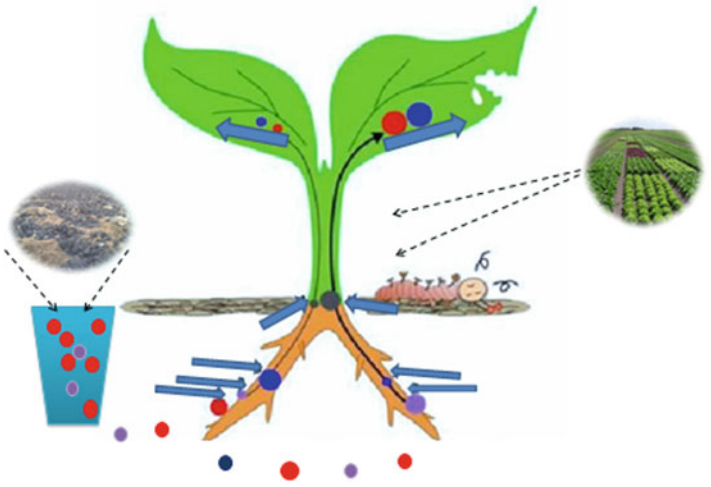


Fig. 3 Absorption of antibiotics in plants from roots to leaves

concentrations of 300 mg L^{-1} reduces the growth of leaves and roots in corn, peas, and millet. Overall, plant species, growth stage, stationary variations, and different antibiotics affect absorption, distribution, and accumulation in plants and soil microorganisms [17, 68].

3 Antibiotics in the Water

Since the 1940s, antibiotics have played an important role in human and animal health [69]. The discharge of wastewater is the major route for antibiotic residues to enter into the aquatic environment [70]. As a consequence, a fraction of these compounds ends up in the sea, which is the main receptor of terrestrial pollutants [71, 72]. Additionally, antibiotics are introduced in aquaculture through feed or water immersion en masse as treatment or prophylaxis of disease in farmed species. In aquatic ecosystems, cyanobacteria, an essential group of prokaryotic organisms which represent for the majority of the phytoplankton mass, are responsible for producing a large amount of free oxygen, in addition to fixing the carbon dioxide in marine habitats, terrestrial habitats, and fixation of atmospheric nitrogen [73, 74]. However, cyanobacteria are very sensitive to antibiotics, which increase the concern about the contamination of drugs in the aquatic habitat [44, 75].

The most commonly found antibiotics in water are quinolones, sulfonamides, and trimethoprim, which exceed 1 μg/L in environmental samples [76]. Those antibiotics have toxic effects even in very low concentrations, both through its active ingredients and additives used in its formulation [77]. It has been reported that the minimum inhibitory concentrations of 122 antibiotics in water ranges from 0.64 to 32,000 μg/L, whereas the unforeseen effect concentrations were between 0.008 and 64 μg/L [12].

The European Medicines Agency suggests that the concentration limit for surface water is of 10 ng/L, and it is not necessary to carry out further drug toxicity tests if the level is lower than 10 ng/L. However, there may be antibiotic resistance even at lower concentrations (<1 ng L^{-1}) [78, 79] (Table 2).

Organisms in the aquatic environment are generally not exposed to individual compounds but rather to mixture of various chemicals [77]. Magdaleno et al. [100] showed that the effects of the mixture were stronger than the effects of a single substance for green algae. Also, Long et al. [101] reported that there are synergistic effects between sulfonamides and sodium cefotaxime and antagonistic effect between sulfonamides and tetracyclines or potassium penicillin V.

4 Strategies to Control the Use of Antibiotics in the Livestock Industry

The use of antibiotics in animals cannot be demonized because they have protected public health and food safety by reducing the transmission of zoonotic pathogens [102], and judicious use of antibiotics is recognized as a way to sustain livestock production [103, 104]. However, the growing world population is leading to increased demand for animal protein, and this in turn is putting pressure on the chains of food supply systems, which have to implement antibiotics to meet their demands [1]. In addition, pharmaceutical manufacturing of antibiotics will continue as long as there is a market need, or until the discovery of new or more efficient alternatives, which in turn will have their challenges [17].

Strategies must be implemented to promote the judicious use of antibiotics in agriculture. Examples of these strategies are prohibiting and reducing the use of specific antibiotics; limiting their use to specific situations or conditions; avoiding use of antibiotics in regular patterns; purchasing and application antibiotics with licenses; developing partnerships between veterinarians and farmers; reducing the need for antibiotics by improving vaccination and using alternative growth enhancers; as well as improving animal husbandry, hygiene, and health management of farms [1, 17]. In the United States, use of in-feed antibiotics for production purpose in livestock is banned starting January 2017. In the state of California, beginning January 2018 medically important antibiotics cannot be administered to livestock unless ordered by a licensed veterinarian.

Application of vaccination programs, good hygiene, higher animal welfare practices, and a better feeding design can reduce the use of antibiotics in livestock production. A clear example being the some French animal nutrition companies, which produced 105,000 finishing pigs in 2016 for the antibiotic-free markets and its plan for 2017, is to increase to 155,000 and even 200,000 in 2018 [105].

The global organizations (FAO, WHO, OIE) as well as international governments work in close collaboration to promote a unique approach to the reduction in the use of antibiotics globally. The international partnerships are also striving to increase the awareness of using antibiotics and the effects of antibiotics on the ecosystems [1].

Table 2 Antibiotics found in water samples (adapted from Välitalo et al. [44])

Family	Name	Concentration (μg/L)	Localization	Country	Bibliography
Quinolones	Enrofloxacin	4.24	River water	China	Wei et al. [80]
		1.09	Effluent	China	Wei et al. [80]
	Ciprofloxacin	5.93	River water	China	Wei et al. [80]
		3.35	Effluent	China	Wei et al. [80]
	Norfloxacin	1.15	River water	Australia	Watkinson et al. [81]
		3.70	Effluent	China	Leung et al. [82]
	Ofloxacin	7.87	Effluent	China	Leung et al. [82]
		0.231	River water	France	Dinh et al. [83]
Sulfonamides	Sulfadiazine	0.002	Seawater	Greek	Alygizakis et al. [84]
		0.108	River water	Vietnam	Giang et al. [85]
		0.560	Effluent	China	Gao et al. [86]
	Sulphapyridine	0.378	Effluent	UK	Kasprzyk-Hordern et al. [87]
		0.142	River water	UK	Kasprzyk-Hordern et al. [88]
	Sulfamethazine	0.360	Groundwater	USA	Barnes et al. [89]
		0.009		China	Gao et al. [86]
		0.472	River water	USA	Bartelt-Hunt et al. [90]
	Sulfadimethoxine	0.002	Effluent	China	Dong et al. [91]
		0.003	River water	Luxemburg	Pailler et al. [92]
	Sulfamerazine	0.160	River water	USA	Yang and Carlson [93]
		0.004	Effluent	China	Dong et al. [91]
	Sulfamethoxazole	0.006	Sea water	Greek	Alygizakis et al. [84]
		13.765	River water	Kenya	Ngumba et al. [94]
		1.11	Groundwater	USA	Barnes et al. [89]
		3.336	Effluent	Kenia	Ngumba et al. [94]
	Sulfatiazole	0.60	Effluent	Australia	Watkinson et al. [81]

(*continued*)

Table 2 (continued)

Family	Name	Concentration (μg/L)	Localization	Country	Bibliography
		0.006	River water	USA	Bartelt-Hunt et al. [90]
	Sulfachloropyridazine	0.13	River water	USA	Yang and Carlson [93]
		0.02	Effluent	USA	Bartelt-Hunt et al. [90]
Trimethoprim		0.029	Sea water	Belgium	Wille et al. [95]
		2.65	River water	Kenya	Ngumba et al. [94]
		3.052	Effluent	UK	Kasprzyk-Hordern et al. [87]
Macrolides	Clarithromycin	0.001	Sea water	Greek	Alygizakis et al. [84]
		0.020	River water	Italy	Calamari et al. [96]
		1.89	Effluent	Slovakia	Birošová et al. [97]
	Erythromycin	3.98	River water	Spain	Rodríguez-Gil et al. [98]
	Erythromycin H_2O	2.841	River water	UK	Kasprzyk-Hordern et al. [87]
		4.33	Effluent	China	Leung et al. [82]
	Azithromycin	1.547	River water	USA	Bartelt-Hunt et al. [90]
		1.220	Effluent	Slovakia	Birošová et al. [97]
Penicillins	Amoxicillin	1.128	Sea water	Greek	Alygizakis et al. [84]
		0.622	River water	UK	Kasprzyk-Hordern et al. [88]

		1.67	Effluent	China	Leung et al. [82]
	Ampicillin	0.29	River water	Kenya	Kimosop et al. [99]
		0.79	Effluent	Kenya	Kimosop et al. [99]
Tetracyclines	Tetracycline	0.81	River water	China	Wei et al. [80]
		1.42	Effluent	China	Leung et al. [82]
	Chlortetracycline	2.42	River water	China	Wei et al. [80]
		0.28	Effluent	UK	Leung et al. [82]
	Oxytetracycline	2.20	River water	China	Watkinson et al. [81]
		0.842	Effluent	China	Yang and Carlson [93]
	Doxycycline	0.40	River water	Australia	Kasprzyk-Hordern et al. [88]
		0.34	Effluent	USA	Kasprzyk-Hordern et al. [87]
Nitroimidazoles	Metronidazole	0.014	River water	UK	Barnes et al. [89]
		0.421	Effluent	UK	Calamari et al. [96]
Lincosamides	Lincomycin	0.320	Groundwater	USA	Watkinson et al. [81]
		0.249	River water	Italy	Wei et al. [80]
		0.30	Effluent	Australia	Leung et al. [82]

5 Conclusion

The primary strategy is to control the use of antibiotics in animal production, followed by increase public and agricultural community's awareness of and promote judicious use of antibiotics in animals. If we start by providing farmers with the correct knowledge of use antibiotics in animals, they will have more alternatives to use antibiotics in animals, thus reducing antibiotics in the ecosystems and agroecosystems.

Acknowledgments This study was supported by the Red de Investigación en cambio climático, producción y salud del ganado bovino, and UAEM-CA-193-Ciencia e Innovación Tecnológica Pecuaria. Miss Robles Jimenez and Angeles Hernandez held a grant from the Consejo Nacional de Ciencia y Tecnologia (CONACyT).

References

1. FAO. Antimicrobial resistance in food and agriculture. (2017). Available from: http://www.fao.org/antimicrobial-resistance
2. Kümmerer K (2003) Significance of antibiotics in the environment. J Antimicrob Chemother 52:5–7. https://doi.org/10.1093/jac/dkg293
3. Kümmerer K (2009) Antibiotics in the aquatic environment – a review – part I. Chemosphere 75:417–434. https://doi.org/10.1016/j.chemosphere.2008.11.086
4. Food and Drug Administration, FDA (2012) The Green Book – FDA approved products. Food and Drug Administration, Rockville. Available from: http://www.accessdata.fda.gov/scripts/animaldrugsatfda
5. Kim KR, Owens G, Kwon SI, So KH, Lee DB, Ok YS (2011) Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. Water Air Soil Pollut 214:163–174. https://doi.org/10.1007/s11270-010-0412-2
6. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Laxminarayan R (2015) Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A 112 (18):5649–5654. https://doi.org/10.1073/pnas.1503141112
7. Li C, Chen J, Wang J, Ma Z, Han P, Luan Y, Lu A (2015) Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment. Sci Total Environ 521:101–107. https://doi.org/10.1016/j.scitotenv.2015.03.070
8. Solliec M, Roy-Lachapelle A, Gasser MO, Coté C, Genéreux M, Sauvé S (2016) Fractionation and analysis of veterinary antibiotics and their related degradation products in agricultural soils and drainage waters following swine manure amendment. Sci Total Environ 543:524–535. https://doi.org/10.1016/j.scitotenv.2015.11.061
9. Zuccato E, Castiglioni S, Bagnati R, Melis M, Fanelli R (2010) Source, occurrence and fate of antibiotics in the Italian aquatic environment. J Hazard Mater 179:1042–1048. https://doi.org/10.1016/j.jhazmat.2010.03.110
10. Cardoso O, Porcher JM, Sanchez W (2014) Factory-discharged pharmaceuticals could be a relevant source of aquatic environment contamination: review of evidence and need for knowledge. Chemosphere 115:20–30. https://doi.org/10.1016/j.chemosphere.2014.02.004
11. Review on Antimicrobial Resistance (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance, London. Available from: https://amr-review.org
12. Bengtsson-Palme J, Larsson DGJ (2016) Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environ Int 86:140–149. https://doi.org/10.1016/j.envint.2015.10.015

13. Tello A, Austin B, Telfer TC (2012) Selective pressure of antibiotic pollution on bacteria of importance to public health. Environ Health Perspect 120:1100–1106. https://doi.org/10.1289/ehp.1104650
14. Dang B, Mao D, Xu Y, Luo Y (2017) Conjugative multi-resistant plasmids in Haihe River and their impacts on the abundance and spatial distribution of antibiotic resistance genes. Water Res 111:81–91. https://doi.org/10.1016/j.watres.2016.12.046
15. Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. Ecol Indic 8 (1):1–13. https://doi.org/10.1016/j.ecolind.2007.06.002
16. Chen YS, Zhang HB, Luo YM, Song J (2012) Occurrence and dissipation of veterinary antibiotics in two typical swine wastewater treatment systems in East China. Environ Monit Assess 184:2205–2217. https://doi.org/10.1007/s10661-011-2110-y
17. Tasho RP, Cho JY (2016) Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. Sci Total Environ 563–564:366–376. https://doi.org/10.1016/j.scitotenv.2016.04.140
18. Alexandrino DAM, Mucha AP, Almeida CMR, Gao W, Jia Z, Carvalho MF (2017) Biodegradation of the veterinary antibiotics enrofloxacin and ceftiofur and associated microbial community dynamics. Sci Total Environ 581–582:359–368. https://doi.org/10.1016/j.scitotenv.2016.12.141
19. Heuer H, Focks A, Lamshöft M, Smalla K, Matthies M, Spiteller M (2008) Fate of sulfadiazine administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in manure and manured soil. Soil Biol Biochem 40(7):1892–1900. https://doi.org/10.1016/j.soilbio.2008.03.014
20. Jechelke S, Heuer H, Siemens J, Amelung W, Smalla K (2014) Fate and effects of veterinary antibiotics in soil. Trends Microbiol 22(9):536–545. https://doi.org/10.1016/j.tim.2014.05.005
21. Dalkmann P, Broszat M, Siebe C, Willaschek E, Sakinc T, Huebner J, Amelung W, Grohmann E, Siemens J (2012) Accumulation of pharmaceuticals, enterococcus, and resistance genes in soils irrigated with wastewater for zero to 100 years in Central Mexico. PLoS One 7:e45397. https://doi.org/10.1371/journal.pone.0045397
22. Tuan XL, Munekage Y (2004) Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Vietnam. Mar Pollut Bull 49:922–929. PMID: 15556177
23. Zhao L, Dong YH, Wang H (2010) Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. Sci Total Environ 408:1069–1075. https://doi.org/10.1016/j.scitotenv.2009.11.014
24. Hu X, Zhou Q, Luo Y (2010) Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environ Pollut 158:2992–2998. https://doi.org/10.1016/j.envpol.2010.05.023
25. Martínez-Carballo E, González-Barreiro C, Scharf S, Gans O (2009) Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environ Pollut 148:570–579. https://doi.org/10.1016/j.envpol.2006.11.035
26. Shi Y, Gao L, Li W, Liu J, Cai Y (2012) Investigation of fluoroquinolones, sulfonamides and macrolides in long-term wastewater irrigation soil in Tianjin. China Bull Environ Contam Toxicol 89:857–861. https://doi.org/10.1007/s00128-012-0761-1
27. Hou J, Wan W, Mao D, Wang C, Mu Q, Qui S, Luo Y (2015) Occurrence and distribution of sulfonamides, tetracyclines, quinolones, macrolides, and nitrofurans in livestock manure and amended soils of northern China. Environ Sci Pollut Res 22:4545–4554. https://doi.org/10.1007/s11356-014-3632-y
28. Ma Y, Chen ZL (2007) Measurement of enrofloxacin remained in hog farms and their circumference. J Tradit Chinese Vet Med 6:11–16
29. Christian T, Schneider RJ, Färber HA, Skutlarek D, Meyer MT, Goldbach HE (2003) Determination of antibiotic residues in manure, soil, and surface waters. Acta Hydrochim Hydrobiol 31:36–44. https://doi.org/10.1002/aheh.200390014
30. Pan X, Qiang ZM, Ben WW, Chen MX (2011) Residual veterinary antibiotics in swine manure from concentrated animal feeding operations in Shandong Province, China. Chemosphere 84:695–700. https://doi.org/10.1016/j.chemosphere.2011.03.022

31. Hamscher G, Sczesny S, Hoper H, Nau H (2002) Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal Chem 74:1509–1518. https://doi.org/10.1021/ac015588m
32. Awad YM, Kim SC, EI-Azeem SAMA, Kim KH, Kim KR, Kim KJ, Jeon C, Lee SS, Ok YS (2014) Veterinary antibiotics contamination in water, sediment, and soil near a swine manure composting facility. Environ Earth Sci 71:1433–1440. https://doi.org/10.1007/s12665-013-2548-z
33. Lin D, Zhou Q, Xu Y, Chen C, Li Y (2012) Physiological and molecular responses of the earthworm (Eisenia fetida) to soil chlortetracycline contamination. Environ Pollut 171:46–51. https://doi.org/10.1016/j.envpol.2012.07.020
34. Xie X, Zhou Q, Lin D, Guo J, Bao Y (2010) Toxic effect of tetracycline exposure on growth, antioxidative and genetic indices of wheat (Triticum aestivum L.). Environ Sci Pollut Res 18 (4):566–575. https://doi.org/10.1007/s11356-010-0398-8
35. Tang X, Lou C, Wang S, Lu Y, Liu M, Hashmi MZ, Liang X, Li Z, Liao Y, Qin W, Fan F, Xu J, Brookes PC (2015) Effects of long-term manure applications on the occurrence of antibiotics and antibiotic resistance genes (ARGs) in paddy soils: Evidence from four field experiments in south of China. Soil Biol Biochem 90:179–187. https://doi.org/10.1016/j.soilbio.2015.07.027
36. Boxall ABA, Johnson P, Smith EJ, Sinclair CJ, Stutt E, Levy LS (2006) Uptake of veterinary medicines from soils into plants. J Agric Food Chem 54:2288–2297
37. Zhang H, Zhou Y, Huang Y, Wu L, Liu X, Luo Y (2016) Residues and risks of veterinary antibiotics in protected vegetable soils following application of different manures. Chemosphere 152:229–237. https://doi.org/10.1021/jf053041t
38. De Liguoro M, Cibin V, Capolongo F, Halling-Sørensen B, Montesissa C (2003) Use of oxytetracycline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. Chemosphere 52:203–212. https://doi.org/10.1016/S0045-6535(03)00284-4
39. Knapp CW, Dolfing J, Ehlert PAI, Graham DW (2010) Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environ Sci Technol 44:580–587. https://doi.org/10.1021/es901221x
40. Ogle M (2013) In meat we trust: an unexpected history of carnivore America. Houghton Mifflin Harcourt, Boton. ISBN 978-0151013401
41. Von Nussbaum F, Brands M, Hinzen B (2006) Medicinal chemistry of antibacterial natural products exodus or revival? Angew Chem Int Ed 45:5072–5129. https://doi.org/10.1002/anie.200600350
42. Zhang QQ, Ying GG, Pan CG, Liu YS, Zhao JL (2015) Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ Sci Technol 49:6772–6782. https://doi.org/10.1021/acs.est.5b00729
43. Wang FH, Ma WQ, Dou ZX, Ma L, Liu XL, Xu JX, Zhang FS (2006) The estimation of the production amount of animal manure and its environmental effect in China. China Environ Sci 26:614–617. https://doi.org/10.3321/j.issn:1000-6923.2006.05.024
44. Välitalo P, Kruglova A, Mikola A, Vahala R (2017) Toxicological impacts of antibiotics on aquatic micro-organisms: a mini-review. Int J Hyg Environ Health 220(3):558–569. https://doi.org/10.1016/j.ijheh.2017.02.003
45. Mitchell SM, Ullman JL, Teel AL, Watts RJ (2014) pH and temperature effects on the hydrolysis of three β-lactam antibiotics: ampicillin, cefalotin and cefoxitin. Sci Total Environ 466:547–555. https://doi.org/10.1016/j.scitotenv.2013.06.027
46. Lin JS, Pan HY, Liu SM, Lai HT (2010) Effects of light and microbial activity on the degradation of two fluoroquinolone antibiotics in pond water and sediment. J Environ Sci Health B 45:456–465. https://doi.org/10.1080/03601231003800222
47. Lamshöft M, Sukul P, Zühlke S, Spiteller M (2010) Behaviour of ^{14}C-sulfadiazine and ^{14}C-difloxacin during manure storage. Sci Total Environ 408:1563–1568. https://doi.org/10.1016/j.scitotenv.2009.12.010

48. Ramaswamy J, Prasher SO, Patel RM, Hussain SA, Barrington SF (2010) The effect of composting on the degradation of a veterinary pharmaceutical. Bioresour Technol 101 (7):2294–2299. https://doi.org/10.1016/j.biortech.2009.10.089
49. Walters E, McClellan K, Halden RU (2010) Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids–soil mixtures in outdoor mesocosms. Water Res 44:6011–6020. https://doi.org/10.1016/j.watres.2010.07.051
50. Zarfl C, Klasmeier J, Matthies M (2009) A conceptual model describing the fate of sulfadiazine and its metabolites observed in manure-amended soils. Chemosphere 77:720–726. https://doi.org/10.1016/j.chemosphere.2009.08.035
51. Pruden A, Arabi M, Storteboom HN (2012) Correlation of upstream human activities with riverine antibiotic resistance genes. Environ Sci Technol 46:11541–11549. https://doi.org/10.1021/es302657r
52. Aydin S, Ince B, Ince O (2016) Assessment of anaerobic bacterial diversity and its effects on anaerobic system stability and the occurrence of antibiotic resistance genes. Bioresour Technol 207:332–338. https://doi.org/10.1016/j.biortech.2016.01.080
53. Liu B, Li Y, Zhang X, Wang J, Gao M (2015) Effects of chlortetracycline on soil microbial communities: comparisons of enzyme activities to the functional diversity via biolog EcoPlates™. Eur J Soil Biol 68:69–76. https://doi.org/10.1016/j.ejsobi.2015.01.002
54. Yan C, Dinh QT, Chevreuil M, Garnier J, Roose-Amsaleg C, Labadie P, Laverman AM (2013) The effect of environmental and therapeutic concentrations of antibiotics on nitrate reduction rates in river sediment. Water Res 47:3654–3662
55. Pan M, Chu LM (2015) Adsorption and degradation of five selected antibiotics in agricultural soil. Sci Total Environ 545–546:48–56. https://doi.org/10.1016/j.watres.2013.04.025
56. Sun M, Ye M, Liu K, Schwab AP, Liu M, Jiao J, Feng Y, Wan J, Tian D, Wu J, Hu F, Jiang X (2017) Dynamic interplay between microbial denitrification and antibiotic resistance under enhanced anoxic denitrification condition in soil. Environ Pollut 222:583–591. https://doi.org/10.1016/j.envpol.2016.10.015
57. Oszaki N, Bester K, Møldruo P, Henriksen K, Komatsu T (2011) Photodegradation of the synthetic fragrance OTNE and the bactericide triclosan adsorbed on dried loamy sand – results from models and experiments. Chemosphere 83:1475–1479. https://doi.org/10.1016/j.chemosphere.2011.03.006
58. Joy SR, Bartelt-Hunt SL, Snow DD, Gilley JE, Woodbury BL, Parker DB, Marx DB, Li X (2013) Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. Environ Sci Technol 47:12081–12088. https://doi.org/10.1021/es4026358
59. Popova IE, Bair DA, Tate KW, Parikh SJ (2013) Sorption, leaching, and surface runoff of beef cattle veterinary pharmaceuticals under simulated irrigated pasture conditions. J Environ Qual 42:1167–1175. https://doi.org/10.2134/jeq2013.01.0012
60. Blackwell PA, Kay P, Ashauer R, Boxall ABA (2009) Effects of agricultural conditions on the leaching behaviour of veterinary antibiotics in soils. Chemosphere 75:13–19. https://doi.org/10.1016/j.chemosphere.2008.11.070
61. Ostermann A, Siemens J, Welp G, Xue Q, Lin X, Liu X, Amelung W (2013) Leaching of veterinary antibiotics in calcareous Chinese croplands. Chemosphere 91:928–934. https://doi.org/10.1016/j.chemosphere.2013.01.110
62. Kumar K, Gupta SC, Baidoo SK, Chander Y, Rosen CJ (2005) Antibiotic uptake by plants from soil fertilized with animal manure. J Environ Qual 34:2082–2085. https://doi.org/10.2134/jeq2005.0026
63. Grote M, Schwake-Anduschus C, Michel R, Stevens H, Heyser W, Langenkämper G, Betsche T, Freitag M (2007) Incorporation of veterinary antibiotics into crops from manured soil. Landbauforschung Völkenrode FAL Agric Res 57(1):25–32
64. Rosendahl I, Siemens J, Groeneweg J, Linzbach E, Laabs V, Herrmann C, Vereecken H, Amelung W (2011) Dissipation and sequestration of the veterinary antibiotic sulfadiazine and its metabolites under field conditions. Environ Sci Technol 45:5216–5222. https://doi.org/10.1021/es200326t

65. Rosendahl I, Siemens J, Kindler R, Groeneweg J, Zimmermann J, Czerwinski S, Lamshöft M, Laabs V, Wilke BM, Vereecken H, Amelung W (2012) Persistence of the fluoroquinolone antibiotic difloxacin in soil and lacking effects on nitrogen turnover. J Environ Qual 41:1275–1283. https://doi.org/10.2134/jeq2011.0459
66. Migliore L, Rotini A, Cerioli NL, Cozzolino S, Fiori M (2010) Phytotoxic sulfadimethoxine elicits a complex hormetic response in the weed Lythrum salicaria L. Dose-Response 8 (4):414–427. https://doi.org/10.2203/dose-response.09-033.Migliore
67. Migliore L, Civitareale C, Brambilla G, Cozzolino S, Casoria P, Gaudio L (1997) Effect of sulphadimethoxine on cosmopolitan weeds (Amaranthus retroflexus L., Plantago major L., and Rumex acetosella L.). Agric Ecosyst Environ 65:163–168. https://doi.org/10.1016/S0167-8809(97)00062-5
68. Wang J, Lin H, Sun W, Xia Y, Ma J, Fu J, Zhang Z, Wu H, Qian M (2016) Variations in the fate and biological effects of sulfamethoxazole, norfloxacin and doxycycline in different vegetable-soil systems following manure application. J Hazard Mater 304:49–57. https://doi.org/10.1016/j.jhazmat.2015.10.038
69. Johnson AC, Keller V, Dumont E, Sumpter JP (2015) Assessing the concentrations and risks of toxicity from the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim and erythromycin in European rivers. Sci Total Environ 1(511):747–755. https://doi.org/10.1016/j.scitotenv.2014.12.055
70. Venkatesan AK, Halden RU (2014) Wastewater treatment plants as chemical observatories to forecast ecological and human health risks of manmade chemicals. Sci Rep 4:37–31. https://doi.org/10.1038/srep03731
71. Zhang R, Tang J, Li J, Cheng Z, Chaemfa C, Liu D, Zheng Q, Song M, Luo C, Zhang G (2013) Occurrence and risks of antibiotics in the coastal aquatic environment of the yellow sea, North China. Sci Total Environ 450-451:197–204. https://doi.org/10.1016/j.scitotenv.2013.02.024
72. Verlicchi P, Al Aukidy M, Zambello E (2012) Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment – a review. Sci Total Environ 429:123–155. https://doi.org/10.1016/j.scitotenv.2012.04.028
73. Berman-Frank I, Lundgren P, Falkowski P (2003) Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. Res Microbiol 154(3):157–164. https://doi.org/10.1016/S0923-2508(03)00029-9
74. Brandt KK, Amézquita A, Backhaus T, Boxall A, Coors A, Heberer T, Lawrence JR, Lazorchak J, Schönfeld J, Snape JR, Zhu YG, Topp E (2015) Ecotoxicological assessment of antibiotics: a call for improved consideration of microorganisms. Environ Int 85:189–205. https://doi.org/10.1016/j.envint.2015.09.013
75. Guo J, Selby K, Boxall AB (2016) Effects of antibiotics on the growth and physiology of chlorophytes, cyanobacteria, and a diatom. Arch Environ Contam Toxicol 71:589–602. https://doi.org/10.1007/s00244-016-0305-5
76. Carvalho IT, Santos L (2016) Antibiotics in the aquatic environments: a review of the European scenario. Environ Int 94:736–757. https://doi.org/10.1016/j.envint.2016.06.025
77. Gonzalez-Pleiter M, Gonzalo S, Rodea-Palomares I, Leganes F, Rosal R, Boltes K, Marco E, Fernandez-Piñas F (2013) Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: implications for environmental risk assessment. Water Res 47:2050–2064. https://doi.org/10.1016/j.watres.2013.01.020
78. Directive 2008/105/EC (2008) Environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and Amending Directive 2000/60/EC. Off J Eur Union L 348: 84–97
79. Grenni P, Ancona V, Anna Barra AC (2017) Ecological effects of antibiotics on natural ecosystems: A review. Microchem J 136:25–39. https://doi.org/10.1016/j.microc.2017.02.006
80. Wei R, Ge F, Chen M, Wang R (2012) Occurrence of ciprofloxacin, enrofloxacin, and florfenicol in animal wastewater and water resources. J Environ Qual 41(5):1481–1486. https://doi.org/10.2134/jeq2012.0014

81. Watkinson AJ, Murby EJ, Kolpin DW, Costanzo SD (2009) The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. Sci Total Environ 407:2711–2723. https://doi.org/10.1016/j.scitotenv.2008.11.059
82. Leung HW, Minh TB, Murphy MB, Lam JC, So MK, Martin M, Lam KSP, Richardson BJ (2012) Distribution, fate and risk assessment of antibiotics in sewage treatment plants in Hong Kong, South China. Environ Int 42:1–9. https://doi.org/10.1016/j.envint.2011.03.004
83. Dinh QT, Alliot F, Moreau-Guigon E, Eurin J, Chevreuil M, Labadie P (2011) Measurement of trace levels of antibiotics in river water using on-line enrichment and triple-quadrupole LC–MS/MS. Talanta 85(3):1238–1245. https://doi.org/10.1016/j.talanta.2011.05.013
84. Alygizakis NA, Gago-Ferrero P, Borova VL, Pavlidou A, Hatzianestis I, Thomaidis NS (2016) Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. Sci Total Environ 541:1097–1105. https://doi.org/10.1016/j.scitotenv.2015.09.145
85. Giang CND, Sebesvari Z, Renaud F, Rosendahl I, Minh QH, Amelung W (2015) Occurrence and dissipation of the antibiotics sulfamethoxazole, sulfadiazine, trimethoprim, and Enrofloxacin in the Mekong Delta. Vietnam PloS one 10(7):e0131855. https://doi.org/10.1371/journal.pone.0131855
86. Gao L, Shi Y, Li W, Niu H, Liu J, Cai Y (2012) Occurrence of antibiotics in eight sewage treatment plants in Beijing. China Chemosphere 86(6):665–671. https://doi.org/10.1016/j.chemosphere.2011.11.019
87. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res 43:363–380. https://doi.org/10.1016/j.watres.2008.10.047
88. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2008) The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res 42:3498–3518. https://doi.org/10.1016/j.watres.2008.04.026
89. Barnes KK, Kolpin DW, Furlong ET, Zaugg SD, Meyer MT, Barber LB (2008) A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States – (I) groundwater. Sci Total Environ 402(2):192–200. https://doi.org/10.1016/j.scitotenv.2008.04.028
90. Bartelt-Hunt SL, Snow DD, Damon T, Shockley J, Hoagland K (2009) The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. Environ Pollut 157(3):786–791. https://doi.org/10.1016/j.envpol.2008.11.025
91. Dong H, Yuan X, Wang W, Qiang Z (2016) Occurrence and removal of antibiotics in ecological and conventional wastewater treatment processes: a field study. J Environ Manag 178:11–19. https://doi.org/10.1016/j.jenvman.2016.04.037
92. Pailler JY, Krein A, Pfister L, Hoffmann L, Guignard C (2009) Solid phase extraction coupled to liquid chromatography-tandem mass spectrometry analysis of sulfonamides, tetracyclines, analgesics and hormones in surface water and wastewater in Luxembourg. Sci Total Environ 407(16):4736–4743. https://doi.org/10.1016/j.scitotenv.2009.04.042
93. Yang S, Carlson K (2003) Evolution of antibiotic occurrence in a river through pristine, urban and agricultural landscapes. Water Res 37:4645–4656. https://doi.org/10.1016/S0043-1354(03)00399-3
94. Ngumba E, Gachanja A, Tuhkanen T (2016) Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River basin, Kenya. Sci Total Environ 539:206–213. https://doi.org/10.1016/j.scitotenv.2015.08.139
95. Wille K, Noppe H, Verheyden K, Bussche JV, De Wulf E, Van Caeter P, Janssen CR, De Brabander HF, Vanhaecke L (2010) Validation and application of an LC-MS/MS method for the simultaneous quantification of 13 pharmaceuticals in seawater. Anal Bioanal Chem 397(5):1797–1808. https://doi.org/10.1007/s00216-010-3702-z
96. Calamari D, Zuccato E, Castiglioni S, Bagnati R, Fanelli R (2003) Strategic survey of therapeutic drugs in the rivers Po and Lambro in northern Italy. Environ Sci Technol 37:1241–1248. https://doi.org/10.1021/es020158e

97. Birošová L, Mackulak T, Bodík I, Ryba J, Škubák J, Grabic R (2014) Pilot study of seasonal occurrence and distribution of antibiotics and drug resistant bacteria in wastewater treatment plants in Slovakia. Sci Total Environ 490:440–444. https://doi.org/10.1016/j.scitotenv.2014.05.030
98. Rodríguez-Gil JL, Catalá M, Alonso SG, Maroto RR, Valcárcel Y, Segura Y, Molina R, Melero JA, Martínez F (2010) Heterogeneous photo-Fenton treatment for the reduction of pharmaceutical contamination in Madrid rivers and ecotoxicological evaluation by a miniaturized fern spores bioassay. Chemosphere 80(4):381–388. https://doi.org/10.1016/j.chemosphere.2010.04.045
99. Kimosop SJ, Getenga ZM, Orata F, Okello VA, Cheruiyot JK (2016) Residue levels and discharge loads of antibiotics in wastewater treatment plants (WWTPs), hospital lagoons, and rivers within Lake Victoria Basin. Kenya Environ Monit Assess 188(9):532. https://doi.org/10.1007/s10661-016-5534-6
100. Magdaleno A, Saenz ME, Juárez AB, Moretton J (2015) Effects of six antibiotics and their binary mixtures on growth of Pseudokirchneriella subcapitata. Ecotoxicol Environ Saf 113:72–78. https://doi.org/10.1016/j.ecoenv.2014.11.021
101. Long X, Wang D, Lin Z, Qin M, Song C, Liu Y (2016) The mixture toxicity of environmental contaminants containing sulfonamides and other antibiotics in Escherichia coli: differences in both the special target proteins of individual chemicals and their effective combined concentration. Chemosphere 158:193–203. https://doi.org/10.1016/j.chemosphere.2016.05.048
102. Hao H, Cheng G, Iqbal Z, Ai X, Hussain HI, Huang L, Dai M, Wang Y, Liu Z, Yuan Z (2014) Benefits and risks of antimicrobial use in foodproducing animals. Front Microbiol 5:288. https://doi.org/10.3389/fmicb.2014.00288
103. Economou V, Gousia P (2015) Agriculture and food animals as a source of antimicrobial resistant bacteria. Infect Drug Resist 8:49–61. https://doi.org/10.2147/IDR.S55778
104. Knoblock-Hahn A, Brown K, Medrow L (2016) A balanced approach to understanding the science of antibiotics in animal agriculture. J Acad Nutr Diet 116(8):1332–1335
105. Guillou D (2017) Key issues in designing feed for antibiotic-free pork. Pig Progress. Available from: https://www.pigprogress.net/. . ./Key-issues-in-designing-feed-for-antibiotic- free-pork-117625E/

Insecticide Residues in Foods

61

Ramesh Kumar Sharma

Contents

Abstract

The conventional agriculture which excessively employs synthetic insecticides for crop protection, sidelining traditionally known agri-friendly environmental factors like biodiversity and soil conservation, of course led to bumper crop yield in farms. But synthetic insecticide molecule residues in foods and water have posed serious threat to biosphere and public health. Therefore traditionally known agri-friendly and environmentally safe natural insecticides like *neem* (*Azadirachta indica*) leaf juice or *neem* oil are again in limelight in India.

The natural insecticides of pyrethrum, derris, ryania, sabadilla, and tobacco sources are worldwide considered to replace synthetic insecticides for crop protection in farms; and a new kind of agriculture called organic farming has

R. K. Sharma (✉)
Food and Environment Issues, Bikaner, Rajasthan, India

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_75

evolved. Still there does not seem ample scope for natural biomolecules of plant and bacterial origin with insecticidal property like azadirachtin, pyrethrins, spinosad, milbemycins, ryanodine, etc. perhaps due to limited legal acceptance to natural insecticides on account of their unfavorable effects in the human body.

Keywords
Anti-insecticide movement · Azadirachtin · Carbamates · Food Safety and Standards Regulation (FSSR) 2011 · Insecticides in food · Integral humanism · Organic farming

1 Introduction

The environmental factors, biodiversity and soil conservation, contribute to agricultural yield and crop or food quality; this traditionally well-known fact had been sidelined since the mid-twentieth century when Swiss scientist Paul Muller noticed in 1939 the insecticidal property of DDT (dichlorodiphenyltrichloroethane), the first discovered synthetic organic insecticide in 1873 by German scientist Ziedler [1]. With the increasing farm-level applications of synthetic insecticides to control pest population and protect crops, their polluting impact on environment – water, soil, and biosphere – and negative effect on public health soon became self-evident. The 1960s is described by environmentalists as the decade of anti-insecticide movement led by Rachel Carson (1907–1964) in the West, particularly the USA and the European countries [2]. Unfortunately that time when the Western world prepared itself for minimum synthetic insecticide applications at farm level, the eastern (including Indian) leadership was perhaps unaware of this fact and continued to use synthetic insecticides. In the same decade (1960s), Deen Dayal Upadhyay (1916–1968) came forward with the theory of integral humanism – humans are the integral part of biosphere, not of robotic network – questioning why synthetic insecticides should be applied in Indian farms, when US agriculture is going to eliminate those as much as possible [3]. Fortunately, nowadays all the governments, the world over, strongly wish to adopt organic farming (or bio farming, as called Jaivik Krushi in India) concerned with avoidance of synthetic insecticides and adoption of natural plant-based insecticides like *neem* (*Azadirachta indica*) leaf juice or *neem* oil. Synthetic insecticide molecules grouped as organochlorines, organophosphorus, and carbamates are nowadays facing stringent legal limitations, but still there does not seem a scope for biomolecules of plant and bacterial nature with insecticidal property like azadirachtin, nicotine sulfate, pyrethrins, rotenone, etc. for farm applications. It is essential for food processing industries to ensure that synthetic as well as natural insecticide contents in their food products are within the limits established by the food law (Food Safety and Standards Regulation, 2011 in India). However low-toxic natural insecticide molecules like azadirachtin, spinosad, and ryanodine are not found capable to get legal tolerance limit advantage of their high LD_{50} values and facilitate organic (or bio) farming.

2 Insecticide Molecules: Configurations

A human utility- and chemical configuration-oriented definition of insecticides, provided by the Condensed Chemical Dictionary (Reinhold Publishing Corporation, New York), is as follows:

> Chemical compounds which are used to control insects which are harmful, directly or indirectly, to man are called insecticides.

However this definition of insecticides is limited to chemical compounds with insecticide controlling property that are synthetically prepared or isolated from natural sources. With due consideration of holistic insecticide application of natural products, the definition might be revised as follows:

> The substances which are used to control insects that are harmful, directly or indirectly, to public health and professions mainly agriculture are called insecticides.

Farming is the essential human activity which utilizes insecticides to control insect population and protect crop yield. Insecticides (insect-controlling substances), fungicides (fungi-controlling substances), and herbicides (weed-controlling substances) are collectively called pesticides. Contrary to global demand for herbicides in high amounts, India's demand more for insecticides is worth mentioning. However India's share in global pesticide market is around 10% in financial year 2017 (CARE rating, 31 May 2017). Indian crop protection (pesticide) market has experienced story growth in the past and is expected to grow further at 12% per annum to reach at 6.8 billion US dollars in financial year 2017 (Tata Strategic Management Group; wap.business-staandard.com). India occupies the fifth place in the list of top 15 pesticide-exporting countries – after Germany USD 4 billion, China USD 3.7 billion, the USA USD 3.4 billion, and France USD 3.4 billion – with exports worth USD 2.1 billion, 6.7% share in global exports in financial year 2016 (World's Top Exporters, 28 July 2017).

The insecticide molecules which can protect crops from insects by killing them or damaging their biological system are capable to harm the entire biosphere due to their toxic nature. The water used in agriculture gets polluted with insecticide molecules and mixed in rivers and underground (well) water. This is considered a big factor in extinction of several creature species.

Despite being both organic and inorganic types, organic synthetic insecticides are more prevalent and might be classified in four main groups, namely, organochlorines, organophosphorus, carbamates, and pyrethroids. The natural insecticide molecules derived from plant and bacterial sources are structurally more complex and diverse in comparison to synthetic insecticides. They mostly are polynuclear compounds.

2.1 Synthetic Insecticide Molecules

The molecular configurations of synthetic insecticide molecules are as follows:

2.1.1 Organochlorines

The biodiversity and soil conservation contribute a lot to agricultural yield and crop quality; it is the traditionally known quintessence. But this important fact had been sidelined since the mid-twentieth century when Swiss scientist Paul Muller noticed in 1939 the insecticidal property of DDT (dichlorodiphenyltrichloroethane), the first discovered synthetic organic insecticide in 1873 by German scientist Ziedler [1].

DDT, BHC (benzene hexachloride), chlordane (octachloro methanoindene), aldrin (hexachloro dimethanonaphthalene), etc. belong to organochlorine class of synthetic insecticides. In the molecular configuration of organochlorine insecticides, there exist at least four chlorine atoms (Fig.1).

Organophosphorus

Since 1968 organophosphorus insecticides have been much more in use when those were claimed to be easily decomposed by Martin [4]. Structurally organophosphorus insecticides are phosphoric or phosphorothionic acid esters of various groups. Parathion (diethyl-p-nitrophenyl monothiophosphate), phosphamidon (dimethyl phosphoric acid ester with chlorodiethyl hydroxycrotonamide), and ethion (phosphorodithioic acid-SS-methylene-oooo-tetra ethyl ester) are some well-known examples of this category of synthetic insecticides (Fig. 2).

Carbamates

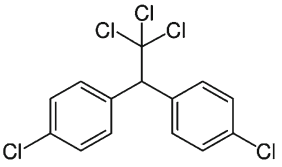
In 1980 Drum found that unlike organophosphorus insecticides, carbamates are insect species specific and reversible in the matter of inhibition of cholinesterase, an important enzyme present in the brain, nerve cells, and red blood cells capable of hydrolyzing acetylcholine to choline and acetic acid [5]. Since then, the carbamates, which are various groups of esters of amino acids, have been increasingly applied in farms. Examples are carbyl (naphthyl N-methylcarbamate), carbofuran (dimethyl benzofuranol methylcarbamate), etc. (Fig. 3).

2.1.2 Pyrethroids

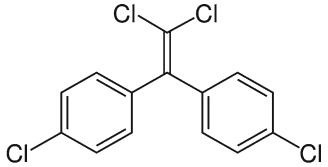
The synthetic analogues of pyrethrum insecticide (natural, obtained from pyrethrum flowers) molecules, called pyrethroids, have also been developed in 1980 onwards. According to Linde, despite powerful insecticidal action, pyrethroids are ecologically more unsafe than pyrethrum insecticides due to susceptibility to photochemical degradation [6]. Well-known pyrethroid structure molecule is 3-(2,2- dibromoethenyl)-2,2-dimethylcyclopropane carboxylic acid cyano (3-phenoxy phenyl) methyl ester known as deltamethrin or decamethrin (Fig. 4).

Miscellaneous Synthetic Insecticide Groups

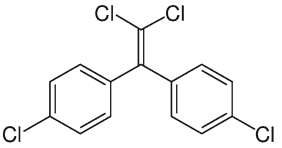
In addition to organochlorines, organophosphorus, carbamates, and pyrethroids, there are some more organic insecticides for crop protection such as phenoxyacetic acid and bipyridyls group of insecticides (Fig. 5).



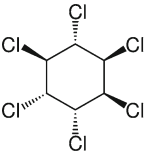
DDT: pp'-dichlorodiphenyltrichloroethane



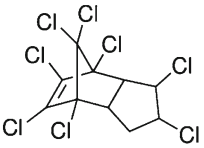
DDD: pp'-dichlorodiphenyldichloroethane



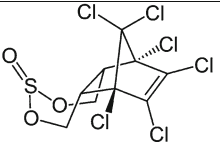
DDE:pp'- dichlorodiphenyldichloroethylene



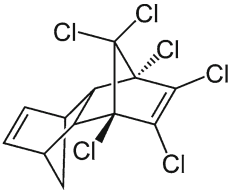
Lindane: gamma isomer of hexachloro-cyclohexane or benzene hexachloride (alpha and beta isomers are also used as insecticides)



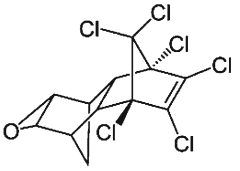
Chlordane:1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-lH-indene.



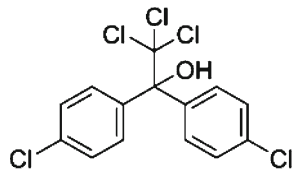
Endosulphan:1,4,5,6,7,7-hexachloro-5-nonbornene -2,3- dimethanol cyclic sulphite.



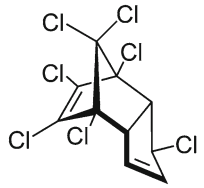
Aldrin:1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8- dimethanonaphthalene



Dieldrin:aldrin-6,7-monooxide

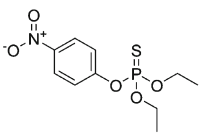


Dicofol: di (p-chlorophenyl) trichloromethyl carbinol (a chlorophenyl alcohol)

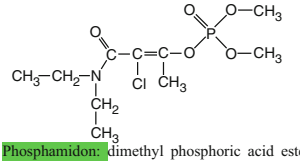


Heptachlor: 1H-1,4,5,6,7,8,8 heptachloro-3a,4,7,7a tetrahydro 4,7 methanoindene

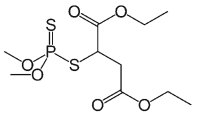
Fig. 1 Selected examples of organochlorine insecticides, with prevalent name and molecular formula/structure



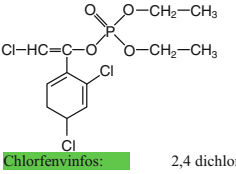
Parathion: di-methyl/ethyl-p-nitrophenyl monothiophosphate; locally known as Folidol



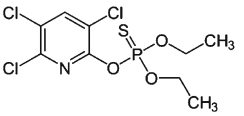
Phosphamidon: dimethyl phosphoric acid ester with 2-chloro-N,N-diethyl-3-hydroxy crotonamide.



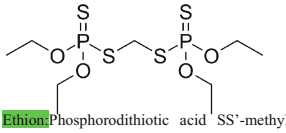
Malathion:diethyl mercaptosuccinate S-ester with O,O dimethyl phosphorothionate.



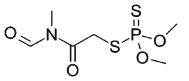
Chlorfenvinfos: 2,4 dichloro (dichloromethylene) benzyl alcohol diethyl phosphate



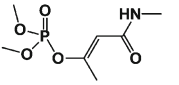
Chlorpyrifos: phosphorothiotic acid O,O diethyl O-(3,5,6 trichloro-2-pyridinyl) ester



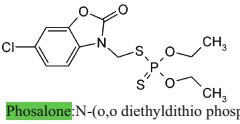
Ethion:Phosphorodithiotic acid SS'-methylene O,O,O',O'- tetra ethyl ester.



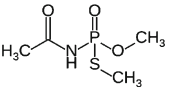
Formothion:Phosphorodithiotic acid, O,O-dimethyl ester, S-ester with N-formyl-2-mercapto-N-methyl acetamide.



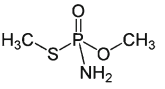
Monocrotophos:Phosphoric acid dimethyl ester, ester with 3-hydrory-N-methyl crotonamide



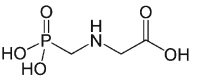
Phosalone:N-(o,o diethyldithio phosphoryl methyl) m-chlorobenzoxazolinone



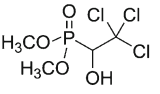
Acephate: acetylphosphoramidothiotic acid o,s-dimethyl ester.



Methamidophos: O,S-dimethyl phosphoramidothioate.

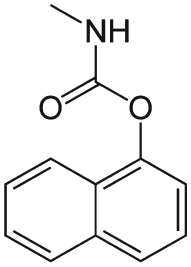


Glyphosate: Phospharomethyl aminoacetic acid.

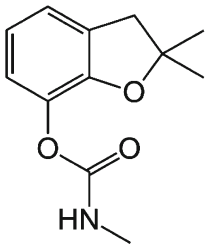


Trichlorfon: (2,2,2 trichloro-1-hydrory ethyl)-phosphoric acid dimethyl ester.

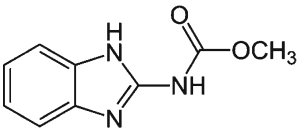
Fig. 2 Some of the leading organophosphorus insecticides with name and molecular formula/ structure



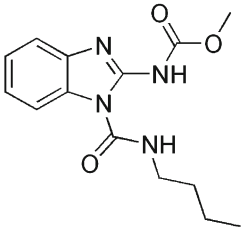
Carbaryl: 1-naphthyl N-methyl-carbamate



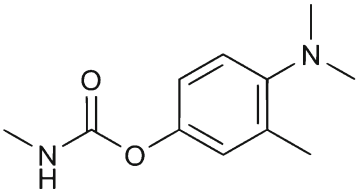
Carbofuran: 3 hydro -2,2 dimethyl -7-benzo furanol methyl carbamate.



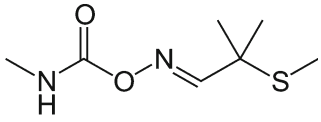
Carbendazim: 2-benzimidazolecarbamic acid methyl ester.



Benomyl: 1-(butylcarbamoyl)-2-benzimidazole carbamic acid methyl ester



Aminocarb: m-methyl p-dimethylamino-phenyl N-methyl earbamate.



Aldicarb: 2-methyl 2-(methylthio) propionaldehyde0-(methylcarbamoyl) oxime $CH_3SC(CH3)_2$ CH=NOCO-$NHCH_3$

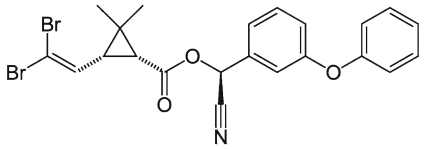
Fig. 3 A few examples of the important carbamate insecticides

2.2 Natural Insecticide Molecules

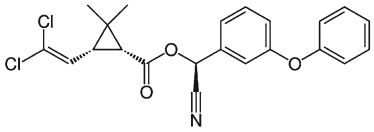
The natural insecticide molecules mostly are polynuclear compounds with structural diversity. Azadirachtin, pyrethrins, spinosads, milbemycins, rotenone, ryanodine, nicotine, etc. are the prominent natural insecticide molecules with configurations as follows:

2.2.1 Azadirachtin

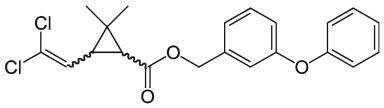
Azadirachtin is the insecticidal active ingredient of neem with oxidized tetra-nortriterpenoid molecular structure (Fig. 6) comprised of enol ether, acetal,



Deltamethrin **(Decamethrin):** 3-(2,2 dibromoethenyl)-2,2 dimethyl-cyclopropane carboxylic acid cyano (3-phenoxy phenyl) methyl ester.

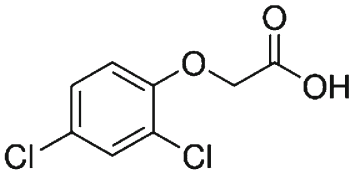


Cypermethrin: 3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropane-carboxylic acid cyano (3-phenoxy phenyl)-methyl ester.

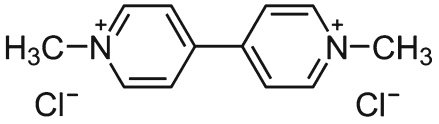


Permethrin: 3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanylacetic acid (3-phenoxy phenyl)-methyl ester.

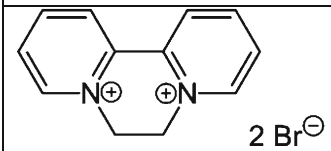
Fig. 4 A few prominent pyrethroid insecticides, developed as synthetic analogues of natural pyrethrum flower's active ingredients



2,4-D: 2,4-dichlorophenoxy acetic acid



Paraquat: 1,1' dimetyl-4,4'-bipyridium dichloride



Diquat: 1,1'-ethenyl -2,2' bipyridium dichloride

Fig. 5 Phenoxyacetic acid and bipyridyls group of insecticides

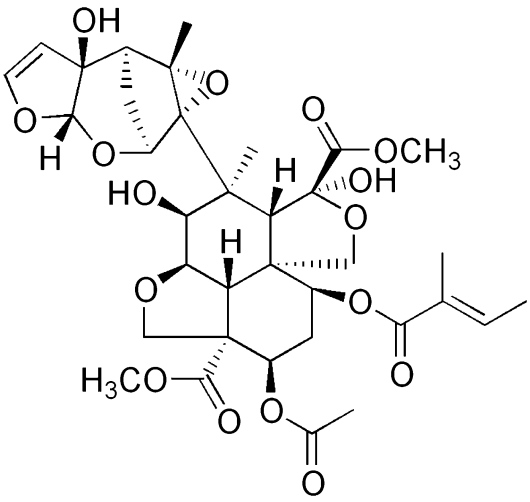


Fig. 6 Azadirachtin obtained from *A.indica*

hemiacetal, and tetra-substituted oxirane as well as a variety of carboxylic acid [7]. Neem leaf juice has been traditionally used in India for crop protection since centuries.

2.2.2 Pyrethrins

The widely legally accepted natural insecticides pyrethrins, extracted from pyrethrum (chrysanthemum cinerariaefolium) flowers, are pyrethrolone esters of chrysanthemum acids (monocarboxylic or dicarboxylic) [8]. These molecules could be structurally represented as follows (Fig.7):

2.2.3 Spinosads

The spinosad molecules derived from soil actinomycete represent tetracylic ring system attached to an amino sugar (D-forosamine) and neutral sugar (tri-O-methyl-L-rhamnose) [9].

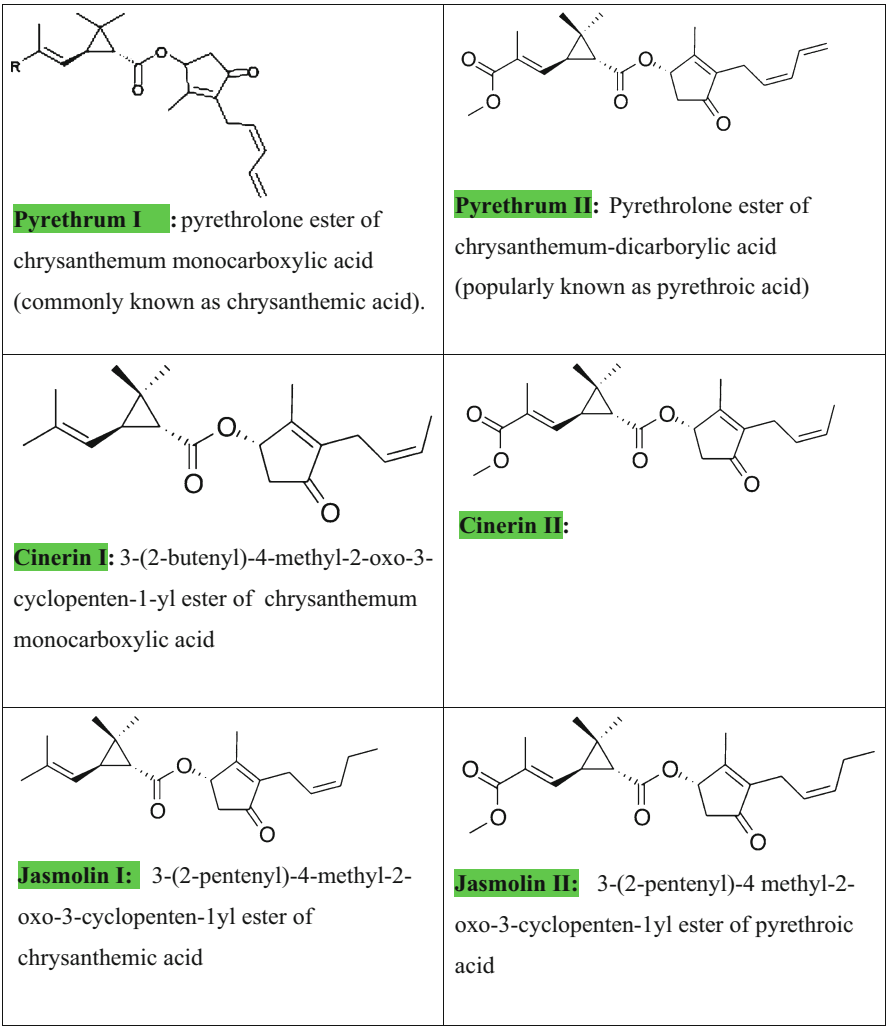
Spinosads, bioactive constituents isolated from the soil actinomycete, *Saccharopolyspora spinosa*, are known as spinosyn A and spinosyn D. These molecules could be structurally represented as follows (Fig.8):

2.2.4 Milbemycins and Avermectins

Avermectins and milbemycins isolated from *Streptomyces* sp. culture lead to diversely substituted pyran trioxy-tetracyclopentacosa tetraene ketonic configuration [10].

Milbemycins and avermectins are also called milbemectins and abamectins in mixtures. Milbemycin A4 has molecular structure as follows (Fig. 9):

6-ethyl-21′,24′ dihydroxy 5, 11′, 13′, 22′ tetramethyl 3, 4, 5, 6- tetrahydro -2h spiro [pyran -2,6′ –(3′, 7′, 19′) trioxy-tetracyclopentacosa (10, 14, 16, 22) tetraene)-2′ one (IUPAC name shortened by the author of the paper).



Pyrethrum I : pyrethrolone ester of chrysanthemum monocarboxylic acid (commonly known as chrysanthemic acid).

Pyrethrum II: Pyrethrolone ester of chrysanthemum-dicarborylic acid (popularly known as pyrethroic acid)

Cinerin I: 3-(2-butenyl)-4-methyl-2-oxo-3-cyclopenten-1-yl ester of chrysanthemum monocarboxylic acid

Cinerin II:

Jasmolin I: 3-(2-pentenyl)-4-methyl-2-oxo-3-cyclopenten-1yl ester of chrysanthemic acid

Jasmolin II: 3-(2-pentenyl)-4 methyl-2-oxo-3-cyclopenten-1yl ester of pyrethroic acid

Fig. 7 Six primary ingredients of pyrethrum flowers having insecticidal property are shown

2.2.5 Rotenone and Deguelin

The rotenone and deguelin molecules isolated from derris root and cube resin have benzopyrano furo benzopyranone and bis benzopyranopyranone configurations, respectively [11].

These molecules could be structurally represented as follows:

Rotenone: 1,2,12a,12b tetrahydro-8,9 dimethoxy-2-(1-methylethenyl)-benzopyrano furo [1] benzopyran-6-one

(IUPAC name shortened by author of paper) (Fig. 10).

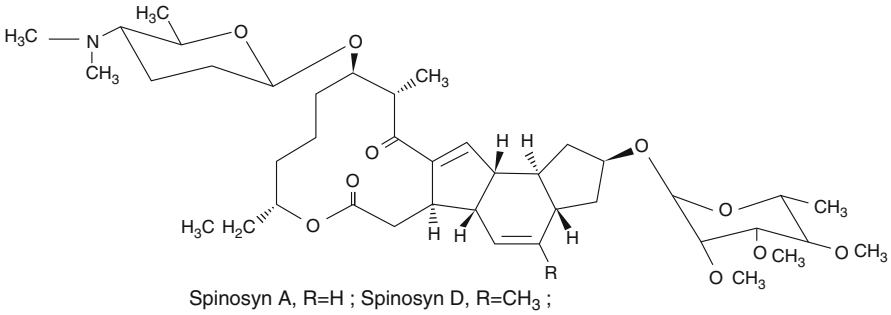
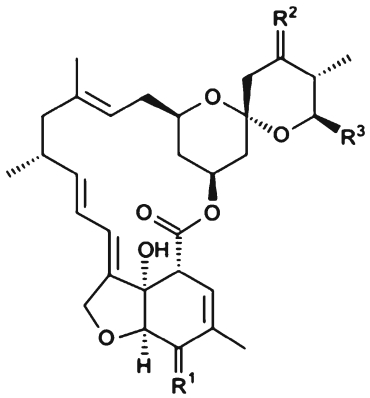


Fig. 8 Spinosad structure



Name	$=R^1$	$=R^2$	$-R^3$
Milbemectin	–H, (β)–OH	–H, –H	$-CH_3 : -CH_2CH_3 = 3:7$[2]
Milbemycin oxime	=NOH	–H, –H	$-CH_3 : -CH_2CH_3 = 3:7$[3]
Moxidectin	–H, (β)–OH	$=NOCH_3$	$(Z)-C(CH_3)=CH-CH(CH_3)_2$[1]
Nemadectin	–H, (β)–OH	–H, (α)–OH	$(Z)-C(CH_3)=CH-CH(CH_3)_2$[1]

Fig. 9 Milbectin and its derivatives

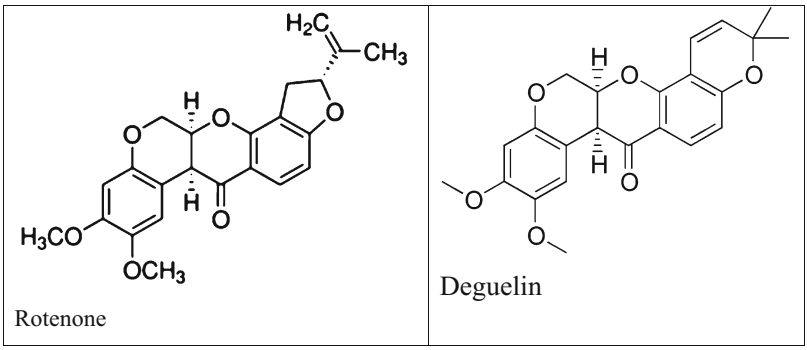


Fig. 10 Structure of rotenone and deguelin

Deguelin: 13a,13b dihydro-3,3-dimethyl-9,10-dimethoxy-3h bis[1] benzopyrano pyran-7-one

(IUPAC name shortened by author of the paper).

2.2.6 Ryanodine

Ryania insecticide obtained from roots and stems of South American shrub *Ryania speciosa* possesses the active ingredient ryanodine representing a complex polycyclic, polyhydroxylic diterpene structure with diversely substituted oxapentacyclo pentadecyl pyrrole-2-carboxylate configuration [12].

Ryanodine, the active ingredient of ryania insecticide derived from South American shrub *Ryania speciosa*, has the following molecular configuration (Fig. 11):

2,6,9,11,13,14-hexahydroxy-11-isopropyl-3,7,10-trimethyl-15-oxapentacyclo-pentadec-12-yl-1h-pyrrole-2′-carboxylate

(IUPAC name shortened by author of paper).

2.2.7 Nicotine

The molecular structure of nicotine derived from tobacco is as follows (Fig.12):

3′-(1-methyl-2-pyrrolidinyl)pyridine

[IUPAC name].

3 Social Concerns

The perception of harm to environment due to insecticides eventually led to anti-insecticide movement in the 1960s when Rachel Carson's popular book *Silent Spring* was published in 1962 [2]. In the same decade, the philosophy of integral humanism was propounded by Pandit Deen Dayal Upadhyay in India, probably in 1966–1967, for the sake of avoidance of anti-biosphere human activities, say use of insecticides [13].

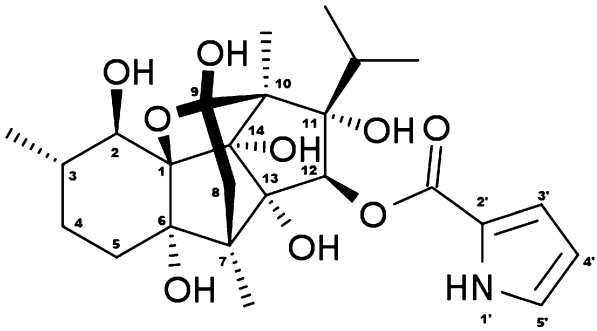


Fig. 11 Ryanodine isolated from *R. speciosa*

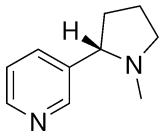


Fig. 12 Nicotine obtained from *Nicotiana tabacum*

3.1 Anti-insecticide Movement

Opposition to synthetic insecticides like DDT is considered to get strength with effect from 1962 when Rachel Carson's book *Silent Spring* was published. It is said that the public reaction to this best seller book, lucidly presenting arguments for how insecticides like DDT endanger wildlife and public health, launched a vigorous anti-insecticide movement. Carson's rationale for anti-biosphere action of insecticides, particularly DDT, was examined by US Science Advisory Committee, when President John F. Kennedy in 1963 ordered to investigate her claims [14]. Madam Carson passed away in 1964, but the anti-insecticide movement, her brain child, continued and led to ban on agricultural use of DDT in Hungary (1968), Cuba (1970), Norway (1970), West Germany (1970), and the USA (1972). Cuba exercised total ban on DDT including contagion control. Later on, total bans on DDT took place in several countries, the USA, Singapore, Chile, the UK, etc., in the 1980s (FAO/UNIP 1991) [15].

Rachel Carson (1907–1964) born on 27 May 1907 in Springdale, Pennsylvania, was a marine biologist, author, and environmentalist. Despite meeting fierce opposition by chemical companies, her book *Silent Spring* succeeded in spurring reversal in pesticide policies in US and Western nations. Carson mentioned a number of cases about toxic pesticide exposure, how exposed people fall ill and painfully die. She proved that these harmful chemicals move to succeeding generations of offspring through mother's milk and other biological processes. Her book also highlights the effects of pesticide spraying on wildlife in the environment. Carson believed in adopting biological control methods for insect population control as alternative to chemical spraying in farms. Her important quote is:

> It is an era dominated by industry in which the right to make dollar is seldom changed. This industry (insecticide industry) is a child of the Second World War. In the course of

> developing agents of chemical warfare some of the chemicals, created in the laboratory, were found lethal to insects. The discovery did not come by chance; insects were widely used to test chemicals as agents of death for men. [2]

Carson died on 14 April 1964 in Silver Spring, Maryland, USA.

3.2 Integral Humanism

In the 1960s, when the Western world began to prepare itself for minimum synthetic insecticide applications at farm level, unfortunately Indian leadership was perhaps unaware of this fact and continued to use synthetic insecticides like DDT and BHC. Questioning why synthetic insecticides be applied in Indian farms when US agriculture was going to eliminate those as much as possible, Pandit Deen Dayal Upadhyay (1916–1968) propounded the theory of integral humanism – humans are the integral part of biosphere, not of robotic network [13]. The postulates of integral humanism are [16]:

i. The humans are integral part of biosphere, not of robotic or mechanical network.
ii. The technical era is the most welcome, provided that innovations and advancements do not invade the biosphere nurtured by nature with the great sense of well-being.
iii. The biosphere – including humans – exists due to cooperation, not due to struggle (contrary to Darwin's survival of the fittest principle).
iv. Avoidance of anti-biosphere activities, say use of insecticides, and follow-up of technical approach toward human integrity to biosphere demand universal consumption patterns. That means humans being integral part of biosphere should consume the same kind (quality) of food and water and air which all the creatures do.

Deen Dayal Upadhyay, born on 25 September 1916 at Dhankya village of Jaipur district in Rajasthan, was a postgraduate in English literature and education. He is regarded as eminent sociologist, economist, historian, journalist, philosopher, and political leader (Bhartiya Jansangh leader). He was perhaps assassinated (thrown from compartment) on 11 February 1968 at Mughalsarai in UP, while traveling on train.

4 Legal Acceptances with Limitations for Insecticides

The Insecticide Act 1968, section 3, permits 890 insecticides for application in Indian farms. On the other hand, the FSSR 2011 enlists 199 insecticides, both synthetic and the natural, with commodity-wise acceptable maximum residue limits in agricultural products. However 219 insecticides (20 more to previously approved insecticides) are proposed in FSSR draft notification (August 2017), calling for suggestions, views, or comments from stakeholders, related to tolerance harmonization

of MRL (maximum residue limits) of insecticides. It means Indian farmers can use 890 insecticides enlisted in section 3 of Insecticide Act for crop protection, but food processing units should procure only those commodities which contain particular insecticide residues within specified limit as listed in Food Safety and Standards (Contaminants, Toxins, and Residues) Regulations 2011. The lethal dosage or LD_{50} value, measure of toxicity of insecticide or any substance, defined as the amount of insecticide at which 50% of test animals expire in the experiment conducted to judge its harmful effect on animals (mostly rats are employed, and LD_{50} is expressed as insecticide dosage milligrams per kilogram weight of test animals), obviously seems to be the basis of deciding the tolerance limit for an insecticide. But in fact, environmentally safe insecticides having high LD_{50} values whether synthetic like benomyl and carbendazim or natural like spinosad and azadirachtin are not capable to get high tolerance limit advantage. On the other hand, a few environmentally unsafe insecticides with low LD_{50} (high toxicity) value like DDT, carbyl, malathion, and dicofol (all synthetic) are advantaged of high tolerance limit in food law and therefore are more worth applying in farms. It means LD_{50} value is not the only criterion in deciding tolerance limit for an insecticide in food law. An attempt is made to understand LD_{50} tolerance limit correlation and analyze the graphical presentation.

4.1 LD_{50} V/S Tolerance Limit

The lethal dosage or LD_{50} value is the measure of toxicity of a substance where its construction kills 50% test animals. LD_{50} value is expressed as milligrams of substance per kilogram weight of animal employed in test. Normally rats, both the male and the female, are employed as test animals. LD_{50} value is experimentally determined by the scientists. Ethically it might be presumed that legally established tolerance limit for insecticide residues in food articles is proportional to their LD_{50} value. But truly it is not so. A graph of LD_{50} V/S tolerance limits (FSSR 2011) is shown here (Fig. 13).

It is worth mentioning that in this attempt of plotting tolerance limit – LD_{50} points – for insecticides, the former parameters belong to FSSR 2011, India, while the latter parameters generally belong to *Merck Index* [17], except in the cases of pyrethrins [18], azadirachtin [19], spinosad [20], rotenone (Agrochemical Handbook 1991, minimum value is taken) [21], nicotine [22], and ryanodine (EPA: archive) [23].

4.2 LD_{50} V/S Tolerance Limit: Analysis

A simple analysis of the graph LD_{50} oral mg/kg animal weight for female rat V/S tolerance limit (FSSR, India) in mg/kg in vegetables of insecticides reveals interesting facts. The graph is divided into four regions: (1) low LD_{50} (high toxicity), low tolerance limit (less worth applying) insecticides; (2) low LD_{50} (high toxicity), high tolerance limit (more worth applying) insecticides; (3) high LD_{50} (low toxicity), low tolerance limit (less worth applying) insecticides; and (4) high LD_{50} (low toxicity),

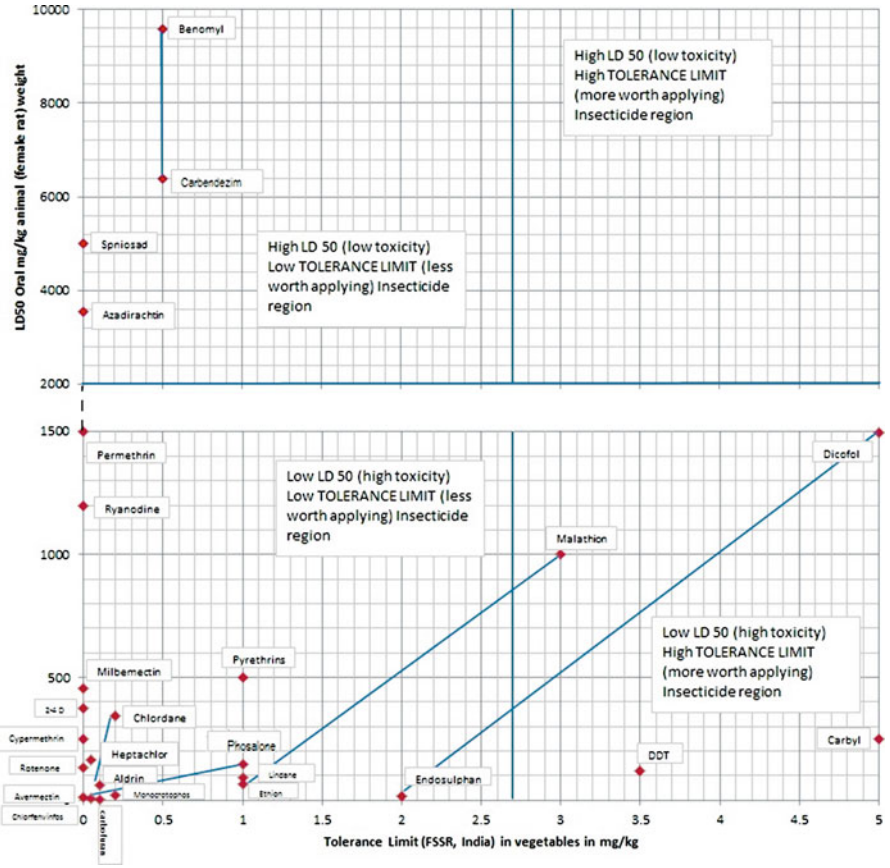


Fig. 13 A graphical representation of LD_{50} and FSSR tolerance limit (for vegetables) values of some of the tabulated insecticides

high tolerance limit (more worth applying) insecticides. The presumed dividing lines are $LD_{50} = 2000$ mg/kg animal weight and tolerance limit = 2.7 (FSSR) in mg/kg of vegetables. The main points of analysis are:

1. Most of the insecticides make a place in low LD_{50}, low tolerance limit region. Not only the most of popular synthetic organochlorines like aldrin, heptachlor, chlordane, and endosulfan, organophosphorus like monocrotophos, ethion, phosalone, and chlorfenvinphos, and pyrethroids like permethrin and cypermethrin but many natural insecticides like avermectins, rotenone, milbemycin, and ryanodine also fall in this region. It means most of the insecticides, both the synthetic and the natural, are much toxic and hence less applicable in the farms.
2. A few organophosphorus like malathion and dicofol and a few carbamates like carbyl and organochlorine DDT are placed in low LD_{50} (high toxicity), high tolerance limit (more worth applying region. Principle-wise high-toxic substances

should not be considered worth applying in farms. But sidelining the principle, Indian food regulation ascertains comparatively high MRL (maximum residue limit) or tolerance limit for these insecticides.

3. Most of the high LD_{50} (low toxicity) insecticides are considered less worth applying in the farms by the food lawmakers. Not only the synthetic environment-friendly carbamates like benomyl and carbendazim but natural low-toxic insecticides like spinosad and azadirachtin also occupy high LD_{50} (low toxicity), low tolerance limit (less worth applying) region.
4. Perhaps there is no such environment-friendly insecticide which is much more worth applying in farms in lawmakers' opinion. As such high LD_{50} (low toxicity), high tolerance limit (more worth applying) graphical region is quite empty.
5. Some of the insecticides are advantaged of tolerance limit proportionately to their LD_{50} values. The organophosphorus insecticides exhibit two prominent linear relationships: one covering ethion and malathion, while the other covering chlorfenvinphos, monocrotophos, and phosalone. However insecticides on former line are more slope-wise advantaged of tolerance limit than those on latter line. Almost parallel to the line joining chlorfenvinphos, ethion, and malathion toward high tolerance limits, there is a line joining organochlorines endosulfan and organophosphorus dicofol. Still more advantaged of tolerance limit to this line of insecticides, DDT (dichlorodiphenyltrichloroethane) makes the place in the graph. The well-known carbamate insecticide carbyl occupies the highest tolerance limit value 5 mg/kg (of vegetables) along with LD_{50} value equal to 250 mg/kg (animal weight).
6. The organochlorines aldrin, heptachlor, and chlordane also exhibit a linear relationship between LD_{50} and tolerance limit. But this line is quite near to LD_{50} axis, and these organochlorines are not advantaged of tolerance limit compared to organochlorines like DDT and endosulfan.
7. Quite near and parallel to LD_{50} axis, there is a line which joins the least toxic insecticides benomyl and carbendazim. Needless to say these carbamate insecticides which have LD_{50} values more than 6000 mg/kg of animal weight are very little advantaged of tolerance limit less than 0.3 mg/kg of vegetables.
8. Most of the natural insecticides, including the safest azadirachtin and spinosad (having high LD_{50} values between 3000 and 6000 mg/kg of animal weight), lie on the LD_{50} axis (tolerance limit being nil in case of vegetables). Avermectins, rotenone, milbemycins, and ryanodine (in increasing LD_{50} – 10–1200 mg/kg – that means decreasing toxicity order) all lie on LD_{50} axis.

5 Low Toxicity Advantage Factor (LTAF) in Tolerance Limits: Discussion

The food law authorities probably do not consider the high LD_{50} or low toxicity data alone while establishing tolerance limits or maximum residue limits for an insecticide in a food product. The biodegradability of an insecticide is also a criterion in this matter with a viewpoint of environmental safety. In general, natural insecticide

molecules possess low toxicity, indicated by low values of $LD_{50,}$ compared to synthetic insecticides. Needless to say, being the part of nature, the natural insecticides are biodegradable too. However there also seems one more criterion, taste agreeability or agreeable effects on human body, which might be prominently considered by lawmakers in assigning tolerance limits for insecticides. Therefore obviously the question arises how much an insecticide is capable of taking advantage of low toxicity (or high LD_{50} data) in tolerance limits. A convenient measure for toxicity consideration in tolerance limit establishment might be the ratio of tolerance limit to LD_{50} value of an insecticide.

LTAF in TLE (low toxicity advantage factor in tolerance limit establishment): It is a term devised by the author of this paper, the ratio of tolerance limit for a particular insecticide in case of a food article established by a food law to its LD_{50} value, to make an idea of positioning of taking advantage of low toxicity or high LD_{50} value in establishing tolerance limits.

Since higher LD_{50} values correspond to lower toxicity of insecticides, the aforementioned ratio is termed as low toxicity advantage factor in tolerance limit establishment or LTAF in TLE.

The well-known insecticides in descending LTAF order in TLE for vegetables are arranged as follows (Table 1):

A perusal of above tabulated data leads to the fact that high LD_{50} (low toxicity) insecticide molecules, most of which are natural including the safest azadirachtin, occupy the bottom positions in the list of insecticides arrayed in descending LTAF order. On the other hand, the top 3 insecticides, all synthetic, possess LD_{50} value less than 20 and hence are highly toxic. The most environment-unfriendly insecticide molecule DDT occupies the fifth position in the list of 34 well-known insecticides in descending LTAF order. It simply means that taste agreeability or agreeable effects on human body play the prominent role in establishment of tolerance limits for insecticides in food products.

6 Conclusion

Dicofol (synthetic organochlorine), malathion (synthetic organophosphorus), and pyrethrums (natural), which possess LD_{50} values more than 500, occupy the mid-positions (14th, 15th, and 16th) in the list of 34 well-known insecticides arranged in descending LTAF order. The least toxic insecticides benomyl and carbendazim (both synthetic carbamates) occupy 22nd and 23rd positions. At 32nd position is the eco-friendliest azadirachtin, active ingredient of *neem.* Spinosad, lesser toxic than azadirachtin, occupies the last position in this list. Azadirachtin, spinosad, pyrethrins, carbendazim, benomyl, malathion, and dicofol perhaps are the insecticides which might be so wisely applied in farms with the viewpoint of safe agriculture that food product does not cross their MRL (maximum residue limit) specified by FSSR. However bio-diversified forests and pastures seem necessary for soil conservation and effective bio-control of insects to facilitate traditional kind of farming utilizing safe natural insecticides for crop protection.

Table 1 Toxicity and LTAF (deceasing order) data

S.NO.	Name and type of insecticide	LD_{50} (female rat) mg/kg animal weight	LTAF in TLE
1.	Parathion (synthetic, organophosphorus)	3.6	1389
2.	Endosulfan (synthetic, organochlorines)	18	1111
3.	Carbofuran (synthetic, carbamates)	2	500 (most deadly toxic aldicarb of carbamate category has also LTAF 500 in potato)
4.	Parathion methyl (synthetic, organophosphorus)	24	417
5.	DDT (synthetic, organochlorines)	118	297
6.	Carbyl (synthetic, carbamates)	250	200
7.	Ethion (synthetic, organophosphorus)	65	154
8.	Lindane (synthetic, organophosphorus)	91	110
9.	Monocrotophos (synthetic, organophosphorus)	20	100
10.	Phosphamidon (synthetic, organophosphorus)	24	83
11.	Phosalone (synthetic, organophosphorus)	147.5 (120–175)	68
12.	Formothion (synthetic, organophosphorus)	350	57
13.	Chlorfenvinphos (synthetic, organophosphorus)	9.66	52
14.	Dicofol (synthetic, organochlorines)	1495	33
15.	Malathion (synthetic, organophosphorus)	1000	30
16.	Pyrethrins (natural)	>500	<20
17.	Chlorpyrifos (synthetic, organophosphorus)	145	14
18	Chlordane (synthetic, organochlorines)	343	6

(*continued*)

Table 1 (continued)

S.NO.	Name and type of insecticide	LD_{50} (female rat) mg/kg animal weight	LTAF in TLE
19.	Paraquat (synthetic, miscellaneous)	100	5
20.	Heptachlor (synthetic, organochlorines)	162	3
21.	Trichlorfon synthetic, organophosphorus)	560	2
22.	Carbendazim (synthetic, carbamates)	6400	0.8
23.	Benomyl (synthetic, carbamates)	9590	0.5
24.	Abamectin (natural)	10	0
25.	Deltamethrin (synthetic, Pyrethroid)	31	0
26.	Rotenone (natural)	132	0
27.	Nicotine (natural)	230, max mice (MAK) varying 3.3–230	0
28.	Cypermethrin (synthetic, Pyrethroid)	250	0
29.	2,4-D (synthetic, miscellaneous)	375	0
30.	Milbemectin (natural)	456	0
31.	Ryanodine (Ryania stem powder) (natural)	1200	0
32.	Azadirachtin (natural)	>3540	0
33.	Glyphosate (synthetic organophosphorus)	4873	0
34.	Spinosad (natural)	>5000	0

References

1. Kroschwitz JI (1998) Kirk-othmer encyclopedia of chemical technology, 4th edn. Wiley, New York
2. Carson R (2002) Silent spring, Fortieth Anniversary edition. Houghton Miffin, Boston
3. Walker R (2011) Hindutva; Indian nationalism and the politics of religious violence. In: Denton PH (ed) Believers in battlespace; religion, ideology and war. Canadian Defence Academy Press, Kingston
4. Martin H. Pesticides manual; basic information on the chemicals used as active components of pesticides. 1st. Farnham; The British Crop Protection Council; 1968

5. Drum C (1980) Soil chemistry of pesticides. PPG Industries, Pittsburg
6. Linde CD (1994) Physico-chemical properties and environmental fate of pesticides. Environmental hazards assessment program. P53. Environmental Protection Agency, Washington, DC
7. Veitch GE, Beckmann E, Burke BJ, Boyer A, Maslen SL, Ley SV (2007) Synthesis of azadirachtin; a long but successful journey. Agnew Chem Int Ed Engl 46:7629–7632
8. Isman MB (2006) Botanical insecticides, deterrent and repellents in modern agriculture and an increasing regulated world. Annu Rev Entomol 51:45
9. Sparks M, Dosdall LM, Keddie BA (2001) Outlooks pest manage. Science 57:896
10. Campbell WC (1989) Avermectin and Abamectin. Springer, New York
11. Fang N, Casida JE (1998) Anticancer action of cube insecticide; correlation for rotenoid constituents between inhibition of NADH ubiquinone Oxidoreductase and induced ornithine decarboxylase activities. Proc Natl Acad Sci USA 95:3380. Fang N, Casida JE (1999) Cube resin insecticide: constituents. J Agric Food Chem 47:2130
12. Crombie L (1971) In: Jecobson M, Crosby DG (eds) Naturally occurring insecticides. Marcel Dekker, New York
13. Sharma RK, Parisi S (2017) Toxins and contaminants in Indian food products; chapter 5, pesticides detection and detrimental chemicals. Springer Nature, Switzerland, p 57
14. Greenberg DS (2009) Pesticides: white house advisory body issues report recommending steps to reduce hazard to public. Science 140(3569):878–879
15. FAO/UNEP DDT. Decision guidance document. Joint FAO/UNEP handbook of toxicology, vol III. National Research Council, Philadelphia; 1959 Programme for the operation of prior informed consent, UNEP/FAO, Rome, Italy 1991
16. Bharat Niti. www.bharatniti.com. Integral humanism can solve world's problems; 25 Oct 2016
17. Budavari S (ed) (1989) Merck Index, An encyclopedia of chemicals drugs and biologicals. Merck & Co, Rahway
18. Shimpkin, Anderson (1936) Reported by John Casida. Pyrethrum, the natural insecticide Google book 2012
19. EXTOXNET (Extension toxicology network). Azadirachtin. www.prnep.cce.cornell.edu
20. WHO (World Health Organization). Specifications and evaluations for public health pesticides: Spinosad
21. Agrochemical Handbook (1991) The Royal Society of Chemistry, Cambridge, UK
22. MAK collection for Occupational Health and Safety. Nicotine 5.1.2. DECOS 2004
23. EPA (United States Environmental Protection Agency) Ryanodine: registration eligibility decision. http://archive.epa.gov/2595fact.pdf

Edible Mushrooms: Cultivation, Bioactive Molecules, and Health Benefits

62

Sachin Gupta, Baby Summuna, Moni Gupta, and Sudheer K. Annepu

Contents

S. Gupta (✉)
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, India
e-mail: sachinmoni@gmail.com

B. Summuna
Division of Plant Pathology, Faculty of Agriculture, SKUAST-Kashmir, Jammu, Jammu and Kashmir, India
e-mail: summunabhat@gmail.com

M. Gupta (✉)
Division of Biochemistry, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu, Jammu and Kashmir, India
e-mail: moniguptaskuast@gmail.com

S. K. Annepu
Divison of Crop Improvement, ICAR-Directorate of Mushroom Research, Solan, Himachal Pradesh, India
e-mail: sudheerannepu@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_86

Abstract

Mushrooms are globally appreciated for their nutritional value and medicinal properties. Their cultivation is an effective bioconversion technology of transforming wastes and woods into potentially valuable resources and could also be an important part of sustainable agriculture and forestry. Although India has the advantage of favorable agroclimate, abundance of agrowastes, relatively low-cost labor, and a rich fungal biodiversity, it has witnessed a lukewarm response in growth of mushroom cultivation. Out of the total mushroom produced in India, white button mushroom share is 73% followed by oyster mushroom (16%), paddy straw mushroom (7%), and milky mushroom (3%). The per capita consumption of mushrooms in India is also very meager and is even less than 100 g per year. Besides low fat and high protein and vitamin contents, mushrooms are rich sources of several minerals and trace elements, as well as dietary fibers. The reported medicinal effects of mushrooms include anti-inflammatory effects, with anti-inflammatory compounds of mushrooms comprising a highly diversified group in terms of their chemical structure. They include polysaccharides, terpenoids, phenolic compounds, and many other low molecular weight molecules. Of late, mushrooms have emerged as wonderful source of nutraceuticals, antioxidants, anticancer, prebiotic, immunomodulating, anti-inflammatory, cardiovascular, antimicrobial, and antidiabetic. Owing to the synergistic action of present bioactive molecules, majority of mushroom products possess beneficial health effects and can be used on a regular basis without harm. Therefore, they are considered as perspective organisms to develop different healthcare biotech product. Mushrooms could potentially be very important in future food supplies and in new dimensions of sustainable agriculture and forestry. In this chapter, an attempt has been made to provide an insight into the various aspects of cultivation of mushroom cultivation in India, nutritional benefits, therapeutic potential, and bioactive components present in edible mushrooms.

Keywords
Mushrooms · Cultivation · Nutrition · Bioactive molecules

1 Mushroom: Structure, Growth, and Composition

In broad sense, "mushroom is a macro fungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand" [1]. It is perhaps the most well-known and documented edible forest product [2]. Mushrooms have been widely used as foods [3, 4] and very often as delicious and nutritious foods [5]. Approximately 14,000 described species of the 1.5 million fungi estimated in the world produce fruiting bodies that are large enough to be considered as mushrooms [6]. Mushrooms belong to basidiomycetes and ascomycetes with a cell cycle including the formation of sexual spores and have two growth phases, i.e., the vegetative phase (mycelia) and the reproductive phase (fruit bodies). The fungal spores are located in a special structure called the basidium (for Basidiomycetes) or the ascus (for Ascomycetes). The mushroom continues its life cycle in three key stages. viz., vegetative growth, reproductive growth, and spore production by fruit bodies of the mushrooms (Fig. 1).

Fungi lack the most important feature of plants, i.e., the ability to use energy from the sun directly through chlorophyll. Thus, fungi depend on other organisms for food, absorbing nutrients from the organic material in which they live. The living body of the fungus is mycelium made out of a tiny web of threads (or filaments) called hyphae. Hyphae absorb digestive products, penetrating the substrate to some extent. Under specific conditions, sexually compatible hyphae will fuse and start to form spores. The larger spore-producing structures are considered as mushrooms. The spores released from the gills again germinate and develop to form hyphae,

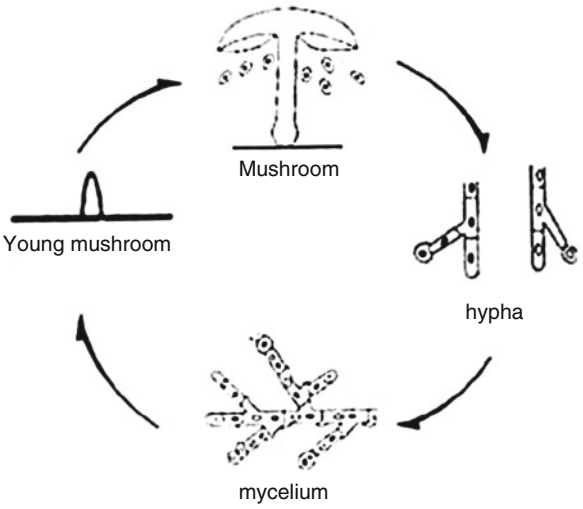


Fig. 1 Basic life cycle of mushroom

which is the main mode of fungal vegetative growth. The mushroom produces several million spores in its life, and this life cycle is repeated each time the spores germinate to form the mycelium. Mycelial growth is generally coupled with increased enzyme production and respiration.

2 Global Trends of Mushroom Cultivation

Mushrooms are the common components in folk medicine, especially in Africa, the Middle East, China, and Japan since ages. Earlier, edible mushrooms were only harvested wild and were difficult to domesticate and cultivate. Collection from wild woodlands is still important in the world and particularly in southern Asia [7, 8] and other developing countries [9]. Mushrooms such as *Auricularia*, *Flammulina*, and *Lentinula* were most likely cultivated for the first time around the year 600–800 AD in China and other Asian countries [10]. Their cultivation at large scale started only at the beginning of the twentieth century when pure cultures of mushroom were prepared from spore and tissue. As the amount of wild mushrooms shrink from both the degraded environment and natural resources and more costly labor, cultivated mushrooms would not only provide food security but also sustainable and more nutritious diets [5]. The commercial production of edible mushrooms represents the unique exploitation of a microbial technology for the bioconversion of agricultural, industrial, forestry, and household wastes into nutritious food (mushrooms). Mushrooms have the capacity to breakdown the lignin and utilize it as a food source, thus exposing the underlying cellulose and hemicellulose for food use by other organisms. Thus, mushroom cultivation represents a very basic natural process of fungal decay.

With the world's increasing population and its decrease in per capita arable land, along with rapid urbanization and industrialization, climate change, and a demand for quality and functional foods, it will be necessary to focus on secondary agriculture and novel crops, such as mushrooms. Mushroom cultivation could also be an important part of sustainable agriculture and forestry. Huge quantities of wide varieties of organic waste are generated from agriculture, forestry, and food processing. The impacts of the mushroom business on livelihoods and poverty reduction are significant and widespread. Mushroom cultivation does not require a lot of land, significant capital investment, but is a viable and attractive activity for both rural farmers and semi-urban dwellers. Mushroom cultivation strengthens the livelihood of poor and marginal farmers by generating constant farm income and reduces the vulnerability to poverty. The scale of cultivation can be large or small based on the capital and labor availability. It can be cultivated on a part-time basis with little maintenance. Indirectly, mushroom cultivation also provides opportunities for improving the sustainability of small farming systems through the recycling of organic matter, which can be used as a growing substrate and then returned to the land as fertilizer. There are hundreds of identified species of fungi which have made a significant contribution to human food and medicine. The total number of described fungi of all kinds is currently 110,000 species [11] of which 16,000

(15%) species are mushrooms [11–13]. Out of these, more than 3000 species from 231 genera are regarded as prime edible mushrooms [13–15] of which only about 200 are experimentally grown, 100 economically cultivated, around 60 commercially cultivated, and more than 10 produced on an industrial scale in many countries [16]. Approximately 700 mushroom species out of the known 16,000 are considered to be safe and have medicinal properties [13]. The number of poisonous mushrooms approximates 500 species. The most acceptable varieties among the cultivated types are *Agaricus bisporus* (button mushroom), *Pleurotus* spp. (oyster mushroom), *Lentinus edodes* (Shiitake), and *Volvariella* spp. (paddy straw mushrooms). In the second half of the twentieth century, there were rapid changes in rate of growth of mushroom production and number of species like shiitake, oyster mushrooms, and wood ear mushroom, and *Flammulina* were brought under commercial cultivation. By the end of the twentieth century, the share of button mushroom in total world production was less than 40%, which in next 10 years became around 30%. Presently shiitake, oyster, wood ear, and button mushrooms contribute 22%, 19%, 18%, and 15%, respectively in terms of total mushroom production in the world [17]. The contribution of medicinal mushrooms in world trade has also increased over last few decades.

Mushroom farming is today being practiced in more than 100 countries, and its production is increasing at an annual rate of 6–7%. Cultivated mushrooms have now become popular all over the world. In 1999, the world production of cultivated edible mushrooms was estimated to be >7 million tons, showing a steady increase over the last two decades. China is the largest producer, consumer, and exporter of mushrooms in the world, followed by the United States and the Netherlands (Table 1). In China, mushroom is the 6th important crop in the country as far as revenue generation for the nation is concerned. Mushroom production in China in 2010 was 21,524,473 t [18]. The last few decades have witnessed a sharp rise in diversification in number of mushroom species that have been cultivated, world mushroom production, commercialization accompanied with mechanization, and in

Table 1 Nutritional value of some commercial edible mushrooms (on dry wt. basis)

	Mushroom			
Nutritional parameters	*Agaricus bisporus*	*Pleurotus* spp.	*Volvariella volvacea*	*Lentinula edodes*
Protein (%)	29.14	19.59	38.10	18.85
Carbohydrates (%)	51.05	64.34	42.30	63.60
Fat (%)	1.56	1.05	0.97	1.22
Vitamin D (IU/g)	984	487	462.04	205
Sodium (mg/kg)	500.8	208.87	345.34	82.49
Potassium (%)	4.21	2.70	4.16	2.10
K:Na	84:1	129:1	120:1	255:1
Iron (mg/kg)	85.86	183.07	72.51	37.55
Manganese (mg/kg)	7.97	6.47	–	17.48
Zinc (mg/kg)	79.64	162.18	94.28	89.63

[37]

many cases automation. Mushroom cultivation and its processing have been beneficial to millions of people in China, India, and other developing countries in terms of financial, social, and health improvement. The global mushroom industry has expanded very rapidly in the last two decades by the addition of newer types of mushrooms for commercial cultivation. In addition, cultivation and development of mushroom industries have positively impacted on economic growth, and this impact of mushroom cultivation and mushroom derivatives and products on human welfare in the twenty-first century can be considered globally as a "nongreen revolution."

3 History and Status of Mushroom Cultivation in India

The history of mushroom cultivation in India goes back to the ages of "Vedas," wherein the mention was made in the classical religious scriptures like "Rig Veda" and "Atharva Veda" about the use of juice from fly agaric mushroom (*Amanita muscaria*) as an intoxicating drink, named "Soma." Vegetarianism is assumed to be the norm of Indian diet and is primarily based on cereals (wheat, rice, and maize) which are deficient in protein. Incorporation of mushrooms in Indian diet has the potential to bridge this protein gap and reduce the problem of malnutrition to a great extent. For people in a developing country like India, the two main issues are the quality food and the level of unemployment, in addition to environmental concerns, which can be very well resolved by popularizing mushroom cultivation among rural masses and the younger generation [19]. Although time to time, small efforts and simple research were conducted to introduce mushroom cultivation in India, scientific and systematic research only started in 1961, when the Indian Council of Agricultural Research, New Delhi, first cultivated *Agaricus bisporus* at Solan in Himachal Pradesh, a hilly state of North India. India, primarily being an agrarian economy, is rich in terms of agrowastes that are not properly utilized by the nation's farmers. India produces nearly 700 million tons of agricultural residues which can profitably be utilized for mushroom cultivation. Even if India uses 2% of its total agroresidues for mushroom production, the production would be 7.0 million tons of fresh mushrooms, which will be equal to current global button mushroom production. Currently, India is using only 0.03% of these residues to produce about 0.13 million tons of mushrooms and contributes $<1\%$ of the total world mushroom production. Though mushroom production in Asian countries started 1000 years ago, cultivation of mushrooms is a relatively new phenomenon in India. There has been significant increase in production of mushrooms in the last few years, especially of the oyster and paddy straw mushrooms in India. By considering the present production data, mushroom industry in India recorded an average annual growth rate of 4.3% per annum. During this period, the productivity has raised from 20% to 24.5% by the releasing of improved strains in commercial edible mushroom. The total white button mushroom produced in India from both seasonal and high-tech cultivation units is estimated at 94,676 mt. There has been significant increase in production and productivity of mushrooms (Fig. 2) in the last few years, especially of the oyster and paddy straw mushrooms in India. The country's production in 2010

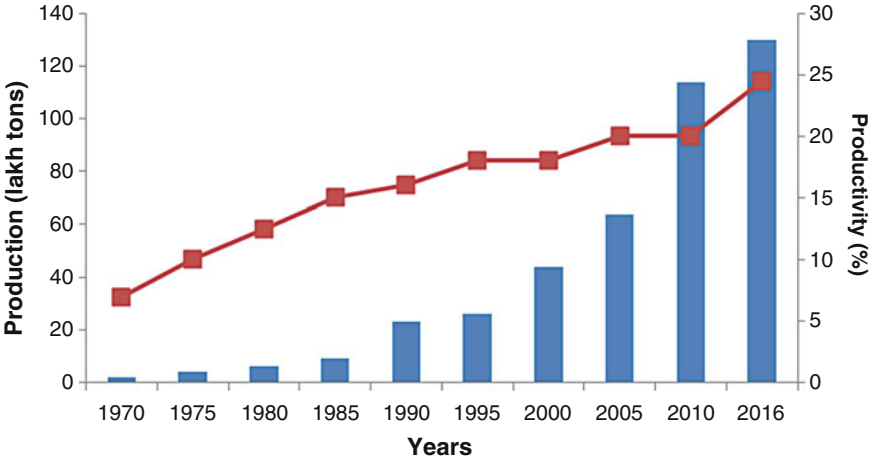


Fig. 2 Production and productivity of mushrooms in India [20]

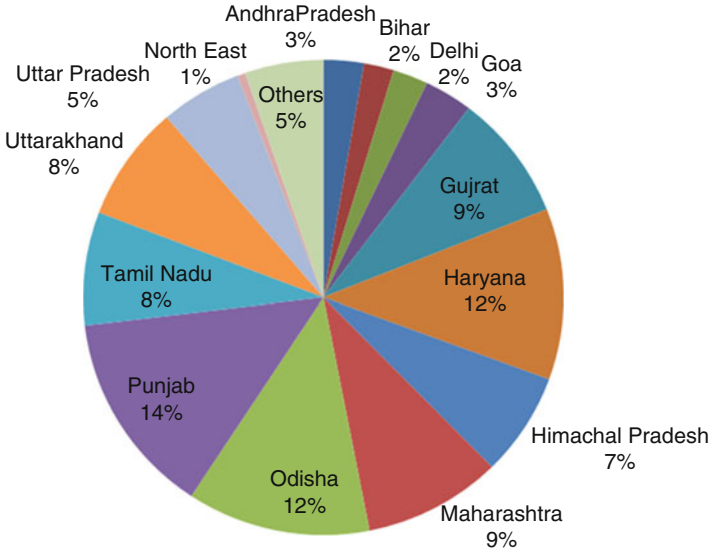


Fig. 3 Share of different states of India in National mushroom production [20]

was 1.00 lakh metric tons, of which button mushroom accounted for 89% of the total production, followed by oyster (6%), milky (1%), and others (4%). Punjab, Uttarakhand, Haryana, Uttar Pradesh, and Tamil Nadu were the leading producers of the mushroom in the country at the time of 2010. The present production status revealed that Punjab, Haryana, and Odisha are emerging as the leading states in mushroom production (Fig. 3).

3.1 Relative Contribution of Different Mushroom Species

In India, there are five mushroom species, viz., white button mushroom (*Agaricus bisporus*), oyster (*Pleurotus* spp.), paddy straw (*Volvariella volvacea*), milky (*Calocybe indica*), and shiitake (*Lentinula edodes*), which are under commercial cultivation. Even though cultivation technologies of many exotic mushrooms have been standardized, the commercial markets are still dominated by *Agaricus bisporus*, *Pleurotus* spp., and *Volvariella volvacea.* These three mushrooms are contributing about 96% of total mushroom produced in India (Fig. 4). Milky mushroom (*Calocybe indica*) is indigenous tropical mushroom of the country [21]. However, the commercial cultivation is restricted to southern states of India and contributing up to 3% to the total mushroom production. Production of paddy straw mushroom became more popular in the states of Odisha and Chhattisgarh, and its production was registered at 7% to the total mushroom production. Two to three crops of button mushroom are grown seasonally in temperate regions with minor adjustments of temperature in the growing rooms, while one crop of button mushroom is raised in the northwestern plains of India seasonally. Oyster, paddy straw, and milky mushrooms are grown seasonally in the tropical/subtropical areas. In Northeastern states, Uttarakhand and Chhattisgarh, oyster mushroom cultivation is emerging as one of the leading cottage industry. Hence, for choosing a species for commercial cultivation, the grower must consider the availability of waste materials to use as a growth medium, the prevailing environmental conditions, available expertise, available resources, and market demand.

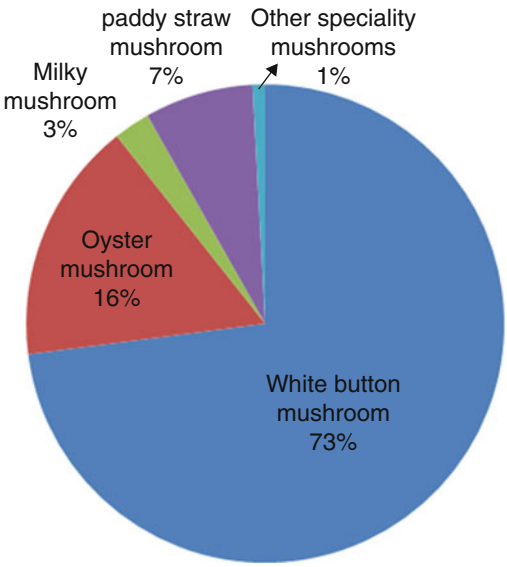


Fig. 4 Relative contribution of different mushroom species in total production [20]

4 Marketing of Mushrooms

Mushroom cultivation provides an opportunity to generate a highly tradable commodity, thereby contributing to income generation. But, majority of the farmers fail to make profits out of their mushroom production activity due to the poor marketing strategy [22]. It is the simple system of producers selling directly to retailer or even to consumer, which has its own limitations like seasonal cultivation of mushrooms and glut of mushrooms at a particular time especially in North India during the winter months forcing the distress sale of the mushrooms. This reinforces that effort to increase the production without solving its marketing problems would be counterproductive. For successful marketing, it is necessary to explore various marketing options for fresh mushrooms and selling them after processing as value-added products like pickles, nuggets, dry powder, etc. which could fetch good returns.

5 Types of Cultivated Mushrooms

The most basic concept of mushroom cultivation is that we need to produce an environment in the substrate that is selectively preferential to the growth of our target mushroom species and less amenable to other types of microorganisms and pests. This involves sterilization or pasteurization. Once the substrate is treated in this way and the majority of the microorganisms are killed, the substrate is suitable for the introduction of our target species of mushroom. Another potential treatment method is simply to soak the substrate in water. It prepares a substrate which is more selective to the higher cellulose-degrading organisms such as oyster mushrooms. This is the simplest method of substrate preparation, but the chemical treatment methods are much more effective and not considerably more difficult or expensive.

5.1 Mushrooms Under Cultivation in India

A brief account of some popularly grown mushrooms in India is as follows:

5.1.1 Button Mushroom

The white button mushroom is the most popular of the cultivated mushrooms. This was the first mushroom to be commercially exploited on an industrial scale and ranks first at the global level.

The genus *Agaricus* has two cultivated species, namely, *A. bisporus* (temperate button mushroom) and *A. bitorquis* (tropical or high temperature-tolerant white button mushroom). A partially decomposed organic matter prepared under aerobic conditions is using as a substrate for growing button mushroom. This substrate is generally termed as compost. The purpose of composting is to prepare a physically and chemically homogeneous medium for the growth of mushroom mycelium. The composting can be done either by long method of composting (LMC) or by short method of composting (SMC). In LMC, the entire composting operation is carried

out in open, and pasteurization required for conditioning of compost is achieved by natural means. SMC is followed by the commercial button mushroom units with environmental control facilities for composting and cropping.

In SMC, the compost is prepared in two phases. During the first phase, outdoor composting for 10–12 days followed by indoor pasteurization and conditioning for 7 days in the second phase. During the phase I, the cereal straw is thoroughly wetted on the cemented floor. This pre-wetted straw is then mixed with the chicken manure and wheat bran and kept as such for 2 days in the form of a pile. After that, the pile is broken and restacked. Four days later, the pile is once again broken and the entire quantity of urea is added. After 2 days, the stack is again turned and watered and gypsum is added in the third turning. During this stage, the temperature of pile raises to 70–80 °C. The final turning is given on the 12th day when the color of the compost changes into dark brown and it starts emitting a strong smell of ammonia. The compost prepared as a result of microbial fermentation process needs to be pasteurized in order to kill undesirable microbes and competitors. The compost from phase I is then filled in the pasteurization tunnels to the height of 2–2.3 m. At this stage, temperature inside the tunnel is set at a range of 45–48 °C which is then raised to 48–52 °C and maintained for 48 h. After 48 h, the compost temperature is raised to 58–62 °C and maintained for 4–6 h by inducting the steam. Thereafter, the temperature is brought down to 48–52 °C, and the compost is retained at this temperature for 3 days. The ammonia level in the compost is monitored regularly and if no ammonia persists in the compost, the compost temperature is brought down to room temperature. At this stage, the final compost should be granular in structure with 65–70% moisture and pH in the range of 7.2–7.5.

Spawning and Spawn Run

After phase II of SMC, the compost is thoroughly mixed with the spawn at 0.5–0.75% (500–750 g) in polypropylene bags and kept in the cropping room where the moisture content of substrate is maintained at 65–70% and temperature at 23 $\pm$ 1 °C. It takes about 12–14 days for the complete colonization of mycelium.

Casing and Case Run

Once mycelium completely colonizes the compost, it can be switched over to fruiting stage by covering the surface of the substrate with a thin layer of casing material. Mixture of 2-year-old FYM and garden soil in 1:1 ratio is most preferable casing material.

Crop Management

Environmental conditions after casing should be the same as during spawn running. Within 3 days of application, the mycelium starts growing into the casing layer. Once mycelial growth is firmly established, the casing is gradually watered up to its optimum moisture-holding capacity.

At this stage, the microclimate in the cropping room which includes substrates and air temperatures in the range of 16–18 °C and humidity to 85% should be adjusted. Besides this, carbon dioxide content of the room should be reduced to less

than 1000 ppm by the induction of fresh air, and the room is lighted on a 12-h on/off cycle as mentioned below to promote the fruiting. This change in environmental parameters helps to the initiation of pinning and subsequent development of pinheads into solid fruit bodies within 3–4 days. The mushroom crop grows in cycles called as "flushes or breaks." The uniform fruit bodies of 4–5 cm in diameter should be handpicked in a twisting motion. Daily watering is required after harvesting the first flush. 18–20 kg of fresh mushrooms can be harvested from 100 kg of prepared compost in a total of three flushes.

5.1.2 Oyster Mushroom

Pleurotus mushroom, which is generally referred to as "oyster mushroom" the world over and as "dhingri" in India, has its origin from the Greek word "pleuro," which means formed laterally or in a sideway position, particularly referring to the lateral position of the stipe (stem) in relation to the pileus (cap). Species of the genus *Pleurotus* are well appreciated for their broad adaptability under varied agroclimatic conditions. The several species of this mushroom occur commonly as wood decomposers in forests throughout the world. *Pleurotus* has always been most attractive to commercial mushroom growers because of the ease by which most of these species can be cultivated. This mushroom consists of species that are suitable for both temperate and subtropical regions. For temperate regions, *Pleurotus ostreatus*, *P. florida* (winter strain), and *P. fossulatus* and *P. eryngii* (king oyster) are ideal. The areas suitable for the button mushroom are equally suitable for the cultivation of these species. The majority of the oyster species can be best grown in the temperature range of 20–30 °C with high relative humidity in excess of 85%. The majority of the *Pleurotus* species require 25–30 °C for spawn runs and 20–25 °C for fruiting. The oyster mushroom is a low-investment, low-risk, low labor-intensive, and moderate-profit enterprise. The greatest advantage of the oyster mushroom is that its production technology is simple and that it can be easily grown at the village level in small makeshift sheds (huts). Growing the oyster mushroom is less expensive and more convenient compared to other species, such as the button mushroom. The method of cultivation is also very simple; unlike the button mushroom, it does not need any compost preparation or precise temperature control. It can be sold both in fresh and dried form.

Substrate Preparation

The oyster mushroom has less specificity toward the substrate. Hence, it can be cultivated on a large number of agrowastes such as cereal straw, sugarcane bagasse, saw dust, dried grasses, discarded wastepaper, etc. The pasteurization of the substrate can be achieved either by steam pasteurization or hot-water treatment. Chemical pasteurization also results in elimination of competitor fungi, but its use is discouraged due to the residual levels in the mushroom fruit bodies.

Spawning and Incubation

Freshly prepared grain spawn is mixed thoroughly at 2–3% of the substrate on wet weight basis, and the spawned substrate is filled in the polythene bags. 10 to 15 small

holes made on all sides of the bag facilitate the aeration and drainage of excess water. During the spawn run stage, the bags are not to be opened, and it doesn't require much ventilation or water sprays.

Crop Management

Optimum temperature for mycelia growth of oyster ranges between 22 °C and 26 °C. Once the mycelium fully colonizes the substrate, it forms a thick mycelial mat indicating the readiness for fruiting. During the fruiting period, a relative humidity of 80–85% needs to be maintained by spraying the water two to three times, and sufficient ventilation should be provided for air circulation. The color of the oyster is also influenced by the light intensity and its duration. Fruit bodies raised in bright light appears in dark brown or gray in color, and the fruit bodies raised in less intensity of light appears in pale yellowish color.

With suitable crop management practices, mushroom fruit bodies are ready for harvesting in 6–8 days after pinhead formation. Mushrooms may be harvested while the edges of the caps are still curled down. Under normal room temperature conditions, fresh mushrooms can be stored for 2–3 days without any deterioration. The fruit bodies can be dried under sunlight to the moisture level of 8–10%. The dried oyster mushroom can be successfully stored for 4–6 months without losing its original properties.

5.1.3 Milky Mushroom

The milky white mushroom grows during the summer, and it is a tropical mushroom known for its nutritional value. Its robust size, sustainable yield, attractive color, delicacy, long shelf life, and lucrative market value have attracted the attention of both mushroom consumers and prospective growers. *Calocybe indica* is rich in protein, lipids, fiber, carbohydrates, and vitamins, and it contains an abundant amount of essential amino acids and is low in fat. These qualities make it suitable as a dietary food supplement. The milky mushroom (*Calocybe indica*) is purely of Indian origin and highly suitable for the tropical climatic conditions of Central India, North India, and South India. Its cultivation is now spreading quickly in many Indian states, including Tamil Nadu, Kerala, Orissa, Haryana, and West Bengal due to its longer shelf life and adaptability to warm and humid conditions. At present, Tamil Nadu is the major producer of the milky mushroom in India.

Milky mushrooms can be grown on a wide range of lignocellulosic residues, but commercial cultivation is done on paddy straw or wheat straw. The cultivation technology of milky mushroom is a mixture of button and oyster mushroom cultivation technologies. It does not require compost for its growth specially as the substrate is prepared as per the technique used for oyster mushroom. Spawning is done at 4–5% of wet weight of substrate, and the spawned bags are then shifted to incubation room at 28–32 °C under dark conditions. It takes 20–25 days for completion of spawn run. However, once the spawn run is complete, a layer of casing material needs to be applied for induction of fruiting. Thereafter temperature of 30–35 °C, R.H. 80–90%, and light intensity at 600–1000 lux is required to be maintained for cropping. Primordia formation and fruit body maturation takes place

within 8–10 days after casing. Mushrooms are harvested when the stipe length reaches 7–8 cm by twisting with hand. Harvested fruit bodies can be consumed fresh or store the clean mushrooms by wrapping in film for 7–10 days at room temperature.

5.1.4 Paddy Straw Mushroom

The paddy straw mushroom *Volvariella* sp. prefers to grow on paddy straw; hence, it is known as paddy straw mushroom. Chinese growers developed its cultivation more than 300 years ago. Therefore, it was named "Chinese mushroom" [23]. The paddy straw mushroom is the sixth most important cultivated mushroom in the world [24]. Paddy straw mushroom (*Volvariella volvacea*) is a world famous edible mushroom variety that enjoys high demand due to its deliciousness and nutritive value. It is the only mushroom which can be cultivated both indoor and outdoor. It is a popular variety among growers because of short cropping season compared to other cultivated mushrooms. No other vegetable or cultivated mushroom can be served as a table dish within a short time after planting, but *V. volvacea* can do this as it comes to harvest 10-days post-planting. The climatic conditions prevailing in India are best suited for the cultivation of this mushroom. Currently, Orissa is the leader in commercial cultivation of this mushroom.

Outdoor Cultivation

The traditional outdoor cultivation is done seasonally under the shades of trees by making mushroom beds on the raised platform made from bricks and bamboo poles. Straw bundles of 45 cm length and 10 cm width are prepared by cutting the top leafy portion and part of thick stalk near the roots by hand or motorized cutter. The bundles are soaked in water or in 2% $CaCO_3$ solution for 12–14 h. Then these bundles are placed side by side followed by placing the mushroom spawn at six to eight spots and covering of spawn with red gram dal powder. 12–15 beds are prepared in the similar manner, and the whole lot of beds prepared in a line are compressed a little and covered with clean plastic sheet.

The spawn run requires a temperature of above 39 °C, and spawn run will be completed in 6–7 days. After the completion of spawn run, the plastic sheet is kept loosely covered over the beds. The mushrooms start coming from all sides of the bed after 12–13 days of spawning. They can be harvested at egg stage by holding between forefinger and thumb followed by twisting clockwise or anticlockwise direction. The harvested mushrooms can be cleaned and packed in polythene bags or in paper bags, but the bags should not to be sealed. These packed mushrooms must be sold for consumption preferably on the same day.

Indoor Cultivation

Indoor cultivation can be done on a substrate/compost prepared by mixing cotton ginning mill waste and paddy straw in 1:1 ratio on weight basis. The substrate after mixing is wetted for 2 days, and later the poultry manure is added at 5%. The substrate is mixed thoroughly and made into a pile. First two turnings are given at an interval of 1 day each, and calcium carbonate at 1.50% is added at third turning, and

the substrate is left for fermentation for next 2 days. After 4 days of outdoor composting, the compost is spread on the shelves to a thickness of 10–15 cm. The steam is introduced inside the cropping room for heat conditioning of the compost. Temperature is maintained at 62 °C for 4–5 h. After a day, the compost is spawned at 1.5% on wet weight basis, and the beds will be covered with the plastic sheet. The room temperature is maintained at 32–34 °C during spawn run period. The compost gets colonized within next 4–5 days, and then the beds are sprinkled with water by removing the plastic sheet. The pinhead starts appearing on 5th–6th day of spawning. After another 4–5 days, the first flush of mushroom gets ready for harvesting. The paddy straw mushroom is not suitable for storing in refrigerator and must be consumed fresh immediately after harvesting or may be stored at room temperature for few hours.

5.1.5 Shiitake Mushroom

Shiitake mushroom is one of the world's most popular edible mushroom species known for its nutrition, flavor, and medicinal properties. It contains a bioactive compound – lentinan – which has anticarcinogenic and tumor-suppressing properties. Traditionally shiitake mushroom was cultivated on fallen wood logs stacked in evergreen forests in China since centuries. But its commercial cultivation gained the momentum after developing the synthetic log cultivation technology.

Synthetic Log Cultivation

Saw dust obtained from the broad-leaved trees such as tuni, poplar, mango, safeda, oak, maple, etc. is the main basal ingredient for the preparation of synthetic logs. For preparation of synthetic logs, saw dust is supplemented with the wheat bran or rice bran in the ratio of 80:20, and calcium carbonate is mixed at 1% on dry weight basis to maintain the pH levels below 7.0. Ingredients are thoroughly mixed and moistened to a level of 60–65%. The prepared substrate is filled in the heat-resistant polypropylene bags and then sterilized for 2 h at 121 °C and 22 psi of pressure. After sterilization, the bags are cooled for a day and thereafter seeded with fresh grain spawn at 45–50 g per bag. Spawning is done under aseptic conditions using laminar airflow chamber. After inoculation, the bags are shifted to incubation room where the temperature is maintained at 22–26 °C. Spawn run is generally completed within 30–35 days, and a thick mycelia coat will develop on the surface of the substrate after 6–8 weeks of spawning. This mycelial coat will eventually turn into brown color with some exudates. Clumps of mycelium will form on the surface of the mycelial coat 10–12 days after mycelial coat formation. These clumps will turn into mushroom fruit bodies at the later stage. The temperature of about 18–20 °C and relative humidity of more than 85% are required to be maintained till the maturity of the fruit bodies. The fruit bodies keep coming for 15–20 days in a flush after each shock treatment of cold water. Cold water treatment should be repeated for the subsequent three to four flushes also till the exhaustion of nutrients in the substrate. The harvested fresh fruiting bodies of shiitake mushroom can be stored for 3–4 days at 18–20 °C, and keeping them at 4–6 °C will extend their shelf life up to 14 days. The shiitake mushroom can also be stored up to 1 year by drying.

6 Nutrition in Mushrooms

Edible mushrooms have been widely utilized as human food for centuries and appreciated for texture, flavor, as well as medicinal and tonic attributes [25]. In general, mushrooms contain 90% water and 10% dry matter [26]. They have a chemical composition, which is attractive from the nutritional point of view [27]. Mushrooms are nutritionally important as they are rich in protein, fibers, and minerals, while poor in fats. The mushroom protein contains all the nine essential amino acids required by humans. Mushrooms are considered as a potential substitute of muscle protein on account of their high digestibility [28]. Besides this, mushrooms are also rich source of vitamin B1, B2, B12, C, D, and E [29, 30] and a relatively good source of nutrients like phosphorus, iron, and vitamins, including thiamine, riboflavin, ascorbic acid, ergosterol, and niacin [31]. Mushrooms are also an excellent source of vitamin D which is otherwise not available in other food supplements [32].

Mushrooms are low in calories, fat-free, cholesterol-free, gluten-free, and very low in sodium. Minerals such as potassium, iron, copper, zinc, and manganese are high in fruit bodies. They also have ash, glycosides, volatile oils, tocopherols, phenolic compounds, flavonoids, carotenoids, folates, organic acids, etc. [33]. Mushrooms are also important from nutraceutical point of view, as they contain several compounds like unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid, and carotenoids. The nutritional attributes of edible mushrooms and the health-benefiting effects of the bioactive compounds they contain make mushrooms a health food [34–36].

Consumers are now deeply interested in food bioactives that provide beneficial effects to humans in terms of health promotion and disease risk reduction. Mushrooms can be considered as functional food which provides health benefits in addition to nutritional value [38]. The concept of "functional foods" was first introduced as a factor in the analysis of foods after nutrients [39].

The most common nutrients of mushrooms are discussed as follows:

6.1 Proteins and Amino Acids

The crude protein content of edible mushrooms is usually high but varies greatly and is affected by factors such as species and stage of development of the mushroom [40]. The free amino acid level of mushrooms is usually low ranging from 7.14 to 12.3 mg/g in dry edible mushrooms and contributes to the main flavor properties of mushrooms [41]. The essential amino acid profiles of mushrooms reveal that the proteins are deficient in sulfur-containing amino acids, including methionine and cysteine. However, these edible mushrooms are comparatively rich in threonine and valine.

6.2 Vitamins

Cultivated mushrooms are a good source of several vitamins, such as riboflavin, niacin, and folates. The vitamin B2 content in mushrooms is higher than that

generally found in vegetables, [30]. Mushrooms contain moderately high amounts of folates, and their bioavailability is as good as that for folic acids [42]. In addition to riboflavin, niacin, and folates, cultivated mushrooms also contain small amounts of vitamin C and vitamin B1 and traces of vitamins B12 and D2 [30].

6.3 Carbohydrates

Edible mushrooms contain high levels of oligosaccharides and only a low level of total soluble sugars [43]. The carbohydrate content of edible mushrooms varies with species and ranges from 35% to 70% DW [44].

6.4 Fatty Acids

The fatty acid level in mushrooms is generally low around 2–8% of distilled water. The level of polyunsaturated fatty acids as compared to saturated fatty acids is quite high, constituting more than 75% of total fatty acids of which oleic and linoleic acids are the most significant, while palmitic acid is the main saturated fatty acid [45].

7 Therapeutic Potential of Mushrooms

The major attribute of mushrooms is their medicinal properties and presence of bioactive compounds. The pharmacological properties of mushrooms include immunity enhancement, maintenance of homeostasis, regulation of biorhythm, and most importantly cure and prevention of various life-threatening diseases such as cancer, stroke, and heart diseases. Medicinal properties of mushrooms including anti-inflammatory, antioxidant, immunomodulatory, anticarcinogenic, antiviral, antibacterial, antifungal, hepatoprotective, antidiabetic, anti-angiogenic, hypoglycemic, etc. have been reported [46]. Immunomodulatory and antitumor activities of polysaccharide–protein complex (PSPC) from mycelial cultures of mushrooms have been extensively studied [47–49]. The pharmacological potential of mushrooms includes the following:

7.1 Mushrooms as Antioxidants

Edible mushrooms possess potent antioxidants. A study of methanolic extracts from black, red, and snow ear mushrooms found that mushrooms possess an inhibitory effect on lipid peroxidation, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and hydroxyl radical scavenging, and a strong reducing power and ability to chelate ferrous ions [50]. Similar studies on other mushrooms, including *D. indusiata*, *G. frondosa*, *H. erinaceus*, *T. giganteum*, *F. velutipes*, *L. edodes*, *P. cystidiosus*, and *P. ostreatus*, *Agrocybe cylindracea* also reported antioxidant properties of these mushrooms [44, 51].

7.2 Mushrooms as Hypocholesterolemic Agents

Regulation of the cholesterol level is important for the prevention and treatment of cardiovascular diseases. Edible mushrooms are an ideal food for the dietetic prevention of atherosclerosis due to their high fiber and low fat content. Initial research on the cholesterol-lowering effects of mushrooms was conducted in Japan in the 1960s, and it was demonstrated that when rats were fed with a high fat and high-cholesterol diet supplemented with 5% water of the fruiting bodies of *L. edodes* for 10 weeks, the plasma cholesterol levels of the animals decreased significantly [52]. Several other studies on *Lentinula* extracts have shown them to cause a significant decrease in serum cholesterol in young women and old people [53]. In another study, dietary fiber extracted from *P. cornucopiae* had a marked in vitro anti-atherosclerotic effect and patients with coronary disease showed a decreased atherogenic activity (20–40%) in their sera after the consumption of this mushroom, which confirms its property of natural cholesterol-lowering agent [54]. It has been suggested that the fruiting bodies of oyster mushrooms could be recommended for consumption as a natural cholesterol-lowering agent in the human diet [55]. Dietary fiber isolated from *Auricularia auricula* and *Tremella fuciformis* significantly decreases the serum total cholesterol (TC) and LDL cholesterol levels [56]. Various studies have shown that *Lentinula* mushrooms can lower both the blood pressure and the free cholesterol level in plasma and can accelerate the accumulation of lipids in the liver by removing them from circulation [57]. Mushroom like *A. auricula-judae* displays anti-coagulation, anti-aggregatory activity in the blood platelets of mice and rats, thus serving to lower their total cholesterol, total triacylglyceride, and lipid levels [58, 59]. *Grifola frondosa* has been reported to reduce blood pressure in rats without changing the plasma high-density lipoprotein (HDL) level or serum cholesterol level [60].

7.3 Mushrooms as Hypoglycemic Agents

Edible mushrooms are an ideal food for the dietetic prevention of hyperglycemia because of their high dietary fiber and protein and low fat content [61]. Lectins isolated from mushrooms (*Agaricus campestris* and *A. bisporus*) have been shown to enhance insulin release in isolated Langerhans rat islets [62]. The presence of a non-lectin-type component in *A. campestris* that displays insulin-releasing and insulin-like activity has also been reported [63]. Guanidine, which is a known hypoglycemic substance related to the biguanide class of oral antidiabetic drugs, has been found in edible mushrooms [64].

7.4 Mushrooms as Antitumor Agents

Cordyceps militaris has been used for a long time in Eastern Asia as a nutraceutical and in traditional Chinese medicine as a treatment for cancer patients. Searching

for new antitumor agents including mushrooms has become a topic of research. It was reported that *Ganoderma lucidum*, *Phellinus rimosus*, *Pleurotus florida*, and *Pleurotus pulmonarius* possess profound antioxidant and antitumor activities [65]. The antitumor activities of the higher basidiomycetes extracts of fruiting bodies of *Boletus edulis* and other Homobasidiomycetes were tested against the Sarcoma 180 line in mice and were found to have significant activity [66]. Calvacin was isolated from the giant puffball (*Calvatia gigantea*), and it was found effective against many experimental tumors, including Sarcoma 180, mammary adeno carcinoma 755, leukemia L-1210, and HeLa cell lines [67]. There are approximately 650 species of higher basidiomycetes that have been found to possess significant antitumor activity [14, 60].

7.5 Mushrooms as Immunomodulators

Immunomodulators are the most important medicinal mushroom drugs used especially in Japan, China, Korea, and other East Asian countries today. Some polysaccharides or polysaccharide–protein complexes from mushrooms are able to stimulate the non-specific immune system and to exert antitumor activity through the stimulation of the host's defense mechanism [68]. These drugs activate effector cells like macrophages, T lymphocytes, and NK cells to secrete cytokines like TNF-a, IFN-g, IL-1b, etc. which are antiproliferative and induce apoptosis and differentiation in tumor cells [68].

7.6 Mushrooms as Antimicrobial Agents

Mushrooms need antibacterial and antifungal compounds for survival in natural environment. Hence, they are rich sources of natural antibiotics. Many of the extracellular secretions by the mushroom mycelium are known to combat bacteria [69] and viruses [70]. Considerable antifungal and antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* has been reported in several compounds extracted from various mushrooms [71]. It was observed that whole extracts of *Ganoderma pfeifferi* inhibit the growth of microorganisms responsible for skin problems [72]. Oxalic acid has been found to be the compound responsible for the antimicrobial effect of *Lentinula edodes* (Berk.) against *S. aureus* and other bacteria [73]. Ethanolic mycelial extracts from *L. edodes* possess antiprotozoal activity against *Paramecium caudatum* [74].

7.7 Mushrooms as Antiviral Agents

Specific drugs are urgently needed for cure of viral diseases as they cannot be treated by common antibiotics. Antiviral effects are described not only for whole extracts of mushrooms but also for isolated compounds. They may act directly by inhibition of

viral enzymes, synthesis of viral nucleic acids, or adsorption and uptake of viruses into mammalian cells. These direct antiviral effects are exhibited especially by smaller molecules. Indirect antiviral effects are the result of the immune-stimulating activity of polysaccharides or other complex molecules [75]. Small molecular compounds with antiviral activities, several triterpenes from *Ganoderma lucidum* (i.e., ganoderiol F, ganodermanon triol, ganoderic acid B), are active as antiviral agents against human immunodeficiency virus type 1 (HIV-1) [76].

7.8 Mushrooms as Antiallergic Agents

Although extracts of many mushrooms can stimulate the immune system, some have been reported to suppress immune responses also. This property could be of benefit for the treatment of allergic diseases that are nowadays increasing worldwide. Ethanolic extracts of the edible Japanese basidiomycetes *H. marmoreus*, *F. velutipes*, *Pholiota nameko*, and *Pleurotus eryngii* show significant antiallergic effects in mice [77]. Some compounds from *G. lucidum* (ganoderic acids C and D) have been shown to inhibit the histamine release from rat mast cells [78, 79]. Eating of *Tricholoma populinum* led to the regression of severe allergic symptoms in a patient with thromboangiitis obliterans and in another patient with urticaria [80]. Hispolon and hispidin, isolated from fruit bodies of *Inonotus hispidus*, have been reported to inhibit the chemiluminescence response of human mononuclear blood cells and the mitogen-induced proliferation of spleen lymphocytes of mice [81].

7.9 Mushrooms as Anti-inflammatory Agents

Whole mushrooms and extracts may show anti-inflammatory activity due to the presence of bioactive compounds. Ethanolic extracts from *P. linteus* and a proteoglycan have been shown to induce anti-inflammatory effect in the collagen-induced arthritis and in the croton oil-induced ear edema test in mice and antinociceptive effect in the writhing test [82]. The edible mushroom *G. frondosa* contains ergosterol; ergosta-4-6-8(14), 22-tetraen-3-one; and 1-oleoyl-2-linoleoyl-3-palmitoyl glycerol which inhibits cyclooxygenases I and II activity [83].

7.10 Mushrooms as Hepatoprotective Agents

Ganoderic acids *R* and *S* and ganosporeric acid A from *G. lucidum* showed in vitro antihepatotoxic activity in the galactosamine-induced cytotoxic test with primary cultured rat hepatocytes [84]. In vivo study of two fractions of total triterpenoids extract of *G. lucidum* protected mice against hepatic necrosis induced by chloroform and D-galactosamine, and these hepatoprotective effects were perhaps related to the ability to promote the activity of scavenging enzymes for hepatic-free radicals in mice and thus to raise the ability of anti-oxidation in mice [85].

8 Bioactive Compounds in Mushroom

Mushrooms are widely used for their high nutritional value as a functional food. Additionally, they have been highly appreciated for their medicinal and therapeutic applications [86]. Interestingly, mushrooms are a rich source of biologically active compounds providing medicinal or health benefits such as the prevention and treatment of diseases to humans [38]. Edible mushrooms produce a vast diversity of bioactive compounds such as polysaccharides, proteoglycans, terpenoids, phenolic compounds, steroids, and lectins. These compounds have a wide range of therapeutic effects and can act as immunomodulatory, anticarcinogenic, antiviral, antioxidant, and anti-inflammatory agents [87].

Specific bioactive compounds in mushrooms are responsible for improving human health in a number of ways. Bioactive compounds can be found in mushrooms as well as their cell wall components as polysaccharides (β-glucans) and proteins or as secondary metabolites such as phenolic compounds, terpenes, and steroids. The concentration and efficacy of bioactive compounds are varied and depend on the type of mushroom, substrate, fruiting conditions, stage of development, age of mushroom, storage conditions, and of course cooking procedures [88]. On the basis of their chemical structure, bioactive compounds of mushrooms may be classified as peptides and proteins, phenolic compounds, polysaccharides, polysaccharides protein complexes, terpenes, terpenoids, etc.

8.1 Peptides and Proteins

Mushrooms produce many bioactive proteins and peptides, primarily lectins which are non-immune proteins or glycol proteins that bind specifically to fungal cell wall carbohydrates and have the ability of cell agglutination. These bioactive proteins possess enzymatic activities such as fungal immunomodulatory proteins (FIPs), ribosome-inactivating proteins (RIPs), and laccases. Anti-inflammatory peptides of different molecular weights have been isolated from mushrooms. Chu et al. [89] isolated an antifungal peptide (pleurostrin) from *P. ostreatus*, which exhibited antifungal activity. Wang et al. [90] isolated a peptide (SU2) from *Russula paludosa*, which showed antiviral properties. Ngai et al. [91] isolated an antifungal peptide (agrocybin) from fresh fruiting bodies of the mushroom *Agrocybe cylindracea* which exhibited antifungal activity against *Mycosphaerella arachidicola*, with an IC50 value of 125 μMat different temperatures up to 80 °C [92]. Cordymin, a low molecular weight peptide (10,906 Da) which shows anti-inflammatory activity, has been purified from *Cordyceps sinensis* [93, 94] and from *Cordyceps militaris* [95]. Liu et al. [96] isolated a xylose-specific lectin showing potent antimitogenic and antitumor activities from fresh fruiting bodies of *Xylaria hypoxylon*. It has been reported that lectins isolated from *Pholiota adiposa* and *H. erinaceum* exhibited antiviral and antitumor activities [97, 98]. Zhang et al. [99] isolated a lectin (32 kDa) from *Russula lepida*, which exhibited antitumor activity. Ribosome-inactivating proteins (RIPs) are enzymes that inactivate ribosomes by eliminating adenosine residues from rRNA. It has been reported that a ribosome-inactivating protein (marmorin) was isolated

from *Hypsizygus marmoreus* and showed antitumor activity [100]. Laccases are phenol oxidases widely diffused in basidiomycete and ascomycete fungi which they use to degrade lignocellulosic substrates. Laccases with antiviral activities have been isolated from *Pleurotus eryngii* [101] to *P. ostreatus* [102]. Zhang et al. [103] purified a laccase from *Clitocybe maxima*, which also showed antitumor activity. Some proteins targeting immune cells known as fungal immunomodulatory proteins (FIPs) are a new of group bioactive proteins and have been isolated from *F. velutipes* [104], *Ganoderma tsugae* [105], and *Volvariella volvacea* [106]. Lin et al. [98] isolated an immunomodulatory protein GM I from *Ganoderma microsporum*, which showed antimetastasis activity. Du et al. [107] purified a water-soluble Se-containing protein Se-GL-P (36 kDa) from the Se-enriched *G. lucidum*, which exhibited antitumor activity. A glycoprotein (PCP-3A), purified from *Pleurotus citrinopileatus*, showed antitumor activity [108]. Kodama et al. [109] isolated a low molecular weight protein fraction from *Grifola frondosa*, which showed antitumor activity.

8.2 Phenolic Compounds

Phenolic compounds are aromatic hydroxylated compounds with one or more aromatic rings and hydroxyl groups. Anti-inflammatory properties of many mushrooms have been attributed to the presence of some phenolic compounds which include phenolic acids, hydroxycinnamic acids, lignans, tannins, flavonoids, hydroxybenzoic acids, stilbenes, and oxidized polyphenols [110, 111]. It has been reported that these compounds exhibited act as free radical inhibitors, peroxide decomposers, metal inactivators, or oxygen scavengers [112, 113]. Palacios et al. [114] studied the antioxidant activity of phenolic compounds in various mushrooms and reported that *C. cibarius* and *C. cornucopioides* exhibited the greatest antioxidant effect with respect to the other mushroom species studied. The phenolic molecule pyrogallol has been extracted from *A. bisporus*, *C. cibarius*, and *L. deliciosus* [115, 116] which has been found to exhibit anti-inflammatory activity. Grifolin and grifolin derivatives are farnesyl phenolic compounds which showed anti-inflammatory properties and have been isolated from the edible mushroom *Albatrellus ovinus* [117]. It has been reported that phenol analogous compounds (hericenones C, D, E, F, G, H) isolated from *H. erinaceus* had antineurodegenerative properties [46] and antioxidant activity [118]. Attarat and Phermthai [119] reported that catechin, a major group of phenolic compounds isolated from *Lentinus squarrosulus*, *Lentinus polychrous*, and *L. edodes*, exhibited antioxidant activity. Chowdhury et al. [120] isolated phenolic compounds and flavonoids from *P. ostreatus*, *L. edodes*, and *Hypsizygus tessellatus*, which showed antioxidant, antifungal, and antibacterial properties. Dai et al. [121] reported that hispidin, a class of polyphenols, is an important medicinal metabolite from *Phellinus* spp. In most of these studies, a positive correlation was found between the total phenolic content in the mushroom extracts and their antioxidative properties, which confirms that edible mushrooms have a potential as natural antioxidants due to the ability of their phenolics to inhibit lipid oxidation.

8.3 Polysaccharides

Polysaccharides are the major class of bioactive compounds found in mushrooms and possess significant immune-stimulating, antitumor, antioxidant, antibacterial, and antiviral activities [122]. Fungal polysaccharides are the most potent mushroom-derived substances with antitumor and immunomodulating properties. They are present in cell wall with different types of glycosidic linkages. Mushroom polysaccharides with anti-inflammatory properties have been reported in crude extracts of *Lentinus polychrous*, *Termitomyces albuminosus*, and *Phellinus linteus*. Fungal pigment melanin possesses antioxidant, immunomodulating, antimutagenic, and radioprotective properties [123]. The polysaccharides of *Flammulina velutipes* are composed of three monosaccharides (glucose, mannose, and xylose) and have been found to have anti-inflammatory activities [124].

8.4 Glucans

β-glucan is one of the key components of several basidiomycetes and ascomycetes cell wall. It is a long-chain polysaccharide with β-D glucose as basic subunit linked to one another by one to three glycosidic chain with one to six glycosidic branches. β-glucans are able to enhance the immune system and prevent and treat several common diseases to promote health [125]. Fruit body extracts of *Pleurotus pulmonarius* showed mixed α-linkages and β-anomeric carbon linkages, whereas polysaccharide from mycelial extracts has mainly α-glucan linkages [126]. Lentinan, the bioactive glucan from *Lentinula edodes* showed immunomodulatory and antitumor activities [127]. Schizophyllan is the active β-glucan from *Schizophyllum commune* [128]. Glucans such as (1,3)-glucopyranosyl from *Pleurotus pulmonarius* have been reported to exhibit anti-inflammatory properties [38]. Lavi et al. [129] reported that ganoderan A and B glucans of *Ganoderma lucidum* fruiting bodies showed hypoglycemic effects. On the other hand, ganopoly, the polysaccharide-containing preparation of *G. lucidum*, exhibited hepatoprotective effects in patients with chronic hepatitis B [130]. A β-glucan isolated from the fruiting bodies of *P. ostreatus* has also been proven to exert antitumor activity against Hela tumor cell [131]. Additionally, glucans such as (1,3)- D-glucopyranosyl from *P. pulmonarius* have been reported to exhibit anti-inflammatory properties [124]. Lavi et al. [129] reported that polysaccharides of *Flammulina velutipes* are found to have anti-inflammatory activities [124].

8.5 Polysaccharide: Protein Complexes

Some polysaccharides such as polysaccharide-K (polysaccharide-Kureha; PSK) also known as krestin isolated from *Trametes versicolor* have been identified as

polysaccharide–protein complexes and showed antimetastatic activity [14]. Coriolan, a β-glucanprotein complex obtained from submerged grown *T. versicolor* biomass, exhibited hypoglycemic effects and ameliorated the symptoms of diabetes [38]. Chatterjee et al. [132] isolated calvacin, a moderately heat stable, nondiffusible basic mucoprotein from *Calvatia gigantea* which showed antitumor activity. On the other hand, ethanolic extracts and a proteoglycan purified from *Phellinus linteus* showed anti-inflammatory properties [82, 133].

8.6 Terpenes and Triterpenoids

Terpenes are the largest group of anti-inflammatory compounds in mushrooms. Several terpenes isolated from *G. lucidum* showed anti-inflammatory activity. Some triterpenes from *G. lucidum* (ganoderic acid C and derivatives) are able to inhibit the biosynthesis of cholesterol [109], while other triterpenes (ganoderic acid F) contribute to atherosclerosis protection [134]. The antioxidative and free radical effects of triterpenoids from *G. lucidum* have also been shown [38]. El-Mekkawy et al. [76] reported that different triterpenes from *G. lucidum* (i.e., ganoderiol, ganodermanontriol, and ganoderic acid) showed antiviral activity. Sterols and triterpenes (lucialdehyde D, ganoderone A, and ganoderone C) have been isolated from the fruiting bodies of *Ganoderma pfeifferi*. Several triterpenes (trametenolic acid; ergosterol peroxide; 3b-hydroxy-8, 24-dien-21-al; ergosterol; and inotodiol) isolated from the sclerotia of *I. obliquus* exhibited anti-inflammatory and anticancer activities [135]. Han et al. [136] isolated five novel cyathane diterpenes (identified as cyathins DH) and three diterpenes (neosarcodonin, cyathatriol, and 11-*O*-acetylcyathatriol) from *Cyathus Africans*, which showed potent anti-inflammatory properties. Chen et al. [137] reported that several triterpenes isolated from *Antrodia camphorata* which showed neuroprotective activity.

9 Mushroom-Based Biotech Products

Mushroom-based healthcare commercial biotech products with preventive and curative effects are today available in dried forms as functional food additives (nutraceuticals) and are largely sold and consumed in the world market. Owing to the synergistic action of bioactive molecules, majority of mushroom products possess beneficial health effects and can be used on a regular basis without undesirable side effects. Healthy food developed from biotechnologically cultivated mycelia of medicinal edible mushrooms *Hericium erinaceus* and *Tremella* spp. in combination with other natural substances (medicinal plants, algae, etc.) possess antioxidant and immune-stimulating activity and regulate the level of blood lipids and sugar [138, 139]. Some mushroom products are able to decrease high glucose and lipid levels in blood and are recommended as neuro- and vasotonics,

hepatoprotective, and thrombolytic agents [87]. Nutritive, anti-inflammatory, regenerative, and antioxidant properties of several mushrooms make their usage perspective in manufacturing of cosmetic products [140]. Mushrooms are currently proposed as highly active ingredients in world production of hair and skin care products. *Tremella* mushrooms contain hydrophilic agent–polysaccharide glucuronoxylomannan (GXM) with anti-inflammatory and wound healing properties largely used in cosmetology [141]. *Tremella* cosmetic products are applicable in treatment of neurodermatitis and sclerodermatitis. They prevent skin pigmentation and stimulate blood circulation. Biological characteristics of mycelia, particularly fast growth and easy reproduction in culture conditions, are assisting biotechnological cultivation of medicinal mushrooms to obtain desired bioactive molecules and biotech products. Thus, mushrooms have significant biotechnological potential.

10 Role of Mushroom Culture Collections

Currently, two different approaches in biotechnological cultivation of mushrooms are used. First is fruit body production which is a long-term process and takes 1–2 months, while cultivation of mycelia takes a few days. Establishment and maintenance of culture collections of different group of mushrooms are of valuable importance to study their biodiversity, genetic resources, and biotechnological potential. The submerged cultivation of mycelia is the best technique to obtain biomass and desired bioactive molecules for further development of consistent and safe healthcare mushroom biotech products and thus has significant industrial potential.

11 Future Trends

Mushrooms can make an important contribution to the livelihoods of rural and semi-urban dwellers through food security and income generation, and mushrooms can make a valuable dietary addition through their protein and micronutrient content as well as their medicinal properties. Its cultivation is highly compatible with a variety of other traditional agricultural and domestic activities and can make a particularly important contribution to the livelihoods of the disabled, of women, and of the landless poor who, with appropriate training and access to inputs, can increase their independence and self. The high benefit/cost ratio, the easily obtained and inexpensive agrowastes, and congenial weather conditions make adoption of mushroom cultivation a lucrative means of societal development. Mushroom cultivation can represent a valuable small-scale enterprise option. The FAO has been actively promoting mushroom cultivation for rural development and food security in developing countries [142].

India has tremendous potential for mushroom production, and all commercial edible and medicinal mushrooms can be easily grown here. Although, India is not a

major producer of any of the mushroom varieties, it does cultivate all edible and medicinal mushrooms in one or other part, due to its diverse climatic conditions. Mushroom cultivation is based on recycling of agricultural residues, which are available in huge quantities in every nook and corner of the country. At present, the areas with rice–wheat cropping system of India are facing tough challenge to handle the mounting crop residues. Mushroom cultivation can effectively utilize these agroresidues for production of protein rich food and plays crucial role in management of these agroresidues. The supply and demand gap in the world trade of mushrooms and the shrinkage of production in western countries due to high labor costs have resulted in better market prices for Indian mushroom producers. With a domestic population of more than 1.2 billion, India itself is a large market for mushrooms. To be successful in both domestic and export market, it is essential to produce quality fresh mushrooms and mushroom fortified value-added products at competitive rates without any agrochemical residues. Efforts should also be made to exploit the commercial utilization of mushroom substrate left after cultivation for preparation of organic manure, vermicompost, briquettes, etc. One final reason for optimism concerning India's potential as a major mushroom producer is its strategic geographical location, making it more convenient to export mushrooms to the Middle East, Southeast Asia, and European countries. To be successful in both domestic and export markets, it is essential to produce quality fresh mushrooms and processed products devoid of pesticide residues and at competitive prices. However, mushroom cultivation is yet to be adopted by Indian farmers and mushroom growers on a large scale. The gap in technology knowledge and its adoption needs to be bridged by providing training to farmers regarding various aspects of mushroom cultivation.

Mushrooms are functional food and are a source of biologically valuable components that offer great therapeutic potential for the prevention and control of several diseases. Further research and clinical trials needs to be carried out to validate that mushrooms are source of bioactive molecules with medicinal applications. They may be used directly in the diet to promote health, taking advantage of the additive and synergistic effects of the bioactive compounds present in them. Research is needed to elucidate the different roles of multiple active compounds and the pathways involved. The potential therapeutic implications of mushrooms are enormous but detailed mechanisms of the various health benefits of mushrooms to humans still require intensive investigation, especially with the emergence of new evidence of their health benefit effects. The exploration of newly cultivated mushrooms and isolation of their active ingredients with mechanism-based potential therapeutic value remains a challenge, and hence mushrooms will keep on to be the foremost spotlight of research in the upcoming prospect as well. Medicinal mushrooms represent a growing segment of today's pharmaceutical industry owing to the plethora of useful bioactive compounds. Conservation and cloning of therapeutic mushrooms is needed for sustainable development. Dedicated research should be undertaken to isolate, purify, and structural1ly investigate of novel anticancer and immune-stimulator compounds.


White button mushroom (*Agaricus bisporus*)

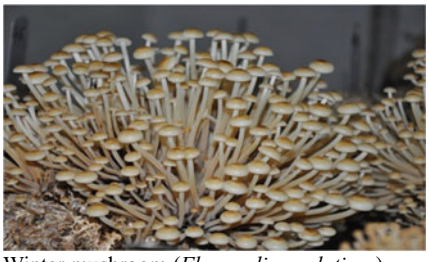

Brown button mushroom (*Agaricus bisporus*)


Milky mushroom (*Calocybe indica*)


Macrocybe mushroom (*Macrocybe gigantea*)


Shiitake mushroom (*Lentinula edodes*)


Paddy straw mushroom (*Volvariella volvacea*)


Winter mushroom (*Flammulina velutipes*)


Reishi mushroom (*Ganoderma lucidum*)

(*continued*)


Gray oyster (*Pleurotus pulmonarius*)


Pink oyster (*Pleurotus djamor*)


Blue oyster (*Hypsizygus ulmarius*)


Yellow oyster (*Pleurotus cornucopiae*)

References

1. Chang ST, Miles PG (1982) Introduction to mushroom science. In: Chang ST, Quimio TH (eds) Tropical mushrooms: biological nature and cultivation methods. Chinese University Press, Hong Kong, pp 3–10
2. Chamberlain J, Bush R, Hammett A (1998) Non-timber forest products: the other forest products. For Prod J 48:10–19
3. Falconer J, Koppell CRS (1990) The major significance of 'minor' forest products: the local use and value of forests in the West African humid forest zone. FAO Community Forestry Note; Food and Agriculture Organization of the United Nations, Roma
4. Gilbert FA, Robinson RF (1957) Food from fungi. Econ Bot 11:126–145
5. Vinceti B, Termote C, Ickowitz A, Powell B, Kehlenbeck K, Hunter D (2013) The contribution of forests and trees to sustainable diets. Sustainability 5:4797–4824
6. Chang ST (2006) The world mushroom industry: trends and technological development. Int J Med Mushrooms 8:297–314
7. Arora D (2008) Notes on economic mushrooms. Econ Bot 62:540–544
8. Yang X, He J, Li C, Ma J, Yang, Xu J (2008) Matsutake trade in Yunnan Province, China: an overview. Econ Bot 62:269–277
9. Fanzo J, Cogill B, Mattei F (2012) Metrics of sustainable diets and food systems. In: Technical brief-Madrid roundtable. Bioversity International and Daniel and Nina Carasso Foundation, Rome
10. Chang ST, Wasser SP (2017) The cultivation and environmental impact of mushrooms. Printed from the oxford Research Encyclopaedia, Environmental Science (c) Oxford University Press, p 43
11. Kirk PM, Cannon PF, David JC, Stalpers JA (2008) Ainsworth & Brisby's dictionary of the fungi, 10th edn. CAB International, Wallingford

12. Hawksworth DL (2012) Global species numbers of fungi: are tropical studies and molecular approaches contributing to more robust estimate? Biodivers Conserv 21:2425–2433
13. Wasser SP (2010) Medicinal mushroom science: history, current status, future trends, and unsolved problems. Int J Med Mushrooms 12(1):1–16
14. Wasser SP (2002) Review of medicinal mushrooms advances: good news from old allies. Herbal Gram 56:28–33
15. Wasser SP, Weis AL (1999) Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives. Int J Med Mushrooms 1:31–62
16. Chang ST, Wasser SP (2017) The cultivation and environmental impact of mushrooms. Agric Environ. https://doi.org/10.1093/acrefore/9780199389414.013.231
17. Singh M, Kamal S, Sharma VP (2017) Status and trends in world mushroom production-I. Mushroom Res 26(1):1–20
18. Li Y (2012) Present development situation and tendency of edible mushroom industry in China. In: Zhang J, Hexiang W, Mingjie C (eds) Proceedings of 18th congress of the international society of mushroom science. China Agriculture Press, Beijing, pp 1–9
19. Gupta S, Summuna B, Moni G, Mantoo A (2016) Mushroom cultivation: a means of nutritional security in India. Asia Pac J Food Saf Secur 2(1):3–12
20. Sharma VP, Sudheer KA, Gautam Y, Singh M, Kamal S (2017) Status of mushroom production in India. Mushroom Res 26(2):111–120
21. Satish K, Sharma VP, Shirur M, Kamal S (2017) Status of milky mushroom (*Calocybe indica*) in India – a review. Mushroom Res 26(1):21–39
22. Shirur M, Shivalingegowda NS (2015) Mushroom marketing channels and consumer behaviour: a critical analysis. Mysore J Agric Sci 49(2):390–393
23. Zhanxi, Zhanhua (2000) Training manual of APEMT China-chapter 11, *Volvariella volvacea* cultivation, pp 100–109
24. Ahlawat OP, Tewari RP (2007) Cultivation technology of paddy straw mushroom (*Volvariella volvacea*). Technical bulletin. National Research Centre for Mushroom (ICAR), Chamaghat, p 36
25. Manzi P, Aguzzi A, Pizzoferrato L (2001) Nutritional value of mushrooms widely consumed in Italy. Food Chem 73:321–325
26. Sánchez C (2010) Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Appl Microbiol Biotechnol 85(5):1321–1337
27. Dundar A, Acy H, Yildiz A (2008) Yield performance and nutritional contents of three oyster mushroom species cultivated on wheat stalk. Afr J Biotechnol 7:3497–3501
28. Pavel K (2009) Chemical composition and nutritional value of European species of wild growing mushrooms: a review. Food Chem 113(1):9–16
29. Heleno SA, Barros L, Sousa MJ, Martins A, Ferreira ICFR (2010) Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. Food Chem 119:1443–1450
30. Mattila P, Konko K, Euvola M, Pihlava J, Astola J, Vahteristo L (2001) Contents of vitamins, mineral elements and some phenolic compound in cultivated mushrooms. J Agric Food Chem 42:2449–2453
31. Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira IC (2008) Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem Toxicol 46:2742–2747
32. Pehrsson PR, Haytowitz DB, Holden JM (2003) The USDA's national food and nutrient analysis program: update 2002. J Food Compos Anal 16:331–341
33. Sánchez C (2004) Modern aspects of mushroom culture technology. Appl Microbiol Biotechnol 64(6):756–762
34. Ferreira ICFR, Barros L, Abreu RMV (2009) Antioxidants in wild mushrooms. Curr Med Chem 16:1543–1560
35. Pereira E, Barros L, Martins A, Ferreira ICFR (2012) Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats. Food Chem 130:394–403
36. Vaz JA, Heleno SA, Martins A, Almeida GM, Vasconcelos MH, Ferreira ICFR (2010) Wild mushrooms *Clitocybe alexandri* and *Lepista inversa*: in vitro antioxidant activity and growth inhibition of human tumour cell lines. Food Chem Toxicol 48:2881–2884

37. Ahlawat OP, Manikandan K, Singh M (2016) Proximate composition of different mushroom varieties and effect of UV light exposure on vitamin D content in *Agaricus bisporus* and *Volvariella volvacea*. Mushroom Res 25(1):1–8
38. Rathee S, Rathee D, Rathee D (2012) Mushrooms as therapeutic agents. Braz J Pharmacogn 22(2):459–474
39. Sadler M, Saltmarsh M (1998) Functional foods: the consumer, the products and the evidence. Royal Society of Chemistry, Cambridge, UK
40. Longvah T, Deosthale YG (1998) Composition and nutritional studies on edible wild mushroom from Northeast India. Food Chem 63:331–334
41. Maga JA (1981) Mushroom flavor. J Agric Food Chem 29:1–4
42. Clifford AJ, Heid MK, Peerson JM, Bills ND (1991) Bioavailability of food folates and evaluation of food matrix effects with a rat bioassay. J Nutr 121:445–453
43. Bano Z, Rajarathnam S (1988) *Pleurotus* mushrooms. Part II. Chemical composition, nutritional value, post-harvest physiology, preservation, and role as human food. Crit Rev Food Sci Nutr 27:87–158
44. Mau JL, Chao GR, Wu KT (2001) Antioxidant properties of methanolic extracts from several ear mushrooms. J Agric Food Chem 49:5461–5467
45. Ribeiroa B, Pinhoa PG, Andradea PB, Baptistab P, Valentao P (2009) Fatty acid composition of wild edible mushrooms species: a comparative study. Microchem J 93:29–35
46. Xu T, Beelman RB (2015) The bioactive compounds in medicinal mushrooms have potential protective effects against neurodegenerative diseases. Adv Food Technol Nutr Sci Open J 1(2):62–65. https://doi.org/10.17140/AFTNSOJ-1-110
47. Yip KP, Fung KP, Chang ST, Tam SC (1987) Purification and mechanism of the hypotensive action of an extract from edible mushroom *Pleurotus sajor-caju*. Neurosci Lett Suppl 28:559
48. Wang HX, Liu WK, Ng TB et al (1996) The immunomodulatory and antitumor activities of lectins from the mushroom *Tricholoma mongolicum*. Immunopharmacology 31(2–3): 205–211. https://doi.org/10.1016/0162-3109(95)00049-6
49. Chang ST, Buswell JA, Chiu SW (1993) Mushroom biology and mushroom products. The Chinese University Press, Hong Kong
50. Kasuga A, Aoyagi Y, Sugahara T (1993) Antioxidative activities of several mushroom extracts. J Jpn Soc Food Sci Technol 40:56–63
51. Mau JL, Lin HC, Song SF (2002) Antioxidant properties of several specialty mushrooms. Food Res Int 35:519–526
52. Sun MT, Xiao JT, Zhang SQ, Liu YJ, Li ST (1984) Therapeutic effect of some foods on hyperlipidermia in man. Acta Nutr Sin 6:127–133
53. Tokita F, Shibukawa N, Yasumoto T, Kaneda T (1972) Isolation and chemical structure of the plasma-cholesterol reducing substance from shiitake mushroom. Mushroom Sci 8:783–788
54. Ryong LH, Tertov VV, Vasiley AW, Tutelyan VA, Orekhov AN (1989) Antiatherogenic and antiatherosclerotic effects of mushroom extracts revealed in human aortic intima cell culture. Drug Dev Res 17:109–117
55. Cimerman NG (1999) Medicinal value of the genus *Pleurotus* (Fr.) P. Karst. (Agaricales S. R., basidiomycetes). Int J Med Mushrooms 1:69–80
56. Cheung PCK (1996) The hypocholesterolemic effect of two edible mushrooms: *Auricularia auricula* (tree ear) and *Tremella fuciformis* (white jelly-leaf) in hypercholesterolemic rats. Nutr Res 16:1721–1725
57. Kabir Y, Kimura S (1989) Dietary mushrooms reduce blood pressure in spontaneously hypertensive rats. J Nutr Sci Vitaminol 35:91–94
58. Chen Q (1989) Antilipemic effect of polysaccharides from *Auricularia auricular, Tremella fuciformis, and Tremella fuciformis spores*. Zhongguo Yaoke Daxue Xuebao 20:344–347
59. Sheng J, Chen Q (1990) Effects of polysaccharides from *Auricularia auricula*, *Tremella fuciformis*, and *Tremella fuciformis* spores on experimental thrombin formation. Zhongguo Yaoke Daxue Xuebae 21:39–42
60. Mizuno T (1995) Bioactive biomolecules of mushrooms: food function and medicinal effect of mushroom fungi. Food Rev Int 11:7–12

61. Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilara-Contreras A, Contreras-Weber CC, Flores-Sanez JL (1998) Study of the antihyperglycemic effect of plants used as antidiabetics. J Ethnopharmacol 61:101–110
62. Ahmad N, Bansal AK, Kidwai JR (1984) Effect of PHA-B fraction of Agaricus bisporus lectin on insulin release and 45Ca2C uptake by islet of Langerhans in vitro. Acta Diabetol 21:63–70
63. Gray AM, Flatt PR (1998) Insulin-releasing and insulin-like activity of *Agaricus campestris* (mushroom). J Endocrinol 157:259–266
64. Windholz M (1983) The Merck index, 10th edn. Merck and Co, Rahway
65. Thekkuttuparambil AA, Kainoor K (2007) Janardhanan. Indian medicinal mushrooms as a source of antioxidant and antitumor agents. J Clin Biochem Nutr 40:157–162
66. Lucas EH, Montesano R, Pepper MS, Hafner M, Sablon E (1957) Tumor inhibitors in *Boletus edulis* and other holobasidiomycetes. Antibiot Chemother 7:1–4
67. Lucas EH, Byerrum M, Clarke DA, Reilly HC, Stevens JA, Stock CC (1958) Production of oncostatic principles in vivo and in vitro by species of the genus *Calvatia*. Antibiot Annu 6:493–496
68. Reshetnikov SV, Wasser SP, Tan KK (2001) Higher basidiomycetes as a source of antitumor and immunostimulating polysaccharides (review). Int J Med Mushrooms 3:361–394
69. Lindequist U, Teuscher E, Narbe G (1990) Neue Wirkstoffe aus Basidiomyceten. Phytothérapie 11:139–149
70. Eo SK, Kim YS, Lee CK, Han SS (1999) Antiviral activities of various water and methanol soluble substances isolated from *Ganoderma lucidum*. J Ethnopharmacol 68:129–136
71. Takazawa H, Tajima F, Miyashita C (1982) An antifungal compound from "shiitake" (*Lentinus edodes*). Yakugaku Zasshi 102:489–491
72. Mothana RAA, Jansen R, Julich WD, Lindequist U (2000) Ganomycin A and B, new antimicrobial farnesyl hydroquinones from the basidiomycete *Ganoderma pfeifferi*. J Nat Prod 63:416–418
73. Bender S, Dumitrache CN, Backhaus J, Christie G, Cross RF, Lonergan GT (2003) A case for caution in assessing the antibiotic activity of extracts of culinary-medicinal shiitake mushroom [*Lentinus edodes* (Berk.) singer] (Agaricomycetidae). Int J Med Mushrooms 5:31–35
74. Badalyan SM (2004) Antiprotozoal activity and mitogenic effect of mycelium of culinary-medicinal shiitake mushroom *Lentinus edodes* (Berk.) singer (Agaricomycetidae). Int J Med Mushrooms 6:131–138
75. Brandt CR, Piraino F (2000) Mushroom antivirals. Recent Res Dev Antimicrob Agents Chemother 4:11–26
76. El-Mekkawy S, Meselhy MR, Nakamura N, Tezuka Y, Hattori M, Kakiuchi N (1998) Anti-HIV-1 and antiHIV-1-protease substances from *Ganoderma lucidum*. Phytochemistry 49(6): 1651–1657. https://doi.org/10.1016/S0031-9422(98)00254-4
77. Sano M, Yoshino K, Matsuzawa T, Ikekawa T (2002) Inhibitory effects of edible higher basidiomycetes mushroom extracts on mouse type IV allergy. Int J Med Mushrooms 4:37–41
78. Kohda H, Tokumoto W, Sakamoto K, Fujii M, Hirai Y, Yamasaki K (1985) The biologically-active constituents of *Ganoderma lucidum* (Fr) Karst-histamine release-inhibitory triterpenes. Chem Pharm Bull 33:1367–1373
79. Tasaka K, Mio M, Izushi K, Akagi M, Makino T (1988) Anti-allergic constituents in the culture medium of *Ganoderma lucidum*. (II). The inhibitory effect of cyclooctasulfur on histamine release. Agents Actions 23:157–160
80. Kreisel H, Lindequist U, Horak M (1990) Distribution, ecology and immunosuppressive properties of *Tricholoma populinum* (Basidiomycetes). Zentralbl Mikrobiol 145:393–396
81. Ali NAA, Pilgrim H, Ludke J, Lindequist U (1996) Inhibition of chemiluminescence response of human mononuclear cells and suppression of mitogen induced proliferation of spleen lymphocytes of mice by hispolon and hispidin. Pharmazie 51:667–670
82. Kim SH, Song YS, Kim SK, Kim BC, Lim CJ, Park EH (2004) Anti-inflammatory and related pharmacological activities of the n-BuOH subfraction of mushroom *Phellinus linteus*. J Ethnopharmacol 93:141–146

83. Zhang Y, Mills G, Nair MG (2002) Cyclooxygenase inhibitory and antioxidant compounds from the mycelia of the edible mushroom *Grifola frondosa*. J Agric Food Chem 50:7581–7585
84. Chen RY, Yu DQ (1993) Studies on the triterpenoid constituents of the spores from *Ganoderma lucidum* Karst. J Chin Pharm Sci 2:91–96
85. Wang MY, Liu Q, Che QM, Lin ZB (2002) Effects of total triterpenoids extract from *Ganoderma lucidum* (Curt.:Fr.) P.Karst. (Reishi mushroom) on experimental liver injury models induced by carbon tetrachloride or D-galactosamine in mice. Int J Med Mushrooms 4:337–342
86. Chang ST, Miles PG (2004) Mushrooms: cultivation, nutritional value, medicinal effect and environmental impact, 1st edn. CRC Press, Boca Raton
87. Badalyan SM (2012) Edible ectomycorrhizal mushrooms. In: Zambonelli A, Bonito G (eds) Edible ectomycorrhizal mushrooms. Soil Biology series, vol 34. Springer, Berlin, pp 317–334. ISBN: 978-3-642-33822-9
88. Guillamón S, García-Lafuente A, Lozano M et al (2010) Edible mushrooms: role in the prevention of cardiovascular diseases. Fitoterapia 81(7):715–723
89. Chu KT, Xia LX, Ng TB (2005) Pleurostrin, an antifungal peptide from the oyster mushroom. Peptides 26(11):2098–2103
90. Wang JB, Wang HX, Ng TB (2007) A peptide with HIV-1 reverse transcriptase inhibitory activity from the medicinal mushroom *Russula paludosa*. Peptides 28(3):560–565
91. Ngai PHK, Zhao Z, Ng TB (2005) Agrocybin, an antifungal peptide from the edible mushroom *Agrocybe cylindracea*. Peptides 26(2):191–196
92. Zhang Y, Mills GL, Nair MG (2003) Cyclooxygenase inhibitory and antioxidant compounds from the fruiting body of an edible mushroom, *Agrocybe aegerita*. Phytomedicine 10(5): 386–390
93. Wang J, Liu YM, Cao W et al (2012) Anti-inflammation and antioxidant effect of cordymin, a peptide purified from the medicinal mushroom *Cordyceps sinensis,* in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. Metab Brain Dis 27(2):159–165
94. Qian GM, Pan GF, Guo JY (2011) Anti-inflammatory and antinociceptive effects of cordymin, a peptide purified from the medicinal mushroom *Cordyceps sinensis*. Nat Prod Res 26(24): 2358–2362
95. Wong JH, Ng TB, Wang H et al (2011) Cordymin, an antifungal peptide from the medicinal fungus *Cordyceps militaris*. Phytomedicine 18(5):387–392
96. Liu QH, Wang HX, Ng TB (2006) First report of a xylose-specific lectin with potent hemagglutinating, antiproliferative and anti-mitogenic activities from a wild ascomycete mushroom. Biochim Biophys Acta 1760(12):1914–1919. https://doi.org/10.1016/j.bbagen.2006.07.010
97. Zhang GQ, Sun J, Wang HX (2009) A novel lectin with antiproliferative activity from the medicinal mushroom *Pholiota adiposa*. Acta Biochim Pol 56(3):415–421
98. Lin CH, Sheu GT, Lin YW et al (2010) A new immunomodulatory protein from *Ganoderma microsporum* inhibits epidermal growth factor mediated migration and invasion in A549 lung cancer cells. Process Biochem 45(9):1537–1542. https://doi.org/10.1016/j.procbio.2010.06.006
99. Zhang G, Sun J, Wang H et al (2010a) First isolation and characterization of a novel lectin with potent antitumor activity from a Russula mushroom. Phytomedicine 17(10):775–781. https://doi.org/10.1016/j.phymed.2010.02.001
100. Wong JH, Wang HX, Ng TB (2008) Marmorin, a new ribosome inactivating protein with antiproliferative and HIV-1 reverse transcriptase inhibitory activities from the mushroom *Hypsizigus marmoreus*. Appl Microbiol Biotechnol 81(4):669–674
101. Wang HX, Ng TB (2006a) Purification of a laccase from fruiting bodies of the mushroom *Pleurotus eryngii*. Appl Microbiol Biotechnol 69(5):521–525
102. El Fakharany EM, Haroun BM, Ng TB et al (2010) Oyster mushroom laccase inhibits hepatitis C virus entry into peripheral blood cells and hepatoma cells. Protein Pept Lett 17(8): 1031–1039. https://doi.org/10.2174/092986610791498948

103. Zhang GQ, Wang YF, Zhang XQ (2010b) Purification and characterization of a novel laccase from the edible mushroom *Clitocybe maxima*. Process Biochem 45(5):627–633. https://doi.org/10.1016/j.procbio.2009.12.010
104. Ko JL, Hsu CT, Lin RH et al (1995) A new fungal immunomodulatory protein, FIP-fve isolated from the edible mushroom, *Flammulina velutipes* and its complete amino acid sequence. Eur J Biochem 228:244–249
105. Lin WH, Huang CH, Hsu CI et al (1997) Dimerization of the N-terminal amphipathic a-helix domain of the fungal immunomodulatory protein from *Ganoderma tsugae* (Fip-gts) defined by a yeast two-hybrid system and site-directed mutagenesis. J Biol Chem 272:2044–2048
106. Hsu HC, Hsu CI, Lin RH et al (1997) Fip-vvo, a new fungal immunomodulatory protein isolated from *Volvariella volvacea*. Biochem J 323:557–565
107. Du M, Zhao L, Li CR (2007) Purification and characterization of a novel fungi Se-containing protein from Se-enriched *Ganoderma lucidum* mushroom and its Se-dependent radical scavenging activity. Eur Food Res Technol 224(5):659–665
108. Chen JN, Wang YT, Wu JS (2009) A glycoprotein extracted from golden oyster mushroom *Pleurotus citrinopileatus* exhibiting growth inhibitory effect against U937 leukemia cells. J Agric Food Chem 57(15):6706–6711. https://doi.org/10.1021/jf901284s
109. Kodama N, Komuta K, Nanba H (2002) Can maitake MD fraction aid cancer patients? Altern Med Rev 7:236–239
110. Cote J, Caillet S, Doyon G (2010) Bioactive compounds in cranberries and their biological properties. Crit Rev Food Sci Nutr 50(7):666–679
111. D'Archivio M, Filesi C, Vari R et al (2010) Bioavailability of the polyphenols: status and controversies. Int J Mol Sci 11:1321–1342
112. Dziezak JD (1986) Antioxidants-the ultimate answer to oxidation. Food Technol 40(9):94
113. Yagi K (1970) A rapid method for evaluation of oxidation and antioxidants. Agric Biol Chem 34(1):142–145
114. Palacios I, Lozano M, Moro C (2011) Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chem 128(3):674–678. https://doi.org/10.1016/j.foodchem.2011.03.085
115. Dugler B, Gonuz A, Gucin F (2004) Antimicrobial activity of the macrofungus *Cantharellus cibarius*. JBS 7(9):1535–1539
116. Witkowska MA, Zujko ME, Mironczuk-Chodakowska I (2011) Comparative study of wild edible mushrooms as sources of antioxidants. Int J Med Mushrooms 13(4):335–341
117. Nukata M, Hashimoto T, Yamamoto I et al (2002) Neogrifolin derivatives possessing antioxidative activity from the mushroom *Albatrellus ovinus*. Phytochemistry 59(7):731–737
118. Mizuno T (1999) Bioactive substances in *Hericium erinaceus* (Bull.:Fr.) Pers. (Yamabushitake), and its medicinal utilization. Int J Med Mushrooms 1:105–119. https://doi.org/10.1615/IntJMedMushrooms.v1.i2.10
119. Attarat J, Phermthai T (2015) Bioactive compounds in three edible *Lentinus* mushrooms. Walailak J Sci Technol 12(6):491–504
120. Chowdhury MMH, Kubra K, Ahmed SR (2015) Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh. Ann Clin Microbiol Antimicrob 14:8. https://doi.org/10.1186/s12941-015-0067-3
121. Dai YC, Zhou LW, Cui BK et al (2010) Current advances in Phellinus sensu lato: medicinal species, functions, metabolites and mechanisms. Appl Microbiol Biotechnol 87(5):1587–1593
122. Elsayed EA, Enshasy HE, Wadaan MAM et al (2014) Mushrooms: a potential natural source of anti-inflammatory compounds for medical applications. Mediat Inflamm 1:1–15
123. Badalyan SM (2014) Potential of mushroom bioactive molecules to develop healthcare biotech products. In: Proceedings of the 8th international conference on mushroom biology and mushroom products (ICMBMP8)
124. Wu DM, Duan WQ, Liu Y, Cen Y (2010) Anti-inflammatory effect of the polysaccharides of Golden needle mushroom in burned rats. Int J Biol Macromol 46(1):100–103. https://doi.org/10.1016/j.ijbiomac.2009.10.013

125. Batbayar S, Lee DH, Kim HW (2012) Immunomodulation of fungal b-glucan in host defense signaling by dectin-1. Biomol Ther 20(5):433–445
126. Lavi I, Levinson D, Peri I et al (2010) Chemical characterization, antiproliferative and antiadhesive properties of polysaccharides extracted from *Pleurotus pulmonarius* mycelium and fruiting bodies. Appl Microbiol Biotechnol 85(6):1977–1990
127. Firenzuoli F, Gori L, Lombardo G (2007) The medicinal mushroom *Agaricus blazei* murrill: review of literature and pharmaco-toxicological problems. Evid Based Complement Altern Med 5(1):3–15
128. Bae AH, Lee SW, Ikeda M et al (2004) Rod-like architecture and helicity of the poly(C)/schizophyllan complex observed by AFM and SEM. Carbohydr Res 339(2):251–258
129. Lavi I, Nimri L, Levinson D et al (2012) Glucans from the edible mushroom *Pleurotus pulmonarius* inhibit colitis-associated colon carcinogenesis in mice. J Gastroenterol 47(5): 504–518
130. Gao Y, Zhou S, Chen G et al (2002) A phase I/II study of a *Ganoderma lucidum* (Curt.:Fr.) P. Karst (LingZhi, Reishi mushroom) extract in patients with chronic hepatitis B. Int J Med Mushrooms 4(4):2321–2327
131. Tong H, Xia F, Feng K et al (2009) Structural characterization and in vitro antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. Bioresour Technol 100:1682–1686. https://doi.org/10.1016/j.biortech.2008.09.004
132. Chatterjee S, Biswas G, Basu SK (2011) Antineoplastic effect of mushrooms: a review. Aust J Crop Sci 5(7):904–911
133. Kim GY, Kim SH, Hwang SY et al (2003) Oral administration of proteoglycan isolated from *Phellinus linteus* in the prevention and treatment of collagen-induced arthritis in mice. Biol Pharm Bull 26:823–831
134. Morigiwa A, Kitabatake K, Fujimoto Y et al (1986) Angiotensin converting enzyme inhibitory triterpenes from *Ganoderma lucidum*. Chem Pharm Bull 34:3025–3028
135. Ma L, Chen H, Dong P et al (2013) Anti-inflammatory and anticancer activities of extracts and compounds from the mushroom *Inonotus obliquus*. Food Chem 139(1–4):503–508. https://doi.org/10.1016/j.foodchem.2013.01.030
136. Han J, Chen Y, Bao L (2013) Anti-inflammatory and cytotoxic cyathane diterpenoids from the medicinal fungus *Cyathus africanus*. Fitoterapia 84:22–31. https://doi.org/10.1016/j.fitote.2012.10.001
137. Chen CC, Shiao YJ, Lin RD et al (2006) Neuroprotective diterpenes from the fruiting body of *Antrodia camphorata*. J Nat Prod 69:689–691
138. Khan MA et al (2013) *Hericium erinaceus*: an edible mushroom with medicinal values. J Complement Integr Med 24:10
139. Standish LJ et al (2008) *Trametes versicolor* mushroom immune therapy in breast cancer. J Soc Integr Oncol 6:122–128
140. Badalyan SM (2001) The main groups of therapeutic compounds of medicinal mushrooms. Probl Med Mycol 3:16–23
141. De Baets S, Vandamme EJ (2001) Extracellular Tremella polysaccharides: structure, properties and applications. Biotechnol Lett 23:1361–1366
142. Marshall E, Nair N (2009) Make money by growing mushrooms. Food and Agriculture Organization of the United Nations (FAO), Roma

Plants Probiotics as a Tool to Produce Highly Functional Fruits

63

Alejandro Jiménez-Gómez, Paula García-Fraile, José David Flores-Félix, and Raúl Rivas

Contents

A. Jiménez-Gómez · J. D. Flores-Félix
Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain

Spanish-Portuguese Institute for Agricultural Research (CIALE), Salamanca, Spain
e-mail: alexjg@usal.es; jdflores@usal.es

P. García-Fraile (✉)
Institute of Microbiology ASCR,v.v.i, Prague, Czech Republic

Lab 210-213, Edificio Departamental de Biología, Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain
e-mail: paulagf81@usal.es

R. Rivas (✉)
Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain

Spanish-Portuguese Institute for Agricultural Research (CIALE), Salamanca, Spain

Associated R&D Unit, USAL-CSIC (IRNASA), Salamanca, Spain

Lab 210-213, Edificio Departamental de Biología, Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain
e-mail: raulrg@usal.es

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_8

Abstract

Plant probiotics are bacteria capable of improving crop yields reducing or even eliminating chemical fertilizers. During the last years, several studies show that many of these bacteria can improve not just production, but also food quality, through the increase of some nutrients as well as some plant bioactive compounds, which are beneficial to human health. This chapter compiles some of the recent research focused on the capabilities of several bacterial plant probiotics to enhance the production of more functional foods, and therefore benefiting our health.

Keywords

Food quality · Biofertilizers · Bacterial inoculants · Nutritional content · Horticultural crops

1 Introduction

According to Shahzad et al. [1], more than 175 million tons of chemical pesticides and fertilizers are applied every year to soils to improve crop yields. Nowadays, farming systems are mainly based on this type of products to satisfy worldwide food demand. However, chemical fertilizers are very costly and produce many environmental and human health problems [2, 3].

Moreover, an increasing percentage of consumers around the world are more aware of the production systems, food safety, and nutritional contents [4]. During last years, food quality and safety are one of the aim issues on the European political agenda [5]. Different production systems are being developed according to the new green politics, which many countries are implementing and including within their legislations.

One of the green new production systems is based on the use of bacterial inoculants, due to their ability to promote plant growth and development and therefor to produce healthier foodstuff. Currently, there are a substantial number of published studies which show significant increased yields and enhanced quality crops [3].

Haas and Keel [6] described the group of beneficial bacteria for plants as plant probiotic bacteria (PPB), according to their effectiveness in niche colonization, ability to induce systemic resistance in their host, improve plant nutritional content, and increase crop quality [7]. Many studies have focused on understanding how these bacteria interact with their plant host and measure the improvements in crop yields [8–10].

Interestingly, apart from the widely known capability to increase crop yields, several studies published in recent years show how bacterial plant probiotics can improve C and increase several plant compounds which augment human health and aid in the prevention of diseases [11]. This is a less-known potential of these bacterial biofertilizers, and therefore, this chapter is focused on the capabilities of plant probiotics to enhance not only crop yields, but also food quality, allowing the production of more functional products benefiting our health.

2 Effects of Microbial Inoculants in Bioactive Compounds Content in Legumes

Legume seeds are undoubtedly one of the most important sources of vegetable proteins in human nutrition [12]. Grains such as soya, beans, chickpeas, lentils, peas are the main source of proteins for many people in developing countries [13–16]. Apart from that, they are excellence source of nutrients such as fiber, vitamins, and minerals [12]. Moreover, several legume grains contain polysaccharides, such as fructooligosaccharides and starch which act as prebiotics [17–21].

Also, some legume seeds, mainly soybeans, but also peanuts, mung beans, lupine grains, green grams, pigeon peas, groundnuts, and bambara, are used to obtain milky drinks, after their extrusion and fermentation with bifidobacteria, lactic bacteria, or yeasts, which are excellent probiotics [22–28].

In addition to their nutritional value, several recent research studies show the importance of the presence of bioactive compounds in legumes, such as flavonoids, tocopherols, carotenoids, fatty acids, and anthocyanins, because of their multiple benefits for human health [12, 29, 30], having been reported how the consumption of legumes reduces the LDL cholesterol levels, preventing heart diseases [31, 32], gastrointestinal cancer [33], hypercholesterolemia [34], diabetes and stroke [32].

Legumes have the capability to establish symbiosis with nitrogen-fixing bacteria. These bacteria, which are currently distributed in several families and genera, are collectively known as rhizobia and have the capability to fix atmospheric nitrogen for the plant [24] inside some organs formed in the roots or stems of the legume plants termed as nodules [25, 26]. The capability of rhizobia to fix nitrogen on legume plants was the first described and characterized bacterial mechanism of plant growth promotion, with the first reports about this symbiosis dating from the beginning of the XIX Century [35]. From that time, many studies have reported yield increments in different legume crops after the application of rhizobial inoculants, reducing or even replacing chemical fertilization [36–38]; thus, the application of rhizobia-based biofertilizers is an efficient agronomic practice to fertilize legume crops in a friendly and sustainable manner, avoiding the environmental contamination and the human health risks derived from chemical fertilization. In these studies, the commonly analyzed parameters are shoot and root weight, nodule number and weight, grain yields, and nutrients content [39].

Moreover, during the last decade, several studies have focused on the analysis of changes in the bioactive substances content of legumes after inoculation with rhizobial strains. Legumes are historically known to contain bioactive compounds such as isoflavones, phenolic acids, and procyanidins, which constitute the major phenolic compounds present in their grains and have been proved to protect against different diseases, such as cancer, obesity, and other metabolic diseases, as well can reduce menopausal symptoms [30, 40, 41]. The molecular structure of isoflavones is similar to that of 17-β-estradiol molecules, so isoflavones can induce similar effects to those of estrogens, but they lack the risks associated with these drugs treatments [42]. Related to the isoflavones content in legumes, several recent studies have found

a positive effect of soybean intake in women for the prevention of cancer diseases related with estrogenic levels, such as endometrial and breast cancers [43, 44]. Soy-based foods consumption has also been correlated with a lower risk of prostate and colorectal cancers [42, 45, 46]. Proanthocyanidins are other phenolic compounds with antioxidant potential present in legume seeds [47–51] and have been related to the prevention of cancer, diabetes, and cardiovascular diseases [52–54]. Tocopherols, compounds with vitamin E activity, are present in important amounts in legume grains [55–58] helping in the prevention of several diseases related with vitamin E deficiency, such as neuromuscular problems [59]. Legume seeds also contain different carotenoids, such as lutein, zeaxanthin, and β-carotene [55], which have been described as potent antioxidants [56] and are precursors of vitamin A, which plays an important role in the prevention of macular degeneration and other eye diseases [60]. Finally, some legume seeds, such as soybeans, peanuts, chickpeas, lentils, beans, and lupins, constitute an important source of unsaturated fatty acids, such as oleic and linoleic [55–57, 61], which have an effect in the lowering of cholesterol levels [62], helping in the prevention of coronary diseases [63, 64].

Most of the research studies on the effects of the inoculation with rhizobial bacteria on the contents of bioactive compounds in legumes have been performed in soybean (*Glycine max*). Silva and collaborators [61] indicated that the inoculation of soybeans with *Bradyrhizobium japonicum* sv *glycinearum* induced an increase in some volatile compounds and organic acids in seeds, confirming the results previously obtained by Couto and collaborators [65], who showed that soybean plants inoculated with *B. japonicum* presented a higher increase in the content of phenolic compounds and organic acids. Besides, grains from inoculated plants presented a higher total fatty acids content, with an increase in both monounsaturated and polyunsaturated fatty acids [61]. On the other hand, the inoculation of faba bean (*Vicia faba*) induced a considerable augmentation in the antioxidant constituents and the total content of flavonoids, phenols, tannins, and proteins [66]. And moreover, a study performed using a *Mesorhizobium* strain to inoculate chickpea (*Cicer arietinum*) showed an increase of flavonoids content in the inoculated plants compared to those ones of the negative control [67].

Finally, some studies have been performed on medicinal legume plants, such as *Psoralea corylifolia* L. The seeds of this plant, known as "Buguzhi," are used in the traditional Chinese medicine for various disorders treatments, particularly vitiligo [68]. *Psoralea corylifolia* contents psoralen [69], a tricyclic furocoumarin, are used for the treatment of hypo-pigmented lesions of the skin for its potent photosensitizing capability [70]. The inoculation of *P. corylifolia* with *Ensifer meliloti* and *Rhizobium leguminosarum* strains has shown an increase in the psoralen content in the seeds of this legume [71].

Considering all these studies and the fact that legumes are regarded as functional foodstuff and recommended as nutraceuticals, we can assume the importance of deepening in the studies of how plant growth-promoting bacteria affect their content in bioactive molecules, not just the mentioned in the already performed studies, but also other not yet considered, which may greatly influence consumers' health.

3 Improvement of Beneficial Substances in Berries by Bacterial Inoculation of Theirs Crops

Berries-type fruits are a heterogeneous group of fruits widely consumed because of their nutraceuticals qualities. These fruits present low energy contents and high antioxidant activity due to high concentrations of bioactive compounds such as vitamins or phenolic compounds [3]. There are several species integrated in the group of the so-called berries-type fruits, i.e., bilberries (*Vaccinuim myrtillus*), lingonberries (*Vaccinium vitis-idaea*), blueberries (*Vaccinium corymbosum),* cranberries (*Vaccinuim macrocarpon* or *Vaccinuim oxycoccos*) strawberries (*Fragaria ananassa),* red raspberries (*Rubus idaeus),* cloudberries (*Rubus chamaemorus*), arctic brambles (*Rubus articus*), loganberies (*Rubus loganobaccus*), honeyberries (*Lonicera caeulea*), rowan berries (*Sorbus* spp.), or crowberries (*Empetrum nigrum*, *E. hermaphroditum*), which present different bioactive compounds profiles, for example, blue and black colored berries show the highest antioxidant activity, which is related to the highest content in anthocyanins [72]. Moreover, berry fruits show good nutritional characteristics, present low amounts of fat contents, high fiber concentration, and an excellent mineral profile [73].

Berries production is usually carried out using conventional agricultural systems, but consumers have started to demand new quality standards of production [74]. In this point, plant growth promotion rhizobacteria (PGPR) could be a key tool to improve their nutraceutical characteristics. However, compared to other type of crops, the number of yield experiences with PGPR is poor yet, but they show good expectations.

Probably, strawberry is the berry which more studies have received due to the relevance of this crop in agriculture. Alvarez-Suarez et al. [75] showed that the consumption of strawberries could improve cardiovascular health and induce an increase on immunological fitness [76]. For this reason, several PGPR studies have been focused on the biofertilizer influence in vitamin contents in strawberries. Different bacteria have been employed to improve nutraceutical qualities of strawberry as *Pseudomonas*, *Bacillus*, *Paenibacillus*, or *Phyllobacterium* [77–79]. The inoculation of *Pseudomonas* BA-8, *Bacillus* OSU-142, and *Bacillus* M-3 produces an increase of vitamin C concentration in strawberry when they are applied by root, foliar, or combined way [77]. Strawberry plants inoculated with *Paenibacillus polymyxa* RC05 produce fruits with high levels of vitamin C concentration [78]. Similar results have been obtained with *Phyllobacterium endophyticum* PEPV15 inoculation under greenhouse conditions. This strain can increase in a 79% the vitamin C concentration comparing with the uninoculated control [79]. Esikten et al. [80] showed that the application of *Pseudomonas* and *Bacillus* strains can increase plant biomass and nutrient content through the production of organic acids from bacteria.

Another important way reported to improve strawberry production is the co-inoculation of PGPR bacteria with mycorrhizal fungi. According to Bona and collaborators, the co-inoculation of *Pseudomonas* strains and an arbuscular mycorrhizal fungi (AMF) produces an increase in vitamin C with respect to the uninoculated control. The authors also show that the vitamin B9 content only

presents in a significant high level in double co-inoculation with AMF and PGPB treatment [81].

Other significant compounds in berries with nutraceutical qualities are flavonoids, which activity is directly related to their concentration [82]. Anthocyanins are the main flavonoids involved in antioxidant activity in berries as strawberry, blackberry or raspberry [83]. Reported results from experiments in strawberry when plants are inoculated with a mixture of plant growth promoting bacterial strains (*Pseudomonas fluorescens* Pf4 and 5Vm1K) and a mycorrhizal fungus (*Glomus* sp.) show how the co-inoculation produces an increment in anthocyanin contents [84]. Orham et al. [85] showed than *Bacillus* OSU-142 and *Bacillus* M3 are also efficient biofertilizers with the ability to improve raspberry production and nutrient quality, including their content in flavonoids.

Basu and Maier [86] showed that all different flavonoids presented in berries crops have antioxidant activity with potential to improve several aspects of human health. Raspberry, blackberry, and other dark berries have a relevant role as flavonoid sources [72]. The inoculation of blackberry plants with the strain *Pseudomonas fluorescens* N21.4 produces an increase in flavonoid content of 22% compared to the uninoculated treatment. These results are especially significant during summer and autumn months [87]. This strain of *Pseudomonas fluorescens* is also able to increase or stabilize total flavonoid content in blackberry fruits under adverse environmental conditions [88], and further experiments suggested that these results are based on differential expression of genes involved in flavonoid synthesis which suffer stimulation when blackberry plants are inoculated with *Pseudomonas fluorescens* N21.4 [89].

4 Increase of Nutrients and Bioactive Compounds in Horticultural Vegetables by PGPR Inoculants

Horticultural crops provide humans with a big variety of essential vitamins and indispensable elements [90]. According to Ramsay et al. [91], the consumption of a wide range of horticultural fruits is linked to overall diet quality.

In addition to be required for many physiological functions, vitamins prevent deficiency syndromes which can affect humans when there is an irregularity of their contents [92]. Scientists are developing different ways and methods to increase the total content of vitamins in horticultural crops. Here, we present some of the studies in which bacterial plant probiotic inoculation has been used to produce an improvement in food quality.

Bona et al. [93] reported that the inoculation of tomato plant with *Pseudomonas* sp. 19Fv1T enhances crop yield and improves vitamin C content in tomatoes, compared to the control treatment. Gül et al. [94] also reported that the highest levels of vitamins content in tomato fruits were obtained after the bacterial inoculation of two *Bacillus amyloliquefaciens* strains (FZB2 and FZB42). Additionally, Shen et al. [95] showed that a mixture of *Bacillus amyloliquefaciens*, *Bacillus megaterium*, and vermicompost also increased tomato yields and vitamin C fruit content. Although Tomato is one of the most horticultural cultivated crops in the

world [96], this fruit is also regarded as an excellent source of antioxidants compounds [97]. Ochoa-Velasco et al. [98] have described an important improvement in vitamin C and total phenols contents with a reduction of nitrogen doses after the inoculation with *Bacillus licheniformis*.

An increase in lycopene antioxidants levels have also been reported in tomato fruits after bacterial inoculation. According to Ordookhani et al. [99], the mix of *Pseudomonas putida*, *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Glomus lipoferum, Glomus mossea*, and *Glomus etunicatum* not only increase lycopene antioxidants levels but also is related to an improvement in potassium fruit contents. The nutrient contents of nitrogen, calcium, magnesium, potassium, and phosphorus were significantly improved in tomato fruits after the inoculation with five combinations of plant growth promotion rhizobacteria such as *Pseudomonas*, *Azotobacter*, and *Azospirillum* [100].

Basil crop (*Ocimum basilicum* L.) is an interesting medicinal plant used worldwide for cooking. Its antioxidant activity has been improved with plant probiotics bacteria. Compared to the uninoculated treatment, the highest antioxidants levels were achieved after the inoculation with a mixture of *Pseudomonas putida*, *Azotobacter chroococcum*, and *Azospirillum lipoferum* strains [99]. Under water stress and after the inoculation with *Pseudomonas* sp., *Bacillus lentus*, and *Azospirillum brasilense*, the antioxidant activity in basil crops also improved [101].

5 Conclusion

Expansion and improvement of a more sustainable and at the same time proficient agriculture, which will guarantee food resources to feed the growing world populations, fighting the hunger in developing countries, with limited land and energy resources while at the same time protecting the environment, is one of the principal challenges of the nowadays human society. There is a plethora of research studies proving that certain bacteria improve yields in agricultural crops, by aiding in nutrients supply, producing growth-stimulating phytohormones, preventing pathogen-induced plant diseases, and inducing plant resistance to biotic or abiotic stresses, so the application of these bacteria as biofertilizers is one of the strategies that could help to achieve the goal.

In parallel, there is an increasing worry about food quality and heathy diets, and many people in developed countries and wealthy families in developing countries demand quality functional foods which improve human health and aid in the prevention of diseases. Consumers' demand of organic foodstuff is increasing, and at the same time most countries are developing several policies to limit or banish the application of chemical fertilizers in crop production.

As shown in this chapter, contrasting chemical fertilizers, the application of plant probiotic bacteria as crops biofertilizers increases not only yields, but also the nutritional quality of seeds, fruits, and horticultural vegetables. Research in this field showed how several bacterial inoculants interact with different plant species, and as a result there is an increase of some plant compounds, which are beneficial to

human health. Thus, the application of probiotic bacteria to reduce chemical fertilizers is an excellent alternative, not just to sustain crop production while limiting chemical fertilizers, but also to improve food quality.

The still scarce number of studies orientated to the application of plant probiotic bacteria to improve the quality of fruits, grains, and horticultural crops has showed an increase in the levels of vitamins, antioxidants, and flavonoids, among other values. Still, there are many other bioactive compounds which could also be enriched by the application of bacterial inoculants. Therefore, new research approaches such as metabolomics, comparing plant foodstuffs produced with and without the application of plant probiotic bacteria, may reveal additional bioactive compounds whose quantity may be enlarged by the applications of bacterial inoculants. Also, more studies on the effects of different bacterial strains on the food quality increase in different plant species are necessary; this would allow for a better selection of plant probiotic bacteria with the potential to increase not just crops yields, but also their quality and health benefiting properties.

Acknowledgments The authors are grateful for the funding received by the Regional Government of Castile and Leon and the Spanish Ministry of Economics and Competitiveness (JCyL SA169U14 and MINECO; AGL2015-70510-R). AJG is funded by the FPU predoctoral fellowship from the Spanish Government. JDFF is funded by the University of Salamanca with a predoctoral fellowship. PGF receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 750795.

References

1. Shahzad SM, Arif MS, Riaz M, Iqbal Z, Ashraf M (2013) PGPR with varied ACC-deamisae activity induced different growth and yield response in maize (Zea mays L.) under fertilized conditions. Eur J Soil Biol 57:27–34
2. García-Fraile P, Menéndez E, Rivas R (2015) Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioeng 2:183–205
3. Jiménez-Gómez A, Celador-Lera L, Fradejas-Bayón M, Rivas R (2017) Plant probiotic bacteria enhance the quality of fruit and horticultural crops. AIMS Microbiol 3:483–501
4. Trienekens J, Zurbier P (2008) Quality and safety standards in the food industry, developments and challenges. Int J Prod Econ 113:107–122
5. García-Fraile P, Menéndez E, Celador-Lera L, Diez-Méndez A, Jiménez-Gómez A, Marcos-García M, Cruz-González XA, Martínez-Hidalgo P, Mateos PF, Rivas R (2017) Bacterial probiotics: a truly green revolution. In: Kumar V, Kumar M, Sharma S, Prasad R (eds) Probiotics and plant health. Springer, Singapore
6. Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. Annu Rev Phytopathol 41:117–153
7. Menéndez E, García-Fraile P (2017) Plant probiotic bacteria: solutions to feed the world. AIMS Microbiol 3:502–524
8. Qin Y, Fu Y, Dong C, Jia N, Liu H (2016) Shifts of microbial communities of wheat (*Triticum aestivum* L.) cultivation in a closed artificial ecosystem. Appl Microbiol Biotechnol 100:4085–4095
9. Young CC, Shen FT, Singh S (2012) Strategies for the exploration and development of biofertilizer. In: Maheshwari DK (ed) Bacteria in agrobiology: plant probiotics. Springer, Berlin/Heidelberg
10. Jiménez-Gómez A, Menéndez E, Flores-Félix JD, García-Fraile P, Mateos PF, Rivas R (2016) Effective colonization of spinach root surface by rhizobium. In: Gonzalez-Andres F, James E

(eds) Biological nitrogen fixation and beneficial plant-microbe interaction. Springer International Publishing, Switzerland
11. García-Fraile P, Carro L, Robledo M, Ramírez-Bahena MH, Flores-Félix JD, Fernández MT, Mateos PF, Rivas R, Igual JM, Martínez-Molina E, Peix A, Velázquez E (2012) Rhizobium promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS One 7:e38122. 57
12. Mudryj AN, Yu N, Aukema HM (2014) Nutritional and health benefits of pulses. Appl Physiol Nutr Metab 9:1197–1204
13. Foyer CH, Lam HM, Nguyen HT, Siddique KHM, Varshney RK, Colmer TD, Mori TA, Hodgson JM, Cooper JW, Cowling W, Bramley H, Miller AJ, Kunert K, Vorster J, Cullis C, Ozga JA, Wahlqvist MK, Liang Y, Shou H, Shi K, Yu J, Fodor N, Kiaser BN, Wong KL, Valliyodan B, Considine MJ (2016) Neglecting legumes has compromised human health and sustainable food production. Nat Plants 2:16112
14. Marventano S, Izquierdo Pulido M, Sánchez-González C, Godos J, Speciani A, Galvano F, Grosso G (2016) Legume consumption and CVD risk: a systematic review and meta-analysis. Public Health Nutr 20:245–254
15. Rebello CJ, Greenway FL, Finley JW (2014) A review of the nutritional value of legumes and their effects on obesity and its related co-morbidities. Obes Rev 15:392–407
16. Singhal P, Kaushik G, Mathur P (2014) Antidiabetic potential of commonly consumed legumes: a review. Crit Rev Food Sci Nutr 54:655–672
17. Johnson CS, Thavaraja D, Combs GF, Thavarajah P (2013) Lentil (*Lens culinaris* L.): a prebiotic-rich whole food legume. Food Res Int 51:107–113
18. Shakuntala S, Mol P, Muralikrishna G (2014) Pectic oligosaccharides derived from chickpea (*Cicer arietinum* L.) husk pectin and elucidation of their role in prebiotic and antioxidant activities. Trends Carbohyd Res 6:29–36
19. Souframanien J, Roja G, Gopalakrishna T (2014) Genetic variation in raffinose family oligosaccharides and sucrose content in black gram [*Vigna mungo* L. (Hepper)]. J Food Legumes 27:37–41
20. Wongputtisin P, Ramaraj R, Unpaprom Y, Kawaree R, Pongtrakul N (2015) Raffinose family oligosaccharides in seed of *Glycine max* cv. Chiang Mai60 and potential source of prebiotic substances. Int J Food Sci Technol 50:1750–1756
21. Karnpanit W, Coorey R, Clements J, Nasar-Abbas SM, Khan MK, Jayasena V (2016) Effect of cultivar, cultivation year and dehulling on raffinose family oligosaccharides in Australian sweet lupin (*Lupinus angustifolius* L.) Food Sci Technol 51:1386–1392
22. Chen KI, Erh MH, Su NW, Liu WH, Chou CC, Cheng KC (2012) Soyfoods and soybean products: from traditional use to modern applications. Appl Microbiol Biotechnol 96:9–22
23. Bensmira M, Jiang B (2015) Total phenolic compounds and antioxidant activity of a novel peanut based kefir. Food Sci Biotechnol 24:1055–1060
24. Kasprowicz-Potocka M, Borowczyk P, Zaworska A, Nowak W, Frankiewicz A, Gulewicz P (2016) The effect of dry yeast fermentation on chemical composition and protein characteristics of blue lupin seeds. Food Technol Biotechnol 54:360–366
25. Parra K, Ferrer M, Piñero M, Barboza Y, Medina LM (2013) Use of *Lactobacillus acidophilus* and *Lactobacillus casei* for a potential probiotic legume–based fermented product using pigeon pea (*Cajanus cajan*). J Food Protect 76:265–271
26. Murevanhema YY, Jideani VA (2013) Potential of bambara groundnut (*Vigna subterranea* (L.) Verdc) milk as a probiotic beverage-a review. Crit Rev Food Sci Nutr 53:954–967
27. Mridula D, Sharma M (2015) Development of non-dairy probiotic drink utilizing sprouted cereals, legume and soymilk. LWT-Food Sci Technol 62:482–487
28. Wu H, Rui X, Li W, Chen X, Jiang M, Dong M (2015) Mung bean (*Vigna radiata*) as probiotic food through fermentation with *Lactobacillus plantarum* B1–6. LWT-Food Sci Technol 63:445–451
29. Cornara L, Xiao J, Burlando B (2016) Therapeutic potential of temperate forage legumes: a review. Crit Rev Food Sci Nutr 56:S149–S161

30. Silva LR, Peix Á, Albuquerque C, Velázquez E (2016) Bioactive compounds of legumes as health promoters. In: Silva LR, Silva BM (eds) Natural bioactive compounds from fruits and vegetables as health promoters, Sharjah, UAE. Science Publishers, Bentham, pp 3–27
31. Bouchenak M, Lamri-Senhadji M (2013) Nutritional quality of legumes, and their role in cardiometabolic risk prevention: a review. J Med Food 16:185–198
32. Afshin A, Micha R, Khatibzadeh S, Mozaffarian D (2014) Consumption of nuts and legumes and risk of incident ischemic heart disease, stroke, and diabetes: a systematic review and meta-analysis. Am J Clin Nutr 100:278–288
33. Zhu B, Sun Y, Qi L, Zhong R, Miao X (2015) Dietary legume consumption reduces risk of colorectal cancer: evidence from a meta-analysis of cohort studies. Sci Rep 5:8797
34. Arnoldi A, Zanoni C, Lammi C, Boschin G (2015) The role of grain legumes in the prevention of hypercholesterolemia and hypertension. Crit Rev Plant Sci 34:144–168
35. Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729–770
36. Mulas D, García-Fraile P, Carro L, Ramírez-Bahena H, Casquero P, Velázquez E, González-Andrés F (2011) Distribution and efficiency of *Rhizobium leguminosarum* strains nodulating *Phaseolus vulgaris* in Northern Spanish soils: selection of native strains that replace conventional N fertilization. Soil Biol Biochem 43:2283–2293
37. Araujo J, Díaz-Alcántara CA, Velázquez E, Urbano B, González-Andrés F (2015) *Bradyrhizobium yuanmingense* related strains form nitrogen-fixing symbiosis with *Cajanus cajan* L. in Dominican Republic and are efficient biofertilizers to replace N fertilization. Sci Hortic 192:421–428
38. Sarr PS,Wase Okon J, Boyogueno Begoude DA, Araki S, Amband Z, Shibata M, Funakawa S (2016) Symbiotic N2-fixation estimated by the 15N tracer technique and growth of *Pueraria phaseoloides* (Roxb.) Benth. Inoculated with Bradyrhizobium strain in field conditions. Scientifica Vol. 2016. ID article 7026859
39. Silva LR, Bento C, Gonçalves AC, Flores-Félix JD, Ramírez-Bahena MH, Peix A, Velazquez E (2017) Legume bioactive compounds: influence of rhizobial inoculation. AIMS Microbiol 3(2):267–278
40. Messina M (2014) Soy foods, isoflavones, and the health of postmenopausal women. Am J Clin Nutr 100:423S–230S
41. Wang Q, Ge X, Tian X, Zhang Y, Zhang J, Zhang P (2013) Soy isoflavone: the multipurpose phytochemical (review). Biomed Rep 1:697–701
42. Hwang KA, Choi KC (2015) Anticarcinogenic effects of dietary phytoestrogens and their chemopreventive mechanisms. Nutr Cancer 21:1–8
43. Messina M (2016) Impact of soy foods on the development of breast cancer and the prognosis of breast cancer patients. Forsch Komplementmed 23:75–80
44. Zhong XS, Ge J, Chen SW, Xiong YQ, Ma SJ, Chen Q (2016) Association between dietary isoflavones in soy and legumes and endometrial cancer: a systematic review and meta-analysis. J Acad Nutr Diet. *in press*.
45. Van Die MD, Bone KM, Williams SG, Pirotta MV (2014) Soy and soy isoflavones in prostate cancer: a systematic review and meta-analysis of randomized controlled trials. BJU Int 113:E119–E130
46. Yu Y, Jing X, Li H, Zhao X, Wang D (2016) Soy isoflavone consumption and colorectal cancer risk: a systematic review and meta-analysis. Sci Rep 6:25939
47. Bittner K, Rzeppa S, Humpf HU (2013) Distribution and quantification of flavan-3-ols and procyanidins with low degree of polymerization in nuts, cereals, and legumes. J Agric Food Chem 61:9148–9154
48. Ojwang LO, Yang L, Dykes L, Awika J (2013) Proanthocyanidin profile of cowpea (*Vigna unguiculata*) reveals catechin-*O*-glucoside as the dominant compound. Food Chem 139:35–43
49. Takahama U, Yamauchi R, Hirota S (2013) Isolation and characterization of a cyaniding-catechin pigment from adzuki bean (*Vigna angularis*). Food Chem 141:282–288

50. Han KH, Kitano-Okada T, Seo JM, Kim SJ, Sasaki K, Shimada KI, Fukushima M (2015) Characterization of anthocyanins and proanthocyanidins of adzuki bean extracts and their antioxidant activity. J Funct Foods 14:692–701
51. Golam Masum Akond ASM, Khandaker L, Berthold J, Gates L, Peters K, Delong H, Hossain K (2011) Anthocyanin, total polyphenols and antioxidant activity of common bean. Am J Food Technol 6:385–394
52. Kruger MJ, Davies N, Myburgh KH, Lecour S (2014) Proanthocyanidins, anthocyanins and cardiovascular diseases. Food Res Int 59:41–52
53. González-Abuín N, Pinent M, Casanova-Marti A, Ardevol A (2015) Procyanidins and their healthy protective effects against type 2 diabetes. Curr Med Chem 22:39–50
54. Lin BW, Gong CC, Song HF, Cui YY (2016) Effects of anthocyanins on the prevention and treatment of cancer. Br J Pharmacol 174:1226–1243.
55. Fernández-Marín B, Milla R, Martín-Robles N, Arc E, Kranner I, Becerril JM, García-Plazaola JI (2014) Side-effects of domestication: cultivated legume seeds contain similar tocopherols and fatty acids but less carotenoids than their wild counterparts. BMC Plant Biol 14:1599
56. Zhang B, Deng Z, Tang Y, Tsao R (2014) Fatty acid, carotenoid and tocopherol compositions of 20 Canadian lentil cultivars and synergistic contribution to antioxidant activities. Food Chem 161:296–304
57. Kalogeropoulos N, Chiou A, Ioannou M, Karathanos VT, Hassapidou M, Andrikopoulos NK (2010) Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries. Food Chem 121:682–690
58. Boschin G, Arnoldi A (2011) Legumes are valuable sources of tocopherols. Food Chem 127:1199–1203
59. Ulatowski L, Parker R, Warrier G, Sultana R, Butterfield DA, Manod D (2013) Vitamin E is essential for Purkinje neuron integrity. Neuroscience 260:120–129
60. Saari JC (2016) Vitamin A and vision. Subcell Biochem 81:231–259
61. Silva LR, Pereira MJ, Azevedo J, Mulas R, Velázquez E, González-Andrés F, Valentao P, Andrade PB (2013) Inoculation with *Bradyrhizobium japonicum* enhances the organic and fatty acids content of soybean (*Glycine max* (L.) Merrill) seeds. Food Chem 141:3636–3648
62. Ramsden CE, Zamora D, Majchrzak-Hong S, Faurot KR, Broste SK, Frantz RP, Davis JM, Ringel A, Suchindran CM, Hibbeln JR (2016) Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota coronary experiment (1968–73). BMJ 353:i1246
63. Zock PL, Blom WA, Nettleton JA, Hornstra G (2016) Progressing insights into the role of dietary fats in the prevention of cardiovascular disease. Curr Cardiol Rep 18:111
64. Huth PJ, Fulgoni VL, Larson BT (2015) A systematic review of high-oleic vegetable oil substitutions for other fats and oils on cardiovascular disease risk factors: implications for novel high-oleic soybean oils. Adv Nutr 6:674–693
65. Couto C, Silva LR, Valentão P, Velázquez E, Peix A, Andrade PB (2011) Effects induced by the nodulation with *Bradyrhizobium japonicum* on Glycine max (soybean) metabolism and antioxidant potential. Food Chem 127:1487–1495
66. Farfour SA, Al-Saman MA, Hamouda RA (2015) Potential activity of some biofertilizer agents on antioxidant and phytochemical constituents of faba bean plant. Glo Adv Res J Agr Sci 4:26–32
67. Singh A, Jain A, Sarma BK, Upadhyay RS, Singh HB (2014) Beneficial compatible microbes enhance antioxidants in chickpea edible parts through synergistic interactions. LWT-Food Sci Technol 56:390–397
68. Chopra B, Dhingra AK, Dhar KL (2013) *Psoralea corylifolia* L. (Buguchi)-Folklore to modern evidence: review. Fitoterapia 90:44–56
69. Liu RM, Li AF, Sun AL, Kong L (2004) Preparative isolation and purification of psoralen and isopsoralen from *Psoralea corylifolia* by high-speed counter-current chromatography. J Chromatogr A 1057:225–228

70. Wolf P (2016) Psoralen-ultraviolet A endures as one of the most powerful treatments in dermatology: reinforcement of this 'triple-product therapy' by the 2016. British guidelines. Br J Dermatol 174:11–14
71. Prabha C, Maheshwari DK, Bajpai VK (2013) Diverse role of fast growing rhizobia in growth promotion and enhancement of psoralen content in *Psoralea corylifolia* L. Pharmacogn Mag 9:S57–S65
72. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J (2015) Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci 16:24673–24706
73. Nile SH, Park SW (2014) Edible berries: bioactive components and their effect on human health. Nutrition 30:134–144
74. Zhao Y (2007) Berry fruit: value-added products for health promotion. CRC press, Boca Raton
75. Alvarez-Suarez JM, Giampieri F, Tulipani S, Casoli T, Santos-Buelga C, Busco F, Quiles JL, Cordero MD, Bompadre S, Mezzetti B, Battino M (2014) One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. J Nutr Biochem 25:289–294
76. Tulipani S, Armeni T, Giampieri F, Alvarez-Suarez JM, Gonzalez-Paramas AM, Santos-Buelga C, Busco F, Principato G, Bompadre S, Mezzetti B, Battino M (2014) Strawberry intake increases blood fluid, erythrocyte and mononuclear cell defenses against oxidative challenge. Food Chem 156:87–93
77. Pırlak L, Köse M (2009) Effects of plant growth promoting rhizobacteria on yield and some fruit properties of strawberry. J Plant Nutr 32:1173–1184
78. Erturk Y, Ercisli S, Cakmakci R (2012) Yield and growth response of strawberry to plant growth-promoting rhizobacteria inoculation. J Plant Nutr 35:817–826
79. Flores-Félix JD, Silva LR, Rivera LP, Marcos-Garcia M, Garcia-Fraile P, Martinez-Molina E, Mateos PF, Velázquez E, Andrade P, Rivas R (2015) Plants probiotics as a tool to produce highly functional fruits: the case of *Phyllobacterium* and vitamin C in strawberries. PLoS One 10:e0122281
80. Esitken A, Yildiz HE, Ercisli S, Donmez MF, Turan M, Gunes A (2010) Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. Sci Hortic 124:62–66
81. Bona E, Lingua G, Manassero P, Cantamessa S, Marsano F, Todeschini V, Copetta A, Massa N, Avidano L, Gamalero E, Berta G (2015) AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. Mycorrhiza 25:181–193
82. Robert P, Fredes C (2015) The encapsulation of anthocyanins from berry-type fruits. Trends in foods. Molecules 20:5875–5888
83. Aaby K, Wrolstad RE, Ekeberg D, Skrede G (2007) Polyphenol composition and antioxidant activity in strawberry purees; impact of achene level and storage. J Agr Food Chem 55:5156–5166
84. Lingua G, Bona E, Manassero P, Marsano F, Todeschini V, Cantamessa S, Copetta A, Gamalero E, Berta G (2013) Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads increases anthocyanin concentration in strawberry fruits (Fragaria x ananassa var. Selva) in conditions of reduced fertilization. Int J Mol Sci 14:16207–16225
85. Orhan E, Esitken A, Ercisli S, Turan M, Sahin F (2006) Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Sci Hortic 111:38–43
86. Basu P, Maier C (2016) In vitro antioxidant activities and polyphenol contents of seven commercially available fruits. Pharm Res 8:258
87. García-Seco D, Bonilla A, Algar E, García-Villaraco A, Gutierrez-Mañero J, Ramos-Solano B (2013) Enhanced blackberry production using *Pseudomonas fluorescens* as elicitor. Agron Sustain Dev 33:385–392

88. Ramos-Solano B, García-Villaraco A, Gutiérrez-Mañero FJ, Lucas JA, Bonilla A, García-Seco D (2014) Annual changes in bioactive contents and production in field-grown blackberry after inoculation with *Pseudomonas fluorescens*. Plant Physiol Biochem 74:1–8
89. García-Seco D, Zhang Y, Gutierrez-Mañero FJ, Martin C, Ramos-Solano B (2015) Application of *Pseudomonas fluorescens* to blackberry under field conditions improves fruit quality by modifying flavonoid metabolism. PLoS One 10:e0142639
90. Drewnowski A (2005) Concept of a nutritious food: toward a nutrient density score. Am J Clin Nutr 82:721–732
91. Ramsay SA, Shriver LH, Taylor CA (2017) Variety of fruit and vegetables is related to preschoolers' overall diet quality. Prev Med 5:112–117
92. Combs JGF, McClung JP (2016) The vitamins: fundamental aspects in nutrition and health. Academic, Amsterdam
93. Bona E, Cantamessa S, Massa N, Manassero P, Marsano F, Copetta A, Lingua G, Gamalero E, Berta G (2017) Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato: a field study. Mycorrhiza 27:1–11
94. Gül A, Kidoglu F, Tüzel Y (2008) Effects of nutrition and *Bacillus amyloliquefaciens* on tomato (*Solanum lycopersicum* L.) growing in perlite. Span J Agric Res 6:422–429
95. Shen F, Zhu TB, Teng MJ, Chen Y, Liu MQ, Hu F, Li HX (2016) Effects of interaction between vermicompost and probiotics on soil nronerty, yield and quality of tomato. Yingyong Shengtai Xuebao 27:484
96. Dorais M, Ehret DL, Papadopoulos AP (2008) Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. Phytochem Rev 7:231–250
97. Martínez-Valverde I, Periago MJ, Provan G, Chesson A (2002) Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). J Sci Food Agr 82:323–330
98. Ochoa-Velasco CE, Valadez-Blanco R, Salas-Coronado R, Sustaita-Rivera F, Hernández-Carlos B, García-Ortega S, Santos-Sánchez NF (2016) Effect of nitrogen fertilization and *Bacillus licheniformis* biofertilizer addition on the antioxidants compounds and antioxidant activity of greenhouse cultivated tomato fruits (*Solanum lycopersicum* L. var. Sheva). Sci Hortic 201:338–345
99. Ordookhani K (2011) Investigation of PGPR on antioxidant activity of essential oil and microelement contents of sweet basil. Adv Environ Biol 5:1114–1120
100. Sharafzadeh S (2012) Effects of PGPR on growth and nutrients uptake of tomato. Int J Adv Eng Technol 2:27
101. Heidari M, Golpayegani A (2012) Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.) J Saudi Soc Agr Sci 11:57–61

Bioactive Compounds of the Wonder Medicinal Mushroom "*Ganoderma lucidum*"

64

Surya Sudheer, Ibrahim Alzorqi, Sivakumar Manickam, and Asgar Ali

Contents

S. Sudheer · I. Alzorqi · S. Manickam (✉)
Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham, Semenyih, Malaysia
e-mail: soorya.m.sudheer@gmail.com; ibrahim.alzorqi@yahoo.com; Sivakumar.Manickam@nottingham.edu.my

A. Ali
Centre of Excellence for Postharvest Biotechnology (CEPB), School of Biosciences, University of Nottingham, Semenyih, Malaysia
e-mail: Asgar.Ali@nottingham.edu.my

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_45

Abstract

Ganoderma lucidum is a highly praised medicinal mushroom having high demand worldwide. Intense research activities are being carried out on the medicinal applications of this mushroom that include anticancer, antiallergic, antioxidant potential, etc. This wonder mushroom contains many bioactive compounds such as polysaccharides, triterpenes, polyphenols, proteins, amino acids, and organic germanium. Ancient Chinese use this mushroom in their medicinal preparations and declared good health and longevity. Health benefits of this mushroom are highly praised in ancient manuscripts. Many pharmaceutical and beauty products made from this mushroom are available in the markets and demands high price. Cultivation and production of this mushroom are limited to certain countries, which leads to an increase in its market value. This chapter describes the details of this special mushroom, including its taxonomy, morphology, ecological and economic status, cultivation, bioactive molecules, and its medicinal applications.

Keywords

Ganoderma lucidum · Bioactive compounds · Cultivation · Therapeutic applications · Polysaccharides · Triterpenes

Abbreviations

C:N	Carbon nitrogen ratio
EPS	Extracellular polysaccharides
G. lucidum	*Ganoderma lucidum*
GA	Ganoderic acid
GTS	Ganoderma triterpenes
HDP	Host Defense Potentiators
IPS	Intracellular polysaccharides
LSC	Liquid state cultivation
LSF	Liquid state fermentation
LZ	Lingzhi
PBP	Protein-bound polysaccharide
RIP	Ribosome inactivating proteins
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SSC	Solid state cultivation
SSF	Solid state fermentation

1 Introduction

Mushrooms are progressively being evaluated in the East and West for their nutritional value. Many of the mushrooms are nutritionally considered as functional foods and some of which are purely evaluated as a source of medicinally beneficial compounds called "nutraceuticals." *Ganoderma lucidum* (Fig. 1) is such a popular mushroom worshipped for its medicinal use and the history of its usage goes back to many thousands of years in the orient. *Ganoderma lucidum* holds a prominent place in traditional Chinese medicine and is well documented in ancient scripts [1, 2]. It has a long record of use in promoting health and longevity in China, Japan, and other Asian countries. Its medicinal activity is usually localized, for example, moderating the immune system by reducing its activity when overstimulated, and proliferating the immune system by increasing the number of active cells. For Chinese people, *G. lucidum* symbolizes the combination of spiritual potency and essence of immortality and thus called as "herb of spiritual potency." Among the cultivated mushrooms, the nutritional and pharmaceutical values of *G. lucidum* are paramount.

Ganoderma lucidum is consumed as a herbal extract like concoctions of tea and tonics. A variety of Ganoderma products are available in the market in various forms such as powders, dietary supplements, soaps, ointments, antiseptic creams, and herbal soaps. The products of this mushroom are available in the market but are expensive as efforts and processes required for the cultivation of this mushroom are intensive. *Ganoderma lucidum* described as a fungal bio-factory by eminent scientists as well as medical practitioners. But the amount of this wild mushroom is not sufficient to meet its demand in the international market. In the mushroom industries through various trial and errors, cultivation of *G. lucidum* has been achieved a considerable progress in the last 40 years. For faster and efficient production, various methods are practiced in these days [3]. Traditional and modern cultivation methods are used for large-scale production. Traditional methods include bag, bottle, and wood log cultivation which acquire more time, whereas modern techniques such as solid and liquid state cultivation could be carried out within a limited period.

Fig. 1 *Ganoderma lucidum*

The benefits of *G. lucidum* are entrusted from generation to generation, as an anticancer, a symbol of divine power, good fortune, good health, and longevity. It is used for various ailments such as allergy, hypertension, cardiac diseases, cancer, viral (HIV), and bacterial infections. It is an annual mushroom belongs to the family of Ganodermataceae. All parts of the mushroom viz., fruiting body, mycelia, and spores, contain bioactive compounds and are useful for the above-mentioned treatments. Polysaccharides, triterpenoids, proteins, amino acids, nucleotides, alkaloids, steroids, lactones, fatty acids, and enzymes are the major components that are responsible for the wide array of bioactive properties shown by this mushroom [4]. A single chemical compound cannot be responsible for the bioactivity which in turn is brought about by a combination of two or more active compounds. Polysaccharides, triterpenoids, and polyphenols are the major active compounds in *G. lucidum* [5]. Polysaccharides are water-soluble fragments which can be extracted with hot water, whereas polyphenols and triterpenoids are more soluble in polar organic solvents such as ethanol and methanol. *Ganoderma lucidum* is considered to be a useful source of protein (7–8%), carbohydrates (3–5%), crude fat (3–5%), crude fiber (59%), ash (1.8%), and other trace elements on a dry weight basis [6, 7]. *Ganoderma lucidum* also contains a higher amount of chitin which makes it hard to chew and digest. Apart from the nutraceutical benefits, it also plays a key role in the environment as decomposers in nutrient cycle. *Ganoderma lucidum* decomposes dead wood of coniferous or hardwood species by producing enzymes such as laccase, manganese peroxidase, and lignin peroxidase which degrade the compounds of lignin and cellulose in the wood. These wood degrading enzymes are of special interest for industrial applications, i.e., bioremediation, biopulping, and biosorption of heavy metals [8].

2 History

The history of *G. lucidum* can be traced back to 2000 years. During ancient times, *G. lucidum* had been worshipped as one of the herbal medicines and believed to heal all kinds of diseases. It was first used by the emperor of China, Shih-Huang of Ch'in Dynasty (221-207 BC), who made the Great Wall of China. Special teas or concoctions made from this mushroom were used by the emperors of Great China and Japan for vitality and healthy life [2]. Those who presented this mushroom to the king were highly rewarded. *Ganoderma lucidum* is popular in folk medicine because of its promising medicinal properties including its potential to extend the lifetime. *Ganoderma lucidum* was first introduced by a famous Chinese medicinal practitioner *Shen Nongs*, who is known as the "Father of Chinese medicine." Medicinal value of this mushroom is well described in his book "Materia Medica" [9, 10]. One of the key features of this mushroom is the absence of side effects. Due to this, *G. lucidum* has attained a reputation as an ultimate health supplement. The popularity of this mushroom has given it a place in "American Pharmacopoeia and Therapeutic Compendium." In addition to its medicinal properties, people of ancient China had a spiritual belief of protecting the people and their homes from evil spirit [11].

3 Scientific Classification

Kingdom	Fungi
Phylum	Basidiomycota
Class	Agaricomycetes
Order	Polyporales
Family	Ganodermataceae
Genus	*Ganoderma*
Species	*lucidum*
Binomial name	*Ganoderma lucidum* (Curtis) P. Karst.

Petter Adolf Karsten in 1881 named the genus as Ganoderma. *Ganoderma lucidum* has several names based on its geographical location and its traditional and historical applications. In Japan, this mushroom is commonly known as Reishi, Mannentake, or 10,000 years mushroom, and symbol to happiness, fortune, immortality, and good health. It has been known as Lingzhi in the Korean and Chinese ancients for more than 4000 years. It has also been called as a "mushroom of herb and immortality." However, the concept of this mushroom as an herb of immortality was described in transcripts of the first ancient empire of China (221-207 B.C) and illustrated in their literature and art perspectives. Botanically, the Latin name is *Ganoderma lucidum*, where lucidum was derived from its shiny surface [2, 9]. There are around 6 species of Ganoderma which are similar to *G. lucidum*. They are *G. atrum, G. tsugae, G. tropicum, G. applanatum, G. capense*, and *G. sinensis*.

4 World Production of *G. lucidum*

Worldwide consumption of *G. lucidum* is several thousand tons, and the market is growing rapidly. A decade ago, more than 90 brands of *G. lucidum* products were registered and marketed internationally [12]. Numerous *G. lucidum* products prepared from various parts of the mushroom are currently available on the market [13]. The simplest type of product available in the market consists of intact fruiting bodies ground to powder and then processed to capsule form. Other products are prepared from the following sources: (1) dried and powdered mycelia harvested from submerged liquid cultures grown in fermentation tanks; (2) dried and powdered combinations of substrate, mycelia, and mushroom primordia, following inoculation and incubation of a semisolid medium with fungal mycelia; and (3) intact fungal spores or spores that have been broken by mechanical means or have had the spore walls removed. Preparations made of spore have been promoted in the recent years because of the faster medicinal effects. It is generally hard to remove the spore wall; therefore, modern techniques, such as ultrasonic extraction, enzyme-based extraction methods are used which represent an additional and often a costly step in the production, are still controversial. Other products that are available in the market are prepared with extracted bioactive compounds such as polysaccharides and triterpenes. These compounds are usually extracted with hot water or ethanol

from fruiting bodies or mycelia and then evaporated to dryness and encapsulated either separately or integrated together in designated proportions. The adoption of supercritical CO_2 extraction technologies has enlarged the spectrum of extracted substances due to the low temperature required during processing [14]. Several other products have been prepared from antler, the deformed type mushroom fruiting body, which is claimed to be highly medicinal.

5 Morphological Characteristics

G. lucidum is a kidney-shaped mushroom with varnished and colored outer surface and has an elegant appearance. They produce pores on the underneath of mushroom cap instead of gills, thereby making them to be differentiated from other polypores which produce gills underneath. But similar to other annual mushrooms they do not grow frequently. This difference makes them rare and special among other mushrooms. The upper side of this mushroom is shiny and dark red. Sometimes it shows varying color depending on the natural habitats and environmental conditions. All the three parts of *G. lucidum* such as mycelium, fruiting body, and spores (Fig. 2a, b, and c) are very important source of bioactive compounds.

5.1 Mycelium

Mycelium of a mushroom is the branching vegetative network which can spread like a mat on the medium or wood logs. Ganoderma mycelium appears to contain many of the bioactive compounds as that of fruiting body [15]. The polysaccharides from the mycelia also have the similar efficacy as that of the fruiting body [16, 17]. The spores of *G. lucidum* shred from the fruiting bodies would land on dead, decaying woods, and under adequate conditions they germinate into mycelium. Mycelia undergo three developmental stages, from primary to secondary and then to tertiary mycelia and eventually the fruiting body will sprout out. In the late phase, formation of basidium and then basidiospores are produced.

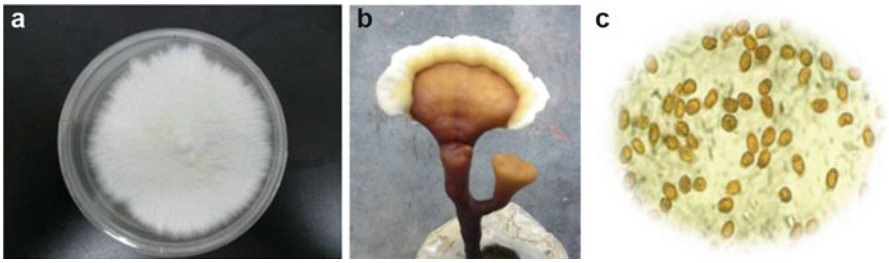


Fig. 2 Mycelium (**a**), fruiting body/basidiocarp (**b**) and spores (**c**) of *G. lucidum*

5.2 Fruiting Body

Basidiocarps or fruiting bodies of the mushroom vary from 2 to 10 cm in diameter. They are kidney-shaped and have shiny outer surface with yellow white striations at the margin which make them beautiful. In their initial stage of growth, they show yellow to white color. Later, with maturity they turn to reddish brown. The stipe (stem) is usually present, but sometimes it maybe with or without a reduced stipe. The length of stipe varies based on the conditions in which they grow as well as the nutrition available in the growth medium. The abaxial side of mushroom cap has several pores which are usually white. The matured mushroom shed spores through these tiny pores [18].

5.3 Spores

The spores of *G. lucidum* are slightly brown in the size range of 1–10 micron. The spore wall is composed of chitin. The spores are reported to contain higher amounts of bioactive compounds than the fruiting body. But the spores are hard to break due to the thicker chitin wall. Possessing a thicker chitin wall is the major characteristic of polypore mushroom as they protect the mushroom from adverse conditions. The sporoderm of *G. lucidum* is made-up of silica (19.01%), calcium (17%), and chitin (52.08–57.64%) [19]. The outer thick coating of sporoderm is resistant to acids giving it an extended shelf-life in the nature.

6 The Ecological Habitat of *Ganoderma lucidum*

This annual mushroom could be found growing on the decaying and dead bodies of a variety of trees. It grows on the decaying logs of deciduous trees like oak, maple, elm, willow, sweet gum, magnolia, and locust. Besides, it grows on the decaying logs of certain coniferous trees such as Larix, ptea, and pinus, in the subtropical regions such as Europe, Asia, and North and South America [20]. In the oriental countries, the mushroom has been found to grow mainly on the plum trees on the stumps near the soil surface and occasionally on soils arising from buried roots [2]. In temperate and subtropical regions of Asia, Europe, and South and North America, this mushroom can be found on the coniferous trees such as Larix, pinus, and picea. They cause white rot disease at the base of trees either alone or in a group. Its natural growth considerably takes longer time, which makes the mushroom very rare, treasured, and exclusive for the rich people and highly ranked powerful individuals. The above limitations push the scientists to find alternative artificial cultivation methods.

7 Nutrition and Growth

Ganoderma lucidum which is used as a folk medicine or nutrition is a saprophyte. They cannot carry out photosynthesis due to the absence of chlorophyll. Mycelia can directly absorb simple sugars, organic acids, and other carbohydrates that are present

in the growth substrate. Mushroom mycelia cannot use inorganic carbon. The growth of mycelium requires the presence of nitrogen approximately 0.016–0.064%. For the production of fruiting body, the requirement of nitrogen is approximately 0.032–0.016%. Growth of mushroom needs the presence of minerals such as P, K, Ca, Mg, S, Fe, Mn, Zn, and other inorganic compounds. Temperature is another crucial factor which determines the growth, quality, and yield of the mushroom. Mushroom at various stages of growth and development requires different temperatures. For the germination of mycelia, the suitable temperature employed is in between 25 °C and 30 °C [21]. Primordia require 15–23 °C, whereas the fruiting bodies require 28–30 °C. Temperatures below 12 °C result in a slower growth, reduced stipe, and thicker flesh. Spores require 22–26 °C for their growth. All the stages of mushroom growth need the presence of water. For the mycelia growth, 45–65% of water is appropriate, but for the fruiting body development a relative humidity of 75–95% is necessary. Humidity more than 95% results in the chances for bacterial contamination. Presence of light is negligible for the growth of mushroom mycelia, whereas spore and primordia formation take place under low intensity of light. Similarly, light is very essential for the better development of fruiting body and its quality [22]. An optimum pH is the other limiting factor which affects the mushroom growth. Almost all the saprophytes including *G. lucidum* grow slightly under an acidic pH of 4–6.

8 Cultivation

Ganoderma lucidum cultivation was first introduced from China in 1980s. Currently there are a diverse range of cultivation methods based on the employed substrates. The three most common traditional methods of cultivation are wood pulp, wooden box, and natural wood log cultivation [23, 24]. Techniques such as wood log or wood chip cultivation are not applicable to produce *G. lucidum* in the commercial practice, as they raise ecological problems such as cutting and processing of trees, and thus there is a concern. It is therefore necessary to find an effective and a reliable way of cultivation as well as to produce in larger-scale to meet the market demands.

8.1 Wood Pulp Cultivation (Bottle Cultivation)

It is the simplest method of cultivation which involves placing the sterilized wood pulp in a glass bottle along with spawns of *G. lucidum*. Mushrooms along with the bottle are incubated for 3 months. This method usually yields smaller-sized mushrooms of relatively inferior quality.

8.2 Box Cultivation

This method involves grafting the fungi into drilled wooden logs (1 m), which are then placed in the wooden boxes. This method takes about 3–6 months for the

generation of fruiting body. Mushrooms of medium-size and moderate quality are generally produced through this method.

8.3 The Natural Wood Log Method

Natural wood log cultivation could be carried out by the following two methods. One utilizes cultivation on longer unsterilized wood logs and the other uses shorter sterilized logs. First method was the common cultivation practice in China until the new development using short logs which was introduced in the late 1980s. Natural wood logs of 1 m were used for long woods without any sterilization. This method takes longer incubation periods (2–3 years) besides requiring more labor to produce matured fruiting bodies. Short wood log cultivation method was introduced from Japan. It is the common cultivation strategy adopted by natural log growers in China, Japan, Korea, and USA. This method offers the production of a larger basidiocarp with superior quality which involves the preparation of wood logs, especially broad-leaf hard woods of 15 cm in diameter with the length of 14–24 cm. The moisture content should be about 35–40%. The wood logs are then enclosed in polypropylene bags and sterilized. They are incubated with precultured mushroom spawns of 5–10 g. Mycelial run is usually carried out with less oxygen and under dark condition. Formation of mushroom primordia occurs during 50–60 days after spawning. The mushroom colonized wood logs are then buried under nutrient rich soil leaving the primordia above the soil in a green house. Regular monitoring is essential to ensure mushrooms of high quality. Harvesting of mushroom takes approximately 25–30 days after the formation of primordia [5].

8.4 Cultivation on Sawdust

The processing of wood logs is expensive and requires special kind of woods and expertise which make the farmers to think about using sawdust as a cultivating medium. Sawdust is the wood dust produced after processing of woods in the mills. In this method, *G. lucidum* is cultivated on sawdust filled in the polypropylene bags along with rice bran (10%) and $CaCO_3$ (3%). They are moistened to 60% and filled into polybags (700–1000 g). Finally, the bags are plugged with a cotton cap. Steaming of the bags is then carried out for 5 h at 95–100 °C. The bags are then kept at room temperature overnight and inoculated with mushroom spawns. About 3–4 weeks is an essential period for the mushroom mycelia to colonize on the substrate. The bags are then opened for the formation of primordia at 28 °C. The relative humidity is maintained at 85–90% during primordia development. Approximately after 2–3 months from the appearance of primordia, matured basidiocarps will be ready for harvest [25].

Following sawdust cultivation, new substrate compositions were proposed by various researchers. Chen [26] recommended a substrate media containing oak sawdust (80%), unprocessed wheat bran (18%), sucrose (1%), calcium carbonate

Table 1 Growth requirements for the cultivation of *G. lucidum*

Mycelial colonization
Temperature – 25 °C (optimum)
Light – not needed
Duration – 3–4 weeks
Primordia formation
Temperature – 28–30 °C
Relative humidity – ~90–95%
Duration – 1 week after mycelia colonization
Light – Reduced light (100 lux)
CO_2 – >0.1%
Mushroom development
Temperature – 28–30 °C
Relative humidity – 80–85%
Duration – 25–30 days after primordia formation
Light – 150–200 lux
CO_2 – >0.1%

(1%), and 60–70% moisture for the cultivation of *G. lucidum*. Substrate media formulation using sunflower seed hulls [27], stillage grains [28] soy residue [29], etc., have been reported. Sawdust cultivation can be carried out in bags, trays or bed [30], bottles, and pots [31] based on the scale of production.

The growth of *G. lucidum* depends on cellulose and lignin that are present in the wood logs. They utilize these compounds by the production of enzymes which break down the bonds in the long cellulose chains into shorter chains so that the carbon become available for the growth of fungi. *Ganoderma lucidum* utilizes carbon resources from compounds such as sugar, starch, cellulose, hemicellulose, and lignin. Mycelia cultivation requires the presence of sugar and nitrogen sources. Compounds such as amino acids, urea, nitrogen can induce the mycelia growth [32]. Sawdust of broadleaf trees [33], cotton seed husk, corn cob, paddy straw, wheat and rice bran, corn powder, ammonium sulfate, and urea are used in the substrate media to produce fruiting body [34]. Carbon to nitrogen ratio is a key factor for the growth of mushroom as well as for the formation of fruiting body. Appropriate ratio of C:N required is 15–45:1 for mycelia and 30–40:1 for fruiting body [35, 36]. The growth requirement of *G. lucidum* on the solid substrates is given in the Table 1.

8.5 Modern Cultivation Practices

In these days, industries are looking forward for faster and cheaper practicing methods for the cultivation of *G. lucidum*. Both solid state fermentation (SSF) and liquid state fermentation (LSF) techniques offer scope for large-scale production of this mushroom using cheaply available agricultural residues. The details obtained from solid state cultivation (SSC) can be applied to study the liquid state cultivation

(LSC) [37, 38]. Cultivation of fruiting bodies of *G. lucidum* takes several months and proper environmental conditions; hence, mycelia-based and culture broth-based products have assumed immense importance due to increased quality control and year-round production [39]. SSF is nowadays used to upgrade the valuable food supplements, enzyme production, and the feed values of waste materials [40]. In submerged state cultivation method, mycelia culture can be standardized easily under controlled conditions and simple processes are required for downstream processing of the bioactive compounds such as polysaccharides and other compounds released into the culture medium. Several researchers have reported the cultivation of mycelia using submerged/liquid state cultivation. Standardizing the culture conditions and medium composition strongly influence mycelial growth and production of bioactive compounds in this mushroom [5, 39, 41]. A novel three-stage light irradiation strategy has been developed in submerged cultures of *G. lucidum* for the efficient production of polysaccharides and ganoderic acid [42].

Development of bidirectional SSF technology is the recent focus in the modern mushroom science. It is a new type of fermentation technique emerged from China in the 1990s. The key of this technology is that the medicinal mushroom strains are cultured in the special substrate, which consisted of Chinese medicinal herbs as substrate instead of the traditionally cultured nutritious substrate. The fermentation products are known as the medicinal fungal substances. During fermentation, these medicinal compounds in the substrate provide nutrients for the fungal growth. At the same time, the fungal strains produce enzymes which change the tissue and components in the substrate to new functional components. Thus, by bidirectional fermentation, medicinal strains produce bioactive compounds of higher quality [43, 44].

Bidirectional SSF was applied by Chen and Chen [45] in *G. lucidum*. They designed three types of medium; *G. lucidum* in an ordinary growth medium, Chinese materia medica (CMM) medium containing *Radix astragali*, and selenium rich CMM-containing medium. Fermentation products from the three types of media were checked at fixed time intervals. Polysaccharide contents of fermentation products from these culture media were 4.65%, 3.76%, and 4.50%, respectively. By observing the changes in the concentration of polysaccharide, protein, and total saponin in fermentation products from the CMM-containing medium at various times, they concluded that the 28th fermentation day was the time during which secondary metabolism was the most active. Similar studies were carried out using *G. lucidum* with different medicinal herbs [46].

Another application of *G. lucidum* is in the functional food research and development. For this, the amount of polysaccharides, reducing sugars, amino acids, and protein contents were determined. The outcome indicates that the optimum production needs specific conditions, i.e., size of the granule, temperature, fermentation time, and inoculum size. The amount of polysaccharide was found to be 21.97 mg/g under optimum culture conditions [47]. Further research in the production of polysaccharides from *G. lucidum* was reported by You [48]. Moreover, *G. lucidum* cultivation opens a door to process forest and agricultural wastes into useful by-products and thus reduces the environmental pollution.

9 Bioactive Compounds in *G. lucidum*

Approximately 400 different bioactive compounds are present in this mushroom. Polysaccharides, triterpenoids, organic germanium, ergosterol, nucleotides, sterols, amino acids, fatty acids, proteins, intracellular/extracellular enzymes, and trace elements [2, 49] are the major active compounds possessing biological activities. All parts of *G. lucidum* viz., fruiting body, mycelia, and spores, are reported to contain these compounds. Mushroom-derived polysaccharides and triterpenes have increased demand in the market. The extracts of this mushroom are given as a health supplement or medicine for anticancer, antiviral, immunomodulating, hepatoprotective, and hypocholesterolemic agents [50, 51]. Absence of side effects and presumed health benefits give reputation for this mushroom to use as herbal medicine [39].

The bioactive compounds from *G. lucidum* have a long history and have received considerable attention in recent years. Intensive work on the therapeutic effects of *G. lucidum* has been reported in the literature.

Ganoderma lucidum possesses unique bioactive molecules which include

- Polysaccharides [52–54]: A variety of polysaccharides that tend to be the components to interact with the immune system and are subdivided into β-1,3-glucans and polysaccharide peptides such as peptidoglycan.
  - Water-soluble polysaccharide peptides include GLPS peptide (GLPP) [55], GLPG [56], GLIS [57], PGY [58] and F3 [59].
  - β-1,3-Glucan (a subset of polysaccharides) sometimes called Curdlan [53] and other Glucan molecules [60].
- Triterpenoids: Over 120 triterpenoids [61] were revealed which can be separated into two classes. Those with a carboxylic side chain (Ganoderma acids) and those without (Ganoderma alcohols). Some are referred to as lucidenic acids [62].
- Nucleotide bases such as thymine, uridine, inosine, guanosine, and adenosine are present in this mushroom. The sum of all ranging from 303 to 1217 mcg/g in the mushroom cap and 22–334 mcg/g in the stem [63].
- Bioactive proteins such as LZ-8 (Lingzhi-8) [64], Ganodermin A and 114 kDa hexameric lectin, a glycoprotein with 9.3% sugar [65] are present.
- A reversible and a highly specific competitive alpha-glucosidase inhibitor known as SKG-3 with an IC_{50} of 4.6 mcg/mL [66].
- Ergostane sterols and ergosterol, also known as pro-vitamin D2 [67].
- C19 fatty acids (nonadecenoic acid and cis-9-nonadecenoic acid) [68, 69].
- Riboflavin.
- Vitamin C.
- Copper and Zinc [70].
- Selenium of up to 72 mcg/g dry weight and can transform selenium into selenium-containing proteins [71].
- Germanium of up to 489 mcg/g based on dry weight.

9.1 Structure and Properties

Polysaccharides, triterpenoids, and peptidoglycans are the main sources that are responsible for the bioactivity of *G. lucidum*. Activities from other molecules have not been studied significantly. Major triterpenoids responsible for the bioactivity are Ganodermic acids, Ganodermic alcohols, and Lucidenic acids.

9.1.1 Polysaccharides

Polysaccharide is a long chain of monosaccharides linked by glycosidic bonds. More than 100 types of polysaccharides have been reported to be present in *G. lucidum*. Most of them belong to a group of β-glucan which consists of a linear backbone of β-(1,3) linked D-glucopyranosyl groups with varying degree of branching from the C-6 position. They are considered as the active agents to fight against cancer. The molecular weight ranges from 4×10^5 to 1×10^6 in the primary structure [2]. β-Glucans of higher molecular mass are more effective than that of glucans with low molecular mass [72, 73]. β-Glucans also exist with heteropolysaccharide chains of xylose, mannose, galactose, uronic acids, and β, D-glucan-protein complexes which are present in the dry fruiting body (10–50%) [74, 75].

Polysaccharides prevent oncogenesis by not attacking the cancer cells directly, but by activating the immune response in the host. Fang and Zhong [76] indicated that active immunomodulatory polysaccharides are water-soluble (β-1,3 and β-1,6) glucans which can be precipitated by ethanol. Other polysaccharides such as glycopeptides [77, 78] and proteoglycans [79, 80] are also reported to have immunomodulatory activities. Purified mushroom polysaccharides have been used worldwide especially in China, Japan, and Korea for years for clinical purposes without any side effects. Polysaccharides are reported to increase the quality of life of cancer patients and offer promising survival rates [81]. Mushroom extracts are used as tonic for the immune system. Polysaccharides are reported to be very effective for wound healing [82].

9.1.2 Triterpenoids

Other class of bioactive compounds are also present abundantly in *G. lucidum*. Approximately 140 types of triterpenes have been identified in *G. lucidum* [49, 83, 84]. Most of them are lanostane type triterpenes. Extract of *G. lucidum* tastes bitter due to the presence of these triterpenoids, especially Ganoderic acid which is the major type of triterpenoid present in this mushroom. There are several types of Ganoderic acids such as, GA-A, B, C, and F. Previous studies indicated that triterpenoids are present more in the spores as compared to other parts of the mushroom. The production of bioactive compounds is also affected by the area and conditions in which they grow [85]. The basic structure of triterpenoid depends on the structure of lanosterol, an important intermediate for the biosynthesis of steroid and triterpene. Triterpenes are divided into three groups based on the number of carbon atoms and functional groups which are C30, C27, and C24 compounds [86]. Many of them find useful as chemotherapeutic agents.

9.1.3 Phenolic Compounds

Phenolic compounds are one of the important and the most commonly extracted bioactive compounds from *G. lucidum*. They can be classified as simple phenols and phenolic acids such as gallic acid, benzoic acid, syringic acid, chlorogenic acid, and polyphenols which are classified into many distinct groups such as flavonoids, tannins, stilbenes. Flavonoids are a group of polyphenolic compounds with known health-beneficial properties that include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory activity [87]. Research studies suggest that the biological activity of these compounds is related to their antioxidant activity. Phenolic compounds have significant biological and pharmacological properties, and some have demonstrated remarkable ability to alter sulfate conjugation. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals [88]. Methanolic extracts of *G. lucidum* have been reported to contain higher antioxidant activity [89, 90], and their radical scavenging mechanism has been revealed (c). Heleno et al. [91] revealed that the phenolic extracts of fruiting body and mycelia have higher antioxidant potential than their corresponding polysaccharide extracts, highlighting a higher contribution of free phenolic compounds than the antioxidants linked to polysaccharides.

9.1.4 Sterol and Ergosterol

Sterols are derivatives of triterpenoids. *Ganoderma lucidum* has been determined to contain ergosterol and 24-methylcholesta-7,22-trien-3-ol. 8,9-epoxyergosta-5,22-dien-3,15-diol from *G. lucidum* was reported as the first isolated free sterol [72].Hajjaj et al. [92] reported the isolation and identification of 26-oxygenosterols such as ganoderol A, ganoderol B, and ganoderic acid Y. They also determined that 26-oxygenosterols could lead to novel therapeutic agents that can lower the blood cholesterol.

Ergosterol is one of the important pharmaceutically relevant compounds. It is a vitamin D precursor. The integrity of fungal cell membrane is maintained by ergosterol. It also generates cellular energy. Measurement of Ergosterol is an important parameter in the biomass production. A new, highly oxygenated sterol, 22E, 24R-ergosta-7,22-diene-3beta, 5alpha, 6beta, 9alpha,14alpha-pentol was reported by Zang et al. [93]. Ergosterol was found to be higher in *G. lucidum* (median content, 705.0 μg/g; range, 189.1–1453.3 μg/g; $n = 19$) as compared to other species (median content, 80.1 μg/g; range, 16.0–409.8 μg/g; $n = 13$) [94].

9.1.5 Proteins

In addition to polysaccharides and triterpenoids, *G. lucidum* is a reservoir of proteins and peptides with biological activities [95]. LZ-8 is one of the proteins isolated from *G. lucidum* which appears to be related to an ancestral protein of the immunoglobulin superfamily. Confirmation of this protein was carried out by sequencing studies, and it resembled with the sequences and secondary structure of heavy chain region of immunoglobulin. The biological activities are almost similar to lectins which have mitogenic capacity [96] towards mouse spleen cells and human peripheral lymphocytes in vitro.

Other proteins are ribosome inactivating proteins (RIP), antimicrobial proteins, ribonucleases, and laccases. All these compounds play essential role in regulating

the body's immune mechanism directly or indirectly. Among these proteins, FIP plays a vital role in antitumor, antiallergic, proliferation of lymphocytes, and transplant rejection activities [97]. Seven FIPs have been identified till now from varied species of Ganoderma. They are LZ-8 (*G. lucidum*), FIP-gts (*G. tsugae*), FIP-fve (*Flammulina velutipes*), FIP-vvo (*Volvoriella volvacea*), FIP-gmi (*G. japonicum*), and FIP-gsi (*G. sinensis*) [98, 99]. One molecule of FIP composed of 110 to 114 amino acids and their molecular weights are around 13 kDa. Among the FIP proteins, LZ-8 contains low levels of carbohydrates, but other FIPs are pure proteins without any carbohydrates. The presence of a lower amount of FIP in *G. lucidum* is a problem for recovery to meet its growing demand. Nowadays it is the major concern among the researchers, and thus the focus is on the genetic engineering to develop FIP protein through cloning FIP genes in eukaryotes and prokaryotes [100, 101].

9.1.6 Nucleotides and Nucleosides

These are the nitrogenous compounds which play important roles in the metabolism and stimulate hemopoieses. Nucleosides include adenosine and 5-deoxy-50 methylsulfinyladenosine. Adenosine of *G. lucidum* suppresses platelet aggregation and prevents heart attacks and thrombosis [102].

9.1.7 Lipids and Fatty Acids

Phosphatidic acids are not the abundant lipid constituents in the living organisms, but they play significant role in the membrane trafficking events and defense mechanisms against infection and tissue damage during inflammation. Presence of these lipids makes this mushroom important among the medicinal species [103]. The main fatty acids in *G. lucidum* are palmitic acid, linoleic acid, oleic acid, and stearic acid. Fatty acids in the spores could inhibit tumor cell proliferation [94]. Nonadecanoic acid is another fatty acid present in this mushroom which has the highest inhibitory activity, followed by heptadecanoic acid, stearic acid, and palmitic acid [69]. Palmitic acid and stearic acid are the strong apoptotic agents [104].

9.1.8 Amino Acids

Nutritional analysis of *G. lucidum* showed the presence of 16 amino acids (Table 2), where glutamic acid (120), aspartic acid (117), glycine, and alanine show the highest relative abundance, whereas methionine shows the least [77].

9.1.9 Alkaloids and Other Compounds

Generally, alkaloid content is relatively less in *G. lucidum*. The alkaloids such as choline and betaine were isolated from the spores of *G. lucidum* [72]. Studies have showed the presence of alkaloids and their chemical allies, i.e., saponins, flavonoids, and tannins [105] in *G. lucidum*.

Studies by Mizuno [106] showed that the extracts of *G. lucidum* (% dry weight) consist of folin-positive material (68.9%), protein (7.3%), glucose (11.1%), and metals such as K, Ca, Ge, and Mg. The outcome of this study agrees well with the reports of other investigations [20, 107]. Moreover, there is a difference in the qualitative and quantitative results in the chemical composition of *G. lucidum* extracts. It is mainly

Table 2 Amino acid composition in *G. lucidum*

Amino acid	Relative abundance
Glutamic acid	120
Aspartic acid	117
Glycine	108
Alanine	100
Threonine	66
Valine	61
Proline	60
Leucine	55
Serine	54
Isoleucine	36
Phenylalanine	28
Arginine	22
Lysine	21
Tyrosine	16
Histidine	12
Methionine	6

affected by the factors such as quality of the strain, origin, cultivation conditions, stages of harvesting, and extraction processes [83]. Elemental analysis of log-cultivated fruiting bodies of *G. lucidum* revealed phosphorus, silica, sulfur, potassium, calcium, and magnesium to be their main mineral components. Iron, sodium, zinc, copper, manganese, and strontium were also detected in lower amounts, as were the heavy metals such as lead, cadmium, and mercury [108]. *Ganoderma lucidum* also contains soluble proteins, oleic acid, cyclo-octasulfur which is an ergosterol peroxide (5,8-epidioxy-ergosta-6,22E-dien-3-ol), and cerebrosides ((4E0,8E)-N-D-20-hydroxystearoyl-1-Ob-D-glucopyranosyl-9-methyl-4-8-sphingadienine, and (4E,8E)-N-D-20-hydroxypamitoyl-1-O-b-D-glucopyranosyl-9-methyl-4-8-sphingadienine [49, 106, 109]. Compounds such as choline, betaine, tetracosanoic acid, stearic acid, palmitic acid, ergosta-22-dien-3-ol, nonadecanoic acid, behenic acid, tetracosane, hentriacontane, ergosterol, and β-sitosterol have been reported in the spores of *G. lucidum* [110].

Some attention has been given to the germanium content of *Ganoderma* spp. Germanium was the fifth highest in terms of concentration (489 μg/g) among the minerals detected in *G. lucidum* fruiting bodies collected from the wild [111]. Germanium is not an essential element at low doses. It has been credited with immunopotentiating, antitumor, antioxidant, and antimutagenic activities [112]. Other compounds that have been isolated from *G. lucidum* include enzymes such as metalloprotease, which delays clotting time, ergosterol (provitamin D_2), nucleosides, and nucleotides (adenosine and guanosine) [2, 50].

9.2 Extraction, Product Recovery, and Analysis of Bioactive Compounds

Ganoderma lucidum is enriched with active compounds such as polysaccharides, triterpenoids, nucleotides, organic germanium, fatty acids, proteins, amino acids,

and sterols. Therefore, it is essential to develop a methodology for the effective recovery and easy analysis of these bioactive compounds. All these components have been reported to have pharmaceutical applications. Separation of individual components is difficult, time-consuming, and expensive; therefore, industries are looking for random isolation of compounds in the form of crude mushroom extract. In the past few years, it is being a challenge to develop a methodology for the elicitation, recovery, extraction, and analysis of bioactive compounds from *G. lucidum*. Triterpenoids appear to be hydrophobic and are present in ethanol or chloroform fractions, whereas the polysaccharides are water-soluble and are the major bioactive compounds in the water-soluble extract of Ganoderma.

9.2.1 Preparation of *G. lucidum* for the Extraction of Bioactive Compounds

Ganoderma lucidum should be first processed after collection. Preparing the raw materials for a process involves cleaning the material from dirt by washing or rinsing and removing the trunks either manually or by using a vegetable slicer. Thereafter, the material should be dried to reduce their moisture content, to avoid probability of degradation, and to facilitate the safe storage and transportation [113]. By removing the moisture content, the efficiency of extraction process will be enhanced remarkably.

9.2.2 Drying (Demoisturization)

Generally, many drying methods are used to dry the mushroom sample. These methods are classified into two groups: heating (baking, ovens, stoves, and infrared) and air-drying (drying chambers with air circulation) [113]. Classically, drying *G. lucidum* in an open air either under shading or direct sunshine is the most commonly used method due to its low-cost and simple preparation set-up. However, choosing the right drying method is limited by the type of desired bio-components and their physical properties. For example, for thermolabile components (thermally nonstable components), such as polyphenols and triterpenes, it is recommended to maintain the drying temperature below 40 °C to avoid thermal degradation of these components [114, 115]. Also, for some of the light sensitive components such as polyphenolics, it is important to keep the raw materials away from the sun light during the drying stage [115]. For industrial production, large-scale batches could be processed by hot air-drying method which is an economically cost-effective but physically may affect the products due to the probability of oxidation damage that can occur when exposed to air stream [116]. In general, to retain most of the bioactive components, a temperature range of 50–60 °C is the most commonly followed for drying the herbal materials [117–119].

Through an extended literature survey, different drying methods were reported to prepare the herbal materials for the extraction of bioactive compounds. *Ganoderma lucidum* under 60 °C for 24 h [120] is suitable to retain the bioactive compounds, while in many other reviews air drying chambers were used to dry *G. lucidum* under 105 °C [54, 121]. The flexibility of using different drying methods could be attributed to the ability of polysaccharides to withstand relatively a wider

range of temperatures, i.e., 30 °C [122], 45 °C [123], 50 °C [124], 90 °C [125], and 105 °C [126], depending on the matrix of the mushroom.

9.2.3 Size Reduction of Herbal Materials (Grinding)

Size reduction is the second important preparatory stage for any extraction process. It was found that cutting the mushroom into small pieces or reducing the particle size of the raw materials before extraction is essential to increase the mass transfer rate of the entire process. Reducing the particle size leads to an increase in the contact surface area between the solvent and the extracted mass, which increases the diffusion of bio-components in the solvent and thus results into an increase in the overall mass transfer rate. The size reduction can be implemented by inserting the mushroom sample into a hammer mill or disc pulverizer provided with sieves in different ranges of particle permeation. Many factors affect the grinding of mushroom samples such as adjusting the speed of the rotor clearance between the hammers and the lining of the grinders, the discharge capacity of the mill, and the sieve sizing which is preferable to be in between 30 and 40 mesh as an optimal range for the extraction of mushroom samples.

For *G. lucidum*, to improve the extraction process of polysaccharides, the fruiting body is preferably to be used in the powder form [121]. A 32 range of particle size (2–5 mm) is reported by several studies on the ultrasonic-assisted extraction of polysaccharides from *G. lucidum*. The fruiting bodies of *G. lucidum* were crushed to pass 2 mm screen [73, 120, 127], 5 mm [118] or cutting into small pieces of 3 to 2 cm of 2 mm thickness [128, 129]. Generally different grinding machines (pulverisers) are used to prepare fine particles in the powder form. Usually these grinding machines are provided with shaker and sifting-sieves of 12–120 mesh [130], such as 70 mesh [131], 40 mesh [132], and 60 mesh [133].

9.2.4 Pretreatment with Aqueous Ethanol

Many studies showed that pretreating the herbal materials with polar organic solvents (ethanol, methanol), or an aqueous alcoholic mixture, helps to exclude large amount of constituents (monosaccharides, fatty acids, amino acids, phenols, and endogenous enzymes), other than macromolecules such as polysaccharides, nucleic acids, and proteins which could be extracted in a later stage by the polar solvent such as water. The aqueous mixture of ethanol, 60–90% (v/v), has been used for this purpose due to its ability to extract a wide range of components with different polarity. The most important stage in the pretreatment with aqueous ethanol is the prior removal of hydrophobic constituents, such as fats that greatly influence the extraction stage and limit the penetration of water into the solid structure [134]. In the extraction of polysaccharides from *G. lucidum,* the raw materials are treated first with 80% ethanol, under shaking, at 30 °C for 24 h, to remove free sugars (monosaccharides), polyphenolics, and lipophilic components [116, 127, 135]. Another composition of aqueous ethanol, i.e., 95%, has been reported in the pre-treatment of *G. lucidum* relatively at a higher temperature (70 °C), to improve the removal of endogenous enzymes and to reduce the long time required as compared to 80% ethanol at room temperature [120, 124].

9.3 Polysaccharides

They are the major contributors of bioactivity in *G. lucidum* with a wide range of physicochemical properties. Major class of polysaccharides are β-1,3 and β-1,6-D glucans. They exist in two forms, i.e., water-soluble and water-insoluble. Polysaccharides are of two types: extracellular (EPS) and intracellular (IPS). Exopolysaccharides or extracellular polysaccharides are secreted by the microorganisms into the surrounding environment. Generally, IPS is extracted with 1 M NaOH at 60 °C for 1 h. Other solvents such as ammonium oxalate (1%) and NaOH (5%) could also be used for the extraction. EPS is usually extracted by precipitation with 3 to 4 volumes of ethanol of 95% [39]. To eliminate smaller molecules such as mono- and oligosaccharides, dialysis of the filtrate is sometimes preferred before applying precipitation using ethanol [76]. In case of solid-state fermentation (SSF), it is difficult to separate EPS from IPS as mycelia will be strongly bound with the substrate and EPS does not dissolve in the liquid phase. To recover the polysaccharides, the fermented substrate along with mycelia should be extracted with cold or hot water [41] for 5 h. However, such a method not only separates EPS and IPS produced by the organisms, but also some polysaccharides from the solid substrate. The resulted polysaccharides are analyzed by phenol-sulfuric acid method [136]. But it has been found out that the detection of polysaccharide by this method is not stable because along with polysaccharides the presence of some of the monosaccharides, oligosaccharides, and protein also give faulty positive result for phenol-sulfuric acid. Therefore, it is important to eliminate other molecules from the samples using techniques such as dialysis, gel exclusion chromatography, or column chromatography. Determination of proteins separately is also necessary to make sure the accuracy of phenol-sulfuric acid assay [39].

To isolate water-soluble polysaccharides from *G. lucidum*, the samples are extracted with hot water (95–100 °C). It is followed by three volumes of ethanol precipitation and the polysaccharide fractions are collected by centrifugation [77].Alkali-soluble polysaccharides are separated by 0.1–1.0 M sodium hydroxide [137, 138]. It has also been reported that to remove the lipids present in the sample, prior to the hot water or alkali extraction it is to be extracted first with ethanol under reflux [53]. This pretreatment also deactivates enzymes which hydrolyze the polysaccharides. Cheong et al. [75] reported that the biological activity of polysaccharide differs based on the types of extracting solvents as well as the fractionation methods employed.

The extracted polysaccharides are further isolated and purified by fractional precipitation, acidic precipitation, ion exchange chromatography, gel filtration, affinity chromatography, and TLC. Electrophoresis and gel filtration chromatography techniques are used as effective tools for determining the homogeneity and molecular weight. Paper chromatography, TLC, GC-MS, and HPLC are used for compositional analysis [72].

9.4 Triterpenoids

More than 130 oxygenated triterpenes have been isolated from *G. lucidum* [139] and most of them are lanostane-type triterpenes. There are two types of extraction

methods for the isolation of triterpenes from *G. lucidum*. One involves the extraction of total triterpenes using the organic solvents and water. Min et al. [85, 140] extracted the triterpenes from spores of *G. lucidum* by refluxing with methanol. Extraction of triterpenes is usually done by means of methanol, ethanol, acetone, chloroform, ether, or a mixture of these solvents.

The second approach is the selective isolation of acidic triterpenes from the fraction of total triterpene. Extraction of fruiting bodies with 95% aqueous ethanol under reflux has been reported [141]. The obtained ethanol fraction was evaporated under reduced pressure to obtain a residue which was then suspended in water followed by extraction with chloroform. 5% $NaHCO_3$ was added to the chloroform extract and the water phase was collected which was again extracted with chloroform after acidified with HCl (6 N) to a pH less than 3 [142]. The collected residue after chloroform evaporation was suspended in absolute ethanol and spectrophotometric measurements were made at 245 nm to determine the Ganoderic acid [143]. Triterpenes could be further purified by silica gel column chromatography and preparative HPLC [144, 145].

10 Therapeutic Effects of Bioactive Compounds of *G. lucidum*

Being worshipped as a herbal medicine, a great deal of work has been carried out on the therapeutic applications of this special mushroom [14]. Medicinal use of *G. lucidum* has been recorded in ancient Chinese manuscripts. Extracts of this mushroom have been included in the treatment of insomnia, dizziness, chronic hepatitis, coronary heart diseases, hypertension, carcinoma, and bronchial cough. One of the promising properties shown by this wonder medicine is the extended lifespan while increasing the vitality. *G. lucidum* has been used in the fields of antiaging, male sexual dysfunction, hypercholesterolemia, chemotherapy induced toxicity, anticarcinogenic, narcotic-induced immunosuppression, antitumor activity, radioprotective, sleep promoting, antiviral, antifibrotic, antiulcer, radical-scavenging, and in the immunostimulation [2]. It is widely used as an active adjuvant in the treatment of carcinoma and related symptoms.

Sliva [146] indicated that in Western medicine one of the major obstacles in accepting the mushroom based natural products is their complexity. Alternatively, this complexity can also bring significant advantages. Some of the components in the natural products could reduce the cytotoxicity of cells, and the interaction between some of the biologically active components may be responsible for their in vivo effects. Besides, different compounds could modulate the unrelated signaling and thereby exhibiting a synergistic effect. The triterpenes of *G. lucidum* directly suppress the growth and invasive behavior of cancer cells, whereas polysaccharides of *G. lucidum* stimulate the immune system resulting in the production of cytokines and activation of anticancer activities of immune cells. The data obtained from the research studies demonstrate the effect of *G. lucidum* only on the molecular level, and thus preclinical and clinical studies are necessary for the validation of this

natural product in the prevention and/or therapy of cancer. The important medicinal applications of *G. lucidum* are described below.

10.1 Anticancer Studies

Cancer is one the most concerned medical conditions among the human population and effective treatments are always sought which have least or no side effects. *Ganoderma lucidum* has been a popular supplement taken by healthy individuals to boost their immune system or by the cancer patients to reduce the side effects of chemotherapy. *Ganoderma lucidum* is considered to be a factory for bioactive compounds which can reduce the lethal effects of cancer. Fruiting body, mycelia, or spores are reported to contain these bioactive compounds. Polysaccharides and triterpenes are the two major classes of components in this mushroom which exhibit chemopreventive and/or tumoricidal effects as proved by numerous in vitro and in vivo studies [147]. Tumor implanted animal models have shown inhibitory effects on angiogenesis and metastasis. However, evidence from well-designed human trials is still scarce. Tomasi et al. [148] tested 58 basidiomycetes mushrooms, of which *G. lucidum* was shown to be the most effective in killing the cancer cells. *Ganoderma lucidum* induced cell-cycle arrest and apoptosis in various human and rodent tumor cells, including murine lymphocytic leukemia L1210 and Lewis lung carcinoma, have been reported [148].

Ganoderma lucidum also exhibits chemo-and radio preventive effects which are attributed to its effects on the immune system. *Ganoderma lucidum* extract showed better effect than Krestin (protein bound β-glucan isolated from *Coriolus versicolor*) in repairing the damage of subset T-cells in the spleen of irradiated mice [149]. One of the polysaccharide peptides from *G. lucidum* reported to restore the immunologic parameters depressed by morphine treatment beyond normal levels [150].

10.2 Antioxidant Activity

Antioxidants are natural or man-made substances which can prevent the activity of other chemicals known as free radicals that cause damage to the cells. *Ganoderma lucidum* is one such mushroom widely used due to its antioxidant activity. Consumption of antioxidants may help prevent cancer and other chronic diseases [151].Antioxidants protect cellular components from oxidative damage, decrease risk of mutations and carcinogenesis, and protect immune cells, allowing them to maintain immune surveillance and responses. The bioactive compounds such as polysaccharides and triterpenoids show antioxidant activity in vitro [58, 152, 153]. Antioxidants from *G. lucidum* were found to be absorbed quickly after ingestion, resulting in an increase in plasma total antioxidant activity [154].

Ooi and Liu [155] reported that protein-bound polysaccharide (PBP) and polysaccharide peptide could mimic the endogenous antioxidant superoxide dismutase (SOD) in cancer-bearing animals in vivo. These polysaccharides were also reported

to protect the immune cells from oxidative damage. The protective effects of *G. lucidum* on DNA strand scission induced by a metal-catalyzed Fenton reaction, ultraviolet irradiation, and hydroxyl radical attack were shown in agarose gel electrophoresis in vitro [156]. Hot water extracts of *G. lucidum* significantly protected Raji cells from hydrogen peroxide (H_2O_2)-induced DNA damage [157]. The aqueous extract protected cellular DNA from oxidative damage, whereas the ethanolic extract damaged the cellular DNA with increased H_2O_2 production and significant cell-killing effects were observed. The results suggested that different effects of *G. lucidum* could be exhibited by different components of extract in bladder chemoprevention. Methanol extracts of *G. lucidum* were reported to prevent kidney damage (induced by the anticancer drug cisplatin) through restoration of the renal antioxidant defense system [158]. In contrast, a fraction of Ganoderma triterpenes (GTS) was found to enhance the intracellular reactive oxygen species (ROS) in HeLa cells, leading to more DNA damage and apoptosis, whereas such a synergism was inhibited by a ROS scavenger [159]. In an animal study (diabetic rats), nonenzymic and enzymic antioxidant levels increased and lipid peroxidation levels decreased with *G. lucidum* treatment [160]. However, a direct link has not been established between the antioxidant properties of *G. lucidum* and its immunomodulatory and anticancer effects.

10.3 Antiallergic Property

Another important application of the fruiting bodies of *G. lucidum* is their application as anti-inflammatory agents for the treatment of asthma or allergy. *Ganoderma lucidum* has unique array of compounds called immunonutraceuticals, which play a leading role in the treatment of histamine-mediated allergic responses [161]. *Ganoderma lucidum* is reported to be as an effective agent to restore the normal balance between the cytokines TH1 and TH2 immune states in patients with histamine-mediated allergic responses [162]. In a case study of hay fever patients, Powell found that patients (male) of age 5 and 39 with different doses viz. 2 tablets × 500 mg a day and 6 tablets × 500 mg per day of *G. lucidum* resulted in a decrease in drowsiness, itchiness and sneezing.

10.4 Antibacterial and Antiviral Activity

Ganoderma lucidum has been widely reported as a good antibacterial agent. Aqueous as well as methanolic extracts of *G. lucidum* inhibited several types of gram-positive and gram-negative bacteria. Studies indicated that a combination of *G. lucidum* extract with different antibiotics has resulted in an additive effect in most of the instances [163]. Active compounds such as triterpenes, ganomycein, and other aqueous extracts have broad spectrum antibacterial activity in vitro [39]. A 15-kDa antifungal protein ganodermin isolated from *G. lucidum* is known to inhibit the mycelia growth of *Botrytis cinerea*, *Fusarium oxysporum*, and *Physalospora piricola* with an IC 50 value of 15.2 μM, 12.4 μM, and 18.1 μM, respectively [65].

10.5 Antidiabetic Effect

Few animal studies of polysaccharide fractions of *G. lucidum* demonstrated that they have the potential hypoglycemic and hypolipidemic activities. The aqueous extract (1000 mg/kg) of *G. lucidum* normalized the blood glucose levels in alloxan-induced diabetes in Wistar rats [164]. Water extract of *G. lucidum* reduced the increase in blood glucose levels in rats following oral glucose test. A clinical study aimed at evaluating the antidiabetic efficacy and safety of polysaccharide fractions extracted from *G. lucidum* (Ganopoly) by a patented technique in 71 patients with confirmed type II diabetes mellitus has been reported by Gao et al. [109]. Treatment with Ganopoly significantly decreased the mean HbA1c from 8.4% at baseline to 7.6% at 12 weeks which demonstrated that Ganopoly is efficacious and safe in lowering the concentration of blood glucose.

11 Conclusions

Ganoderma lucidum is a well-known medicine with remarkable range of applications. Though the global consumption of *G. lucidum* is high, the production rate is not satisfactory. The bioactive compounds in *G. lucidum* make it very special and are classified under Host Defense Potentiators (HDP) which can have immune system enhancement properties. Various products such as capsules, creams, tonics, and syrups are available in the market, which offer high health benefits. Many researches have been performed in this mushrooms on its cultivational and medicinal aspects. Its positive health benefits include anticancer effects, antioxidant, antibacterial, blood glucose regulation, and antiviral effects and protection against liver and gastric injury. Though many studies are performed on animal models, successful studies on human cell models are least reported. Human experimental studies have often been small, and the results are not always supportive of the in vitro findings. Therefore, Ganoderma research on its clinical aspects need more supportive clarifications for the dosage and side effects in the human beings. Similarly, the mechanism of action of different bioactive molecules isolated from this mushroom is yet to be elucidated.

References

1. Wachtel-Galor S, Buswell JA, Tomlinson B, Benzie IFF (2004a) Lingzhi polyphorous fungus. In: Herbal and traditional medicine: molecular aspects of health. Marcel Dekker, New York, pp 179–228
2. Wasser SP (2005) Reishi or Lingzhi (*G. lucidum*). In: Coates P, Blackman MR, Cragg G, Levine M, Moss J, White J (eds) Encyclopaedia of dietary supplements. Marcel Dekker, New York, pp 603–622
3. Hou RH, Liao ST (2009) Research development of the artificial cultivation of *G. lucidum* in China. Guangdong Agric Sci 11:29–32. (In Chinese)
4. Hirose K, Niimura K, Ohara M, Oguchi Y, Matsunaga K, Kadochi J, Sugita N, Furushu T, Yoshikumi C (1988) Antiviral agent. J. P. Patent No.63316734

5. Boh B, Berovic M, Zhang J, Zhi-Bin L (2007) *G. lucidum* and its pharmaceutically active compounds. Biotechnol Annu Rev 13:265–301
6. Borchers AT, Stern JS, Hackman RM, Keen CL, Gershwin ME (1999) Mushrooms, tumours, and immunity. Proc Soc Exp Biol Med 221(4):281–293
7. Mau JL, Lin HC, Ma JT, Song SF (2001a) Non-volatile taste components of several specialty mushrooms. Food Chem 73:461–466
8. Joo SS, Ryu IW, Park JK, Yoo YM, Lee DH, Hwang KW, Choi HT, Lim CJ, Lee DI, Kim K (2008) Molecular cloning and expression of a laccase from *G. lucidum*, and its antioxidative properties. Mol Cell 25(1):112–118
9. Leung SWS, Yeung KY, Ricky YLS, Man YK (2002) Lingzhi (Ganoderma) research the past, present and future perspectives. In: Lin ZB (ed) Ganoderma: genetics, chemistry, pharmacology and therapeutics. Beijing medical University Press, Beijing, pp 1–9
10. Kim HW, Kim B (2002) Recent advances on the biologically active triterpenoids of *G. lucidum*. In: Zhi-Bin Lin (ed) Ganoderma: genetics, chemistry, pharmacology and therapeutics. Proceedings of international symposium on Ganoderma research, Shanghai, 21–23 Oct, Beijing, Medical University Press, pp 10–19
11. Chang ST, Buswell J (1999) *G. lucidum* (Curt.: Fr.) P. Karst. (Amphyllophoromycetidae) – a mushrooming medicinal mushroom. Int J Med Mush 1(2):139–146
12. Lin SC (2000) Medicinal fungi of China-production and products development. Chinese Agricultural Press, Beijing
13. Chang ST, Buswell JA (2008) Safety quality control and regulational aspects relating to mushroom nutriceuticals. In: Proceedings of 6th international conference on mushroom biology and mushroom products, GAMU Gmbh, Krefeld, pp 188–95
14. Wachtel-Galor S, Yuen J, Buswell JA, Benzie IFF (2011) Chapter 9: *G. lucidum* (Lingzhi or Reishi) a medicinal mushroom. In: Herbal medicine: biomolecular and clinical aspects, 2nd edn. CRC Press, Boca Raton
15. Wang JL, Li YB, Liu RM (2010) A new ganoderic acid from *G. lucidum* mycelia. J Asian Nat Prod Res 12(8):727–730
16. Hanaoka R, Ueno Y, Tanaka S (2011) The water-soluble extract from cultured medium of *G. lucidum* (Reishi) mycelia (Designated as MAK) ameliorates murine colitis induced by trinitrobenzene sulphonic acid. Scand J Immunol 74(5):454–462
17. Yang XJ, Liu J, Ye LB, Yang F, Ye L, Gao JR, Wu ZH (2006) In vitro and in vivo protective effects of proteoglycan isolated from mycelia of *G. lucidum* on carbon tetrachloride induced liver injury. World J Gastroenterol 12(9):1379–1385
18. Arora D (1986) Mushrooms demystified, 2nd edn. Ten Speed Press, Berkeley, pp 169–164. ISBN 0-89815. Available at http://www.mushworld.com/sub_en.html
19. Jungjing MA, Zhenjyi FU, Peiyan MA, Yanli SU, Qingjie Z (2007) Breaking and characteristics of *G. lucidum* spores by high speed centrifugal shearing pulveriser. J Wuhan Univ Technol Mat Sci Edit 22:617–621
20. Chen AW (1999) Cultivation of the medicinal mushroom *G. lucidum* (Curtis: Fr.) P. Karst (Reishi) in North America. Int J Med Mush 1(3):263–282
21. Jayasinghe C, Imtiaj A, Hur H, Lee GW, Lee TS, Lee UY (2008) Favourable culture conditions for mycelial growth of Korean wild strains in *G. lucidum*. Mycobiology 36(1):28–33
22. Magday JC Jr, Bungihan ME, Dulay RMR (2014) Optimization of mycelial growth and cultivation of fruiting body of Philippine wild strain of *G. lucidum*. Curr Res Environ Appl Mycol 4(2):162–172
23. Han JR, An CH, Yuan JM (2005) Solid-state fermentation of cornmeal with the basidiomycete *G. lucidum* for degrading starch and upgrading nutritional value. J Appl Microbiol 99(4):910–915
24. Erkel EI (2009) The effect of different substrate mediums on yield of *G. lucidum* (Fr.) Karst. J Food Agric Environ 7:841–844
25. Royse DJ (1996) Specialty mushrooms. In: Janick J (ed) Progress in new crops. ASHS Press, Arlington, pp 464–475

26. Chen AW (2003) A fresh look at an ancient mushroom *G. lucidum* (Reishi). MushWorld – Cultivation, 18 Mar 2003. Available at http://www.mushworld.com
27. Gonzalez-Matute R, Figlas D, Devalis R, Delmastro S, Curvetto N (2002) Sunflower seed hulls as a main nutrient source for cultivating *G. lucidum*. Micol Apl Int 14:19–24
28. Yang FC, Hsieh C, Chen HM (2003a) Use of stillage grain from a rice-spirit distillery in the solid state fermentation of *G. lucidum*. Process Biochem 39:21–26
29. Hsieh C, Yang F (2004) Reusing soy residue for the solid-state fermentation of *G. lucidum*. Bioresour Technol 91(1):105–109
30. Chen AW (2004) Growing *Ganoderma* mushrooms. Chapter 11. Mushrooms for the tropics. Part III. Mushroom worldwide. In: Mushroom grower's handbook 1, Oyster Mushroom Cultivation. pp 224–234. Available at https://www.scribd.com/document/265831668/mushroom-growers-handbook-1-mushworld-com-chapter-11-pdf
31. Kim HK (2001) Comparison of characteristics of *G. lucidum* according to geographical origins: consideration of growth characteristics. World Mushroom. Available at http://www.mushworld.com/sub_en.html
32. Zhou XW, Siu K, Zhang YM (2012) Applied modern biotechnology for cultivation of Ganoderma and development of their products. Appl Microbiol Biotechnol 93:941–963
33. Xia ZN, Jiang JA, He CZ, Liu DY (2003) Preliminary researchers on high yield cultivation techniques of Ganoderma. Hunan Agric Sci Technol 6:56–58
34. Yan MH (2000) Cultivation of *G. lucidum* using oak leaf and waste tea. Edible Fungi 2:22–23
35. Han XH, Wang HZ, He B (2003) A preliminary study on nutritional conditions for the strain mycelium growth of several cultivars of Ganoderma. J Hainan Univ 16:88–92
36. Wu BF, Liu LL, Fang ZH, Liu XY (2008) Effect of nutrition factors on mycelium growth of 51427 in *G. lucidum*. Anhui Agric Sci Bull 14:57–58
37. Maldonado MC, Saad AMS (1998) Production of pectinesterase and polygalacturonase by *Aspergillus niger* in submerged and solid state systems. J Ind Microbiol Biotechnol 20:34–38
38. Mahapatra S, Banerjee D (2009) Extracellular tannase production by endophytic Hyalopus sp. J Gen Appl Microbiol 55:255–259
39. Sanodiya BS, Takur GS, Baghel RK, Prasad GB, Bisen PS (2009) *G. lucidum*: a potent pharmacological macrofungus. Curr Pharm Biotechnol 10(8):717–742
40. Pandey A, Soccol CR, Mitchell D (2000) New developments in solid state fermentation: I- bioprocesses and products. Process Biochem 35:1153–1169
41. Habijanic J, Berovic M (2000) The relevance of solid-state substrate moisturing on *G. lucidum* biomass cultivation. Food Technol Biotechnol 38(3):225–228
42. Zhang W, Tang YJA (2008) Novel three-stage light irradiation strategy in the submerged fermentation of medicinal mushroom *G. lucidum* for the efficient production of ganoderic acid and Ganoderma polysaccharides. Biotechnol Prog 24:1249–1261
43. Zhuang Y, Hong J (2006) Two-way pattern solid fermentation engineering in medicinal mushrooms and further development of Chinese medicine residue. Zhong Yao Cai 31:1918–1919
44. Zhuang Y, Pan Y, Xie XM, Zhang LY (2007) The origin, development and its advantage and potential of the bi-directional solid fermentation for medicinal fungi. Edible Fungi China 26:3–6
45. Chen HZ, Chen J (2004) A preliminary report on solid-state fermentation of *G. lucidum* with *Radix astragali* containing medium. Chin J Integr Med 2:216–218
46. Gu SR, Qin JZ, Chen H (2005) New solid fermentation of 14 Ganoderma strains and the study on polysaccharose content of the fermentation product. Lishizhen Med Mater Med Res 16:313–314
47. Gao Y, Gao H, Chan E, Tang W, Xu A, Yang H, Huang M, Lan J, Li X, Duan W, Xu C, Zhou S (2005) Antitumor activity and underlying mechanisms of ganopoly, the refined polysaccharides extracted from *G. lucidum* in mice. Immunol Investig 34:171–198
48. You JJ (2009) The present status and future aspect of medicinal fungi in China. Edible Fungi Chin 28:3–5

49. Mckenna DJ, Jones K, Hughes K (2002) Reishi, botanical medicines. The desk reference for major herbal supplements, 2nd edn. The Haworth Herbal Press, New York/London/Oxford, pp 825–855
50. Paterson RRM (2006) Ganoderma – a therapeutic fungal biofactory. Phytochemistry 67(18):1985–2001
51. Zheng WF (2011) Drug discovery from fungal metabolites: a review of the papers in this monographical issue of mycosystema concerned with the natural resources, problems and strategies. Mycosystema 30:151–157
52. Bao XF, Dong Q, Fang JN (2000) Structure and conformation behaviour of a glucan from spores of *G. lucidum* (Fr.) Karst. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 32(6):557–561
53. Bao XF, Liu CP, Fang JN, Li XY (2001) Structural and immunological studies of a major polysaccharide from spores of *G. lucidum* (Fr.) Karst. Carbohydr Res 332:67–74
54. Bao XF, Wang XS, Dong Q (2002) Structural features of immunologically active polysaccharides from *G. lucidum*. Phytochemistry 59(2):175–181
55. Ho YW, Yeung JS, Chiu PK, Tang WM, Lin ZB, Man RY, Lau CS (2007) *G. lucidum* polysaccharide peptide reduced the production of proinflammatory cytokines in activated rheumatoid synovial fibroblast. Mol Cell Biochem 301(1–2):173–179
56. Li Z, Liu J, Zhao Y (2005) Possible mechanism underlying the antiherpetic activity of a proteoglycan isolated from the mycelia of *G. lucidum* in vitro. J Biochem Mol Biol 38(1):34–40
57. Ji Z, Tang Q, Zhang J, Yang Y, Jia W, Pan Y (2007) Immunomodulation of RAW264.7 macrophages by GLIS, a proteopolysaccharide from *G. lucidum*. J Ethnopharmacol 112(3):445–450
58. Wu Y, Wang D (2009) A new class of natural glycopeptides with sugar moiety-dependent antioxidant activities derived from *G. lucidum* fruiting bodies. J Proteome Res 8:436–442
59. Chien CM, Cheng JL, Chang WT (2004) Polysaccharides of G. lucidum alter cell immunophenotypic expression and enhance CD56+ NK-cell cytotoxicity in cord blood. Bioorg Med Chem 12:5603–5609
60. Dong SF, Chen JM, Zhang W, Sun SH, Wang J, Gu JX, Boraschi D, Qu D (2007) Specific immune response to HBsAg is enhanced by β-glucan oligosaccharide containing α-(1→3)-linked bond and biased towards M2/Th2. Int Immunopharmacol 7:725–733
61. Fatmawati S, Kondo R (2011) Structure-activity relationships of ganoderma acids from *G. lucidum* as aldose reductase inhibitors. Bioorg Med Chem Lett 21(24):7295–7297
62. Weng CJ, Chau CF, Chen KD, Chen DH, Yen GC (2007) The anti-invasive effect of lucidenic acids isolated from a new *G. lucidum* strain. Mol Nutr Food Res 51:1472–1477
63. Gao JI, Leung KS, Wang YT, Lai CM, Li SP, Hu LF, Lu GH, Jiang ZH, Yu ZL (2007) Qualitative and quantitative analyses of nucleosides and nucleobases in Ganoderma spp. by HPLC-DAD-MS. J Pharm Biomed Anal 44(3):807–811
64. Van Der Hem LG, Van Der Vliet JA, Bocken CF, Kino K, Hoitsma AJ, Tax WJ (1995) Lingzhi-8: studies of a new immunomodulating agent. Transplantation 60:438–443
65. Wang H, Ng TB (2006) Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *G. lucidum*. Peptides 27(1):27–30
66. Kim SD, Nho HJ (2004) Isolation and characterization of alpha-glucosidase inhibitor from the fungus *G. lucidum*. J Microbiol 42(3):223–227
67. Liu JJ, Huang WH, Lv ML, Si JP, Guo BL, Li SJ (2011) Determination of Ergosterol in *G. lucidum* from different varieties and cultured tree species by HPLC. Zhong Yao Cai 34:187–190
68. Gao P, Hirano T, Chen Z, Yasuhara T, Nakata Y, Sugimoto A (2012) Isolation and identification of C-19 fatty acids with anti-tumor activity from the spores of *G. lucidum* (Reishi mushroom). Fitoterapia 83:490–499
69. Fukuzawa M, Hide I, Chen Z, Hirai Y, Sugimoto A, Yasuhara T, Nakata Y (2008) Possible involvement of long chain fatty acids in the spores of *G. lucidum* (Reishi Houshi) to its anti-tumor activity. Biol Pharm Bull 31:1933–1937

70. Matute RG, Serra A, Figlas D, Curvetto N (2011) Copper and zinc bioaccumulation and bioavailability of *G. lucidum*. J Med Food 14(10):1273–1279
71. Falandysz J (2008) Selenium in edible mushrooms. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 26(3):256–299
72. Huie CW, Di X (2004) Chromatographic and electrophoretic methods for Lingzhi pharmacologically active components. J Chromatogr B 812:241–257
73. Chang YW, Lu T (2004) Molecular characterization of polysaccharides in hot water extracts of *G. lucidum* fruiting bodies. J Food Drug Anal 12:59–67
74. Gao Y, Zhou S, Chen G, Dai X, Ye J, Gao H (2002) A phase I/II study of a *G. lucidum* (Curt.: Fr.) P. Karst. (Lingzhi, Reishi mushroom) extract in patients with chronic hepatitis B. Int J Med Mush 4:321–327
75. Cheong J, Jung W, Park W (1999) Characterization of an alkali-extracted peptidoglycan from Korean *G. lucidum*. Arch Pharm Res 22:515–519
76. Fang QH, Zhong JJ (2002a) Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *G. lucidum*. Process Biochem 37:769–774
77. Wang YY, Chen ST, Lin CC, Wong CH, Lin CH (2002) Studies on the immunomodulation and antitumor activities of *G. lucidum* (Reishi) polysaccharides: functional and proteomic analysis of fucose-containing glycoprotein fraction responsible for the activities. Bioorg Med Chem 10:1057–1062
78. Lin SQ, Wang SZ, Lin ZB, Lin YX (2002) Purification and identification of glycopeptides from *G. lucidum* fruit bodies cultivated with grass and wood log. In: Zhi-Bin Lin (ed) Ganoderma: genetics, chemistry, pharmacology and therapeutics. Proceedings of international symposium on Ganoderma research, Shanghai, 21–23 Oct, Beijing, Medical University Press, pp 109–114
79. Zhang QH, Lin ZB (1999) Effect of G. lucidum polysaccharides B on TNF-a and INF-g production and their mRNA expression. Beijing Yi Ke Da Xue Xue Bao 31:179–183
80. Ooi LSM, Ooi VEC, Fung MC (2002) Induction of gene expression of immunomodulatory cytokines in the mouse by a polysaccharide from *G. lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae). Int J Med Mush 4:27–35
81. Kidd PH (2000) The use of mushroom glucans and proteoglycans in cancer treatment. Altern Med Rev 5:4–27
82. Kang YK, Yoon SI, Lee SY, Kim DD, Lee SJ, Park WH, Hudson SM (2010) Chitosan-coated poly (vinyl alcohol) nanofibers for wound dressings. J Biomed Mater Res Part B Appl Biomater 92(2):568–576
83. Wasser SP, Weis AL (1997) Reishi mushroom [*G. lucidum* (Curt.: Fr.) P. Karst.] In: Nevo E (ed) Medicinal mushrooms. Peledfus, Haifa, p 39
84. Hobbs CH (1995) Medicinal mushrooms: an exploration of tradition, healing and culture, 2nd edn. Botanica Press, Santa Cruz, p 252
85. Min BS, Gao JJ, Hattori M, Lee HK, Kim YH (2001) Anticomplement activity of terpenoids from the spores of *G. lucidum*. Planta Med 67(9):811–814
86. Luo J, Lin ZB (2002) Advances of pharmacological effects of triterpenes from *G. lucidum*. Acta Pharm Sin B 37:574–578
87. Frankel EN (1997) Nutritional benefits of flavonoids. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T (eds) Food factors for cancer prevention. Springer, Tokyo, pp 6l3–616
88. Decker EA (1997) Phenolics: prooxidants or antioxidants? Nutr Rev 55:396–407
89. Mau JL, Lin HC, Song SF (2002) Antioxidant properties of several specialty mushrooms. Food Res Int 35(6):519–526
90. Kumari K, Prakash V, Rana S, Sagar A (2016) In vitro antioxidant activity of methanolic extract of *G. lucidum* (Curt.) P. Karst. IJASR 1(5):51–54
91. Heleno SA, Barros L, Martins A, Ferreira ICFR (2012) Fruiting body spores and in vitro produced mycelium of *G. lucidum* from Northeast Portugal: a comparative study of the antioxidant potential of phenolic and polysaccharidic extracts. Food Res Int 46:135–140

92. Hajjaj H, Mace M, Roberts M, Niederberger P, Fay LB (2005) Effect of 26-oxygenosterols from *G. lucidum* and their activity as cholesterol synthesis inhibitors. Appl Environ Microbiol 71:3653–3658
93. Zhang CR, Yang SP, Yue JM (2008) Sterols and triterpenoids from the spores of *G. lucidum*. Nat Prod Res 22(13):1137–1142
94. Lv GP, Zhao J, Duan JA, Tang YP, Li SP (2012) Comparison of sterols and fatty acids in two species of Ganoderma. Chem Cent J 6(1):10
95. Xu XF, Yan HD, Chen J, Zhang XW (2011) Bioactive proteins from mushrooms. Biotechnol Adv 29:667–674
96. Kawagishi H, Yamawaki M, Ido M, Shimada A, Kinoshita T, Murata T, Usui T, Kimura A, Chiba S (1997) A lectin from mycelia of the fungus *G. lucidum*. J Phytochem 44:7–10
97. Li YG, Ji DF, Zhong S, Zhu JX, Chen S, Hu GY (2011a) Anti-tumor effects of proteoglycan from *Phellinus linteus* by immunomodulating and inhibiting Reg IV/EGFR/Akt signalling pathway in colorectal carcinoma. Int J Biol Macromol 48:511–517
98. Kino K, Yamashita A, Yamaoka K, Watanabe J, Tanaka S, Ko K, Shimizu K, Tsunoo H (1989) Isolated and characterization of a new immunomodulatory protein Lingzhi-8 (LZ-8), from *G. lucidum*. J Biol Chem 264:472–478
99. Li QZ, Wang XF, Zhou XW (2011b) Recent status and prospects of the fungal immunomodulatory protein family. Crit Rev Biotechnol 31:365–375
100. Tsai LL (2007) Immunomodulatory protein cloned from *G. microsporum*. US Patent US 7601808 B2
101. Huang J, Zhang LX, Hu SN, Yu LJ (2008) Optimizing expression of LZ-8 protein and primary assaying of its immunomodulatory activity. Zhejiang Univ (Agric & Life Sci) 34:7–12
102. Shimizu A, Yano T, Saito Y, Inada Y (1985) Isolation of an inhibitor of platelet aggregation from a fungus, *G. lucidum*. Biol Pharm Bull 33(7):3012–3015
103. Gao Y, Zhou SH, Huang M, Xu A (2003) Antibacterial and antiviral value of the genus Ganoderma P. Karst. species (Aphyllophoromycetideae): a review. Int J Med Mush 5(3):235–246
104. Hardy S, Przybytkowski E, Joly E, Prentki M, Langelier Y (2003) Saturated fatty acid-induced apoptosis in MDA-MB-231 breast cancer cells. A role for cardiolipin. J Biol Chem 278:31861–31870
105. Ihayere CA, Oghenekaro AO, Osemwegie OO, Okhuoya JA (2010) Chemical nature of *G. lucidum* (Curtis) Karsten from woodlands of Edo state, Nigeria. C J Biol Sci 3:8–15
106. Mizuno T (1995) *G. lucidum* and *G. tsugae*: bioactive substances and medicinal effects. Food Rev Int 11(1):151–166
107. Stamets P (2000) Growing gourmet and medicinal mushrooms, 3rd edn. Ten Speed Press, Berkeley, p 339
108. Chen DH, Shiou WY, Wang KC, Hwang SY, Shie YT, Tsai CM, Shie JF, Chen KD (1999) Chemotaxonomy of triterpenoid pattern of HPLC of *G. lucidum* and *G. tsugae*. J Chin Inst Chem 46:47–51
109. Gao JJ, Nakamura N, Min BS, Hirakawa A, Zuo F, Hattori M (2004) Quantitative determination of bitter principles in specimens of *G. lucidum* using high-performance liquid chromatography and its application to the evaluation of Ganoderma products. Chem Pharm Bull 52(6):688–695
110. Liu GT (1999) Recent advances in research of pharmacology and clinical applications of Ganoderma P. Karst. species (Aphyllophoromycetideae) in China. Int J Med Mush 1(1):63–68
111. Chiu SW, Wang ZM, Leung TM, Moore D (2000) Nutritional value of Ganoderma extract and assessment of its genotoxicity and antigenotoxicity using comet assays of mouse lymphocytes. Food Chem Toxicol 38:173–178
112. Kolesnikova OP, Tuzova MN, Kozlov VA (1997) Screening of immunoactive properties of alkanecarbonic acid derivatives and germanium-organic compounds in vivo. Immunologiya 10:36–38
113. Li P, Yi L, Liu HJ (2011) Chapter 2: Collection and identification of raw herbal materials. In: Liu JH (ed) Traditional herbal medicine research methods: identification, analysis,

bioassay, and pharmaceutical and clinical studies. John Wiley & Sons, Inc., Hoboken, New Jersey, pp 27–79
114. Krishnaswamy N (2010) Chemistry of natural products: the terpenes. Taylor & Francis Group, New York, pp 1–10
115. Kim DO, Lee CY (2003). Extraction and isolation of polyphenolics. In: Current protocols in food analytical chemistry, I:I1:I1.2 (Online)
116. Fan L, Li J, Deng K, Ai L (2012) Effects of drying methods on the antioxidant activities of polysaccharides extracted from *G. lucidum*. Carbohydr Polym 87(2):1849–1854
117. Liu GQ, Zhao Y, Wang XL, Zhu CY (2011) Response surface methodology for optimization of polysaccharides extraction by mild alkaline hydrolysis from fruiting body of medicinal mushroom, *G. lucidum*. J Med Plant Res 5(10):2064–2070
118. Chin SK, Law C, Cheng P (2011) Effect of drying on crude ganoderic acids and water soluble polysaccharides content in *G. lucidum*. Int J Pharm Pharm Sci 3:38–43
119. Chin SK, Law CL, Supramaniam CV, Cheng PG (2009) Thin-layer drying characteristics and quality evaluation of air-dried *Ganoderma tsugae* Murrill. Dry Technol 27(9):975–984
120. Huang SQ, Ning ZX (2010) Extraction of polysaccharide from *Ganoderma lucidum* and its immune enhancement activity. Int J Biol Macromol 47(3):336–341
121. Liu W, Xu J, Jing P, Yao W, Gao X, Yu L (2010) Preparation of a hydroxypropyl *G. lucidum* polysaccharide and its physicochemical properties. Food Chem 122(4):965–971
122. Chen W, Wang WP, Zhang HS, Huang Q (2012) Optimization of ultrasonic-assisted extraction of water-soluble polysaccharides from *Boletus edulis* mycelia using response surface methodology. Carbohydr Polym 87(1):614–619
123. Wen L, Lin L, You L, Yang B, Jiang G, Zhao M (2011) Ultrasound-assisted extraction and structural identification of polysaccharides from *Isodon lophanthoides* var. gerardianus (Bentham) H. Hara. Carbohydr Polym 85(3):541–547
124. Zhao Q, Kennedy JF, Wang X, Yuan X, Zhao B, Peng Y et al (2011) Optimization of ultrasonic circulating extraction of polysaccharides from *Asparagus officinalis* using response surface methodology. Int J Biol Macromol 49(2):181–187
125. Chen X, Wang W, Li S, Xue J, Fan L, Sheng Z et al (2010) Optimization of ultrasound-assisted extraction of Lingzhi polysaccharides using response surface methodology and its inhibitory effect on cervical cancer cells. Carbohydr Polym 80(3):944–948
126. Hromadkova Z, Ebringerova A, Valachovič P (1999) Comparison of classical and ultrasound-assisted extraction of polysaccharides from *Salvia officinalis L*. Ultrason Sonochem 5(4):163–168
127. Huang SQ, Li JW, Wang Z, Pan HX, Chen JX, Ning ZX (2010) Optimization of alkaline extraction of polysaccharides from *G. lucidum* and their effect on immune function in mice. Molecules 15(5):3694–3708
128. Xu DJ, Xia Q, Wang JJ, Wang PP (2008) Molecular weight and monosaccharide composition of *Astragalus polysaccharides*. Molecules 13(10):2408–2415
129. Zhao L, Dong Y, Chen G, Hu Q (2010) Extraction, purification characterization and antitumor activity of polysaccharides from *G. lucidum*. Carbohydr Polym 80:783–789
130. Wakte P, Sachin B, Patil A, Mohato D, Band T, Shinde D (2011) Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from *Curcuma longa*. Sep Purif Technol 79(1):50–55
131. Claver IP, Zhang H, Li Qin Z, Huiming Z (2010) Optimization of ultrasonic extraction of polysaccharides from Chinese malted sorghum using response surface methodology. Pak J Nutr 9(4):336–342
132. Kozarski M, Klaus A, Nikšić M, Vrvić MM, Todorović N, Jakovljević D, Van Griensven LJ (2012) Antioxidative activities and chemical characterization of polysaccharide extracts from the widely used mushrooms *G. applanatum*, *G. lucidum*, *Lentinus edodes* and *Trametes versicolor*. J Food Compos Anal 26(1):144–153
133. Cheung YC, Siu KC, Liu YS, Wu JY (2012) Molecular properties and antioxidant activities of polysaccharide–protein complexes from selected mushrooms by ultrasound-assisted extraction. Process Biochem 47(5):892–895

134. Azmi AF, Mustafa S, Hashim DM, Manap YA (2012) Prebiotic activity of polysaccharides extracted from *Gigantochloa levis* (Buluh beting) shoots. Molecules 17(2):1635–1651
135. Li YQ, Fang L, Zhang KC (2007) Structure and bioactivities of a galactose rich extracellular polysaccharide from submergedly cultured *G. lucidum*. Carbohydr Polym 68(2):323–328
136. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
137. Miyazaki T, Nishijima M (1982) Structural examination of an alkali-extracted, water-soluble hetero glycan of the fungus *G. lucidum*. Carbohydr Res 109(1):290–294
138. Sone Y, Okuda R, Wada N, Kishida E, Misaki A (1985) Structure and antitumor activities of the polysaccharide isolated from fruiting body and the growing culture of mycelium of *G. lucidum*. Agric Biol Chem 49:2641–2653
139. Kim HW, Kim BK (1999) Biomedical triterpenoids of *G. lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae). Int J Med Mush 1(2):121–138
140. Min BS, Nakamura N, Miyashiro H et al (1998) Triterpenes from the spores of G lucidum and their inhibitory activity against HIV-1 protease. Chem Pharm Bull 46(10):1607–1612
141. Fang QH, Zhong JJ (2002b) Submerged fermentation of higher fungus *G. lucidum* for production of valuable bioactive metabolites – ganoderic acid and polysaccharide. Biochem Eng J 10:61–65
142. Ma J, Ye Q, Hua Y, Zhang D, Cooper R, Chang MN, Chang JY, Sun HH (2002) New lanostanoids from the mushroom *G. lucidum*. J Nat Prod 65:72–75
143. Tang YJ, Zhong JJ (2002) Exopolysaccharide biosynthesis and related enzyme activities of the medicinal fungus, *G. lucidum*, grown on lactose in a bioreactor. Biotechnol Lett 24:1023–1026
144. Kikuchi T, Kanomi S, Kadota S, Murai Y, Tsubono K, Ogita Z (1986) Constituents of the fungus *G. lucidum* (Fr.) Karst. I. Structures of ganoderic acids C2, E, I, and K, lucidenic acid F and related compounds. Chem Pharm Bull 34:3695–3712
145. Hirotani MA, Furaya T, Shiro M (1985) A ganoderic acid derivative, a highly oxygenated lanostane-type triterpenoid from *G. lucidum*. Phytochemistry 24(9):2055–2061
146. Sliva D (2004) Cellular and physiological effects of *G. lucidum* (Reishi). Mini-Rev Med Chem 4:873–879
147. Yuen JW, Gohel MD (2005) Anticancer effects of *G. lucidum*: a review of scientific evidence. Nutr Cancer 53:11–17
148. Tomasi S, Lohezic-Le DF, Sauleau P, Bezivin C, Boustie J (2004) Cytotoxic activity of methanol extracts from Basidiomycete mushrooms on murine cancer cell lines. Pharmazie 59:290–293
149. Chen WC, Hau DM, Wang CC, Lin IH, Lee SS (1995) Effects of *G. lucidum* and krestin on subset T-cell in spleen of gamma-irradiated mice. Am J Chin Med 23:289–298
150. Lu ZW (1995) Psycho-neuroimmunological effects of morphine and the immunoprotection of Ganoderma polysaccharides peptide in morphine dependent mice. Chin J Phys 26:45–49
151. Benzie IFF, Wachtel-Galor S (2009) Biomarkers of long-term vegetarian diets. Adv Clin Chem 47:169–220
152. Yuen JW, Gohel MD (2008) The dual roles of Ganoderma antioxidants on urothelial cell DNA under carcinogenic attack. J Ethnopharmacol 118:324–330
153. Saltarelli R, Ceccaroli P, Lotti M, Zambonelli A, Buffalini M, Casadei L, Vallorani L, Stocchi V (2009) Biochemical characterisation and antioxidant activity of mycelium of *G. lucidum* from Central Italy. Food Chem 116:143–151
154. Wachtel-Galor S, Szeto YT, Tomlinson B, Benzie FIF (2004b) *G. lucidum* (Lingzhi): acute and short-term biomarker response to supplementation. Int J Food Sci Nutr 1:75–83
155. Ooi VEC, Liu F (2000) Immunomodulation and anti-cancer activity of polysaccharide–protein complexes. Curr Med Chem 7:715–729
156. Lee JM, Kwon H, Jeong H, Lee JW, Lee SY, Baek SJ, Surh YJ (2001) Inhibition of lipid peroxidation and oxidative DNA damage by *G. lucidum*. Phytother Res 15:245–249

157. Shi YL, James AE, Benzie IF, Buswell JA (2002) Mushroom-derived preparations in the prevention of H_2O_2-induced oxidative damage to cellular DNA. Teratog Carcinog Mutagen 22:103–111
158. Sheena M, Ajith A, Janardhanan K (2003) Prevention of nephrotoxicity induced by the anticancer drug cisplatin, using *G. lucidum*, a medicinal mushroom occurring in South India. Curr Sci 85:478–482
159. Yue QX, Xie FB, Guan SH, Ma C, Yang M, Jiang BH, Liu X, Guo DA (2008) Interaction of Ganoderma triterpenes with doxorubicin and proteomic characterization of the possible molecular targets of Ganoderma triterpenes. Cancer Sci 99:1461–1470
160. Jia J, Zhang XI, Hu YS, Wu Y, Wang QZ, Li NN, Guo QC, Dong XC (2009) Evaluation of in vivo antioxidant activities of *G. lucidum* polysaccharides in STZ-diabetic rats. Food Chem 115:32–36
161. Calder PC (2003) Immunonutrition: may have beneficial effects in surgical patients. BMJ 327(7407):117–118
162. Powell M (2006) *G. lucidum* (Reishi) in the management of histamine-mediated allergic responses. Townsend Letter pp 78–81
163. Yoon SY, Eo SK, Kim YS, Lee CK, Han SS (1994) Antimicrobial activity of *G. lucidum* extract alone and in combination with some antibiotics. Arch Pharm Res 17:438–442
164. Mohammed A, Adelaiye AB, Abubakar MS, Abdurahman EM (2007) Effects of aqueous extract of *G. lucidum* on blood glucose levels of normoglycemic and alloxan induced diabetic wistar rats. J Med Plant Res 1:34–37

Minas Frescal Cheese as a Probiotic Carrier 65

Tatiana Colombo Pimentel, Vanessa Aparecida Marcolino, Carlos Eduardo Barão, Suellen Jensen Klososki, and Michele Rosset

Contents

Abstract

Minas Frescal cheese is one of the most popular cheeses consumed in Brazil, has mild and pleasant flavor and aroma, is slightly acidic and whitish in color, and presents soft texture. It has characteristics that allow the incorporation of probiotic cultures and maintenance of the viability until the end of the shelf life.

T. C. Pimentel (✉) · V. Aparecida Marcolino · C. Eduardo Barão · S. Jensen Klososki
Federal Institute of Paraná (IFPR), Paranavaí, Parana, Brazil
e-mail: tatiana.pimentel@ifpr.edu.br; vanessa.marcolino@ifpr.edu.br; carlos.barao@ifpr.edu.br; suellen.jensen@ifpr.edu.br

M. Rosset
Federal Institute of Paraná (IFPR), Colombo, Parana, Brazil
e-mail: michele.rosset@ifpr.edu.br

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_66

However, the addition of probiotic cultures can have a negative impact on the physicochemical and sensory characteristics of the products. The purpose of this chapter is to present the technology involved in the development of probiotic Minas Frescal cheese. First, it presented the starter and probiotic cultures commonly used in Minas Frescal cheese, the criteria to classify a strain as probiotic, and the health-related benefits. Then, it discussed the technological process and the forms of addition of the probiotic cultures to the cheeses. Finally, it demonstrated the effect of probiotic addition on the physicochemical and sensory properties, the probiotic stability during storage, and the main challenges to maintain the viability.

Keywords
Fresh cheese · Probiotic culture · Sensory properties · Physicochemical · Viability

Abbreviations

Cfu	Colony-forming unit
HDL-C	High-density lipoprotein cholesterol
HTST	High-temperature short time
IgA	Immunoglobulin A
LDL-C	Low-density lipoprotein cholesterol
LTLT	Low-temperature long time
NK	Natural killer
SCFA	Short-chain fatty acids
VLDL	Very low-density lipoprotein

1 Introduction

Probiotics are living microorganisms, which, when administered in adequate amounts, confer benefits to the host health [1]. Probiotic cultures are usually added to dairy products, such as fermented milks, yogurts, and cheeses. This is because dairy products are regarded as healthy products by consumers, assist in the digestion process, are essential for bones, and help the immune system. Furthermore, the consumers are used to the application of microorganisms in the preparation of these types of products.

Minas Frescal cheese is one of the most popular cheeses consumed in Brazil, because of the high yield, relatively low production costs, easy manufacturing process, and acceptable sensory characteristics [2, 3]. It has mild and pleasant flavor and aroma, is slightly acidic and whitish in color, and presents soft texture. The presence of holes and a thin crust can be observed in some commercialized products. According to the Technical Identification and Quality Regulation [4], Minas Frescal cheese is a fresh cheese obtained by enzymatic coagulation with rennet and/or other appropriate coagulant enzymes, supplemented or not with the action of specific lactic acid bacteria. It is classified as a semi-fat cheese (25–44.9%

fat in the dry matter) and has very high moisture content (over 55%) [5]. Due to the characteristics of moisture content and pH, it has a short shelf life (20–30 days) and should be stored under refrigeration.

The production of Minas Frescal cheese requires the pre-pasteurization of the milk for the elimination of pathogens. However, some producers insist on the use of raw milk for the manufacture of this type of cheese, which can compromise the food safety. The pasteurization of the milk also promotes the reduction of the spoilage microbiota, reducing the competition between these cultures and the health cultures, improving the environment for the development and maintenance of the probiotic cultures. The Minas Frescal cheese has characteristics that allow the incorporation of probiotic cultures and maintenance of the viability until the end of the shelf life, such as the presence of nutrients that can be assimilated by the probiotic cultures, high pH (in the range of 5–6), high moisture content, low salt content (1.4–1.6%), medium fat content, capability of protecting the microorganisms from the adverse conditions during the digestion process, refrigerated storage, and short shelf life.

Considering the technology of manufacture of the Minas Frescal cheese, its chemical composition, the method of preservation, and the interest in functional foods, this product has the potential to be added with probiotic cultures. The purpose of this chapter is to present the technology involved in the development of probiotic Minas Frescal cheese. First, it presented the starter and probiotic cultures commonly used in Minas Frescal cheese, the criteria to classify a strain as probiotic, and the health-related benefits. Then, it discussed the technological process and the forms of addition of the probiotic cultures to the cheeses. Finally, it demonstrated the effect of probiotic addition on the physicochemical and sensory properties, the probiotic stability during storage, and the main challenges to maintain the viability.

2 Probiotic Cultures

Probiotics are living microorganisms, which, when administered in adequate amounts, confer benefits to the health of the host [1]. A possible probiotic culture should be identified and characterized, and its infective potential and related interference with the host metabolism evaluated. Up to now, infective capacity and resistance to antibiotics have comprised the main focus in traditional safety criteria [6]. Furthermore, the microorganism should be able to remain viable during technological processing and storage and throughout the shelf life of the product. In addition, when the food is consumed, this microorganism must be able to survive during the passage through the gastrointestinal tract, supporting the pH of the stomach and the bile salts in the small intestine, and, finally, it needs to be able to populate the large intestine of the host and confer the expected health benefits.

In the preparation of Minas Frescal cheeses, some microorganisms can be used, which could be single or mixed strains, such as *Lactococcus lactis* spp. *lactis* [7, 8]; *Lactococcus lactis* spp. *lactis* and *Lactococcus lactis* spp. *cremoris* [7–10];

Lactobacillus acidophilus [11, 12]; *Bifidobacterium lactis* [11]; *Bifidobacterium lactis* and *Lactobacillus acidophilus* [11]; *Lactobacillus plantarum* [8]; *Enterococcus faecium* [8]; *Lactobacillus acidophilus*, *Bifidobacterium animalis*, and *Streptococcus thermophilus* [9]; and *Lactobacillus casei*, *Lactococcus lactis* spp. *lactis*, and *Lactococcus lactis* spp. *cremoris* [9]. Some of the cultures mentioned above present probiotic potential, which can impart functional properties to the product. The species used as starter cultures in the manufacture of Minas Frescal cheese (*Lactococcus lactis*, *Streptococcus thermophilus*, and others) do not generally grow in the gastrointestinal tract and, therefore, would have low probiotic potential.

The insertion of probiotic strains in Minas Frescal cheese can transform it into a functional food, conferring health benefits to individuals with a habit of consuming it. Table 1 shows the main potential probiotic strains used in previous studies with Minas Frescal cheese. The combination of some strains showed synergism and improved probiotic survival throughout the product's shelf life. However, the use of probiotic cultures can lead to changes in the characteristics of the products, reducing their acceptance by consumers by causing changes in the quality parameters of the product. These interferences will be discussed in this chapter.

The addition of probiotic cultures does not ensure the transformation of the Minas Frescal cheese into a functional food. The product, at the time of consumption, must present viable microorganisms in suitable amounts, and, preferably, these microorganisms should resist along the gastrointestinal tract during the digestion process, so that they can exert the probiotic effect on the individual's intestine. This latter characteristic is advised, but there are probiotic cultures with health benefits not related to the colonization of the intestine.

Table 1 Potential probiotic cultures used in Minas Frescal cheese

Probiotic culture	References
Bifidobacterium animalis ssp. *lactis*	[13–15]
Bifidobacterium lactis	[11]
Bifidobacterium lactis and *Lactobacillus acidophilus*	[11]
Lactobacillus acidophilus, *Bifidobacterium animalis*, and *Streptococcus thermophilus*	[9]
Enterococcus faecium	[8]
Lactococcus lactis ssp. *lactis*[a]	[7, 8]
Lactococcus lactis ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*	[7–9]
Lactobacillus acidophilus	[11, 12, 16, 17]
Lactobacillus acidophilus and *Streptococcus thermophilus*	[16]
Lactobacillus acidophilus and *Lactococcus lactis*	[18]
Lactobacillus casei	[2]
Lactobacillus casei and *Lactococcus R704*	[2]
Lactobacillus casei, *Lactococcus lactis* ssp. *lactis*, and *Lactococcus lactis* ssp. *cremoris*[a]	[9]
Lactobacillus plantarum	[8]

[a]Starter cultures. Low probiotic potential

A probiotic food should have counts of 10^6–10^7 colony-forming unit (cfu) g^{-1} or mL^{-1} in the final product or 10^8–10^9 cfu in a portion of 100 g or mL [19]. The Brazilian legislation does not establish the number of microorganisms that must be present in the product. It requires an analysis report to establish the minimum viable quantity of the microorganism to exert functional property at the end of the shelf life of the product and under the conditions of use, storage, and distribution. Therefore, the studies of functionality should be based on robust scientific evidence, constructed through randomized, double-blind, and placebo-controlled clinical studies whose outcomes demonstrate the proposed relationship between the product consumption and the functional effect [20].

3 Health Benefits of Probiotic Cultures

Probiotic culture ingestion is associated with many health benefits, such as reduction in gastrointestinal infections; antimicrobial activity; improvement in lactose metabolism; reduction in serum cholesterol; stimulation of the immune system; antimutagenic, anticarcinogenic, and antidiarrheal properties; improvement in the symptoms of irritable bowel syndrome; suppression of *Helicobacter pylori* infections; and reduction of obesity and atopic dermatitis, among others. The type of health benefit is associated with the strain; therefore, there is no strain capable of providing all the benefits suggested for probiotics. Figure 1 presents the target organs of probiotic action and the health-related benefit.

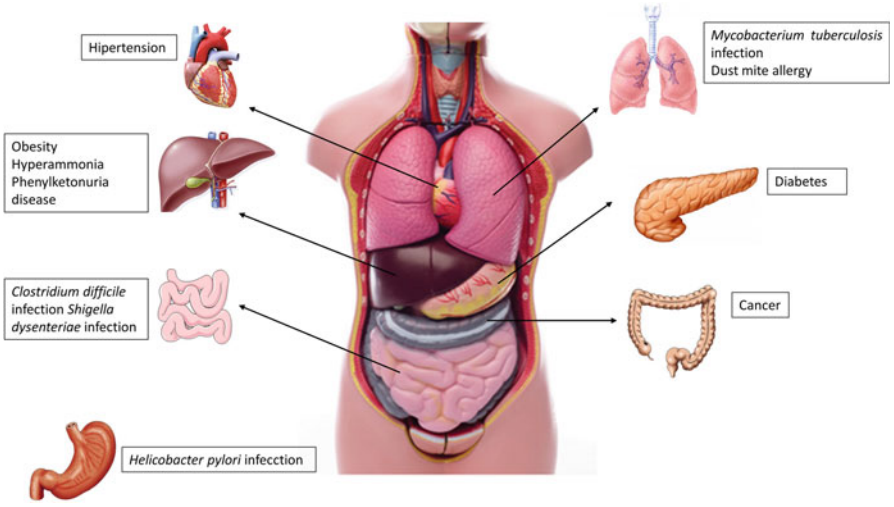


Fig. 1 Target organs of probiotic action and the health-related benefit (Adapted from Chua et al. [21])

3.1 Modulation of the Intestinal Microbiota

The human gastrointestinal tract is a kinetic micro-system that enables the normal performance of the physiological functions of the host, unless harmful and potentially pathogenic microorganisms dominate the microbiota [22].

The human gastrointestinal microbiota plays a key role in nutrition and health. By the fermentation process, the intestinal bacteria metabolize various substrates (mainly dietary components) forming end products, such as short-chain fatty acids (SCFA) and gases. This metabolism contributes positively to the daily energy needs of the host. The human large intestine is a metabolically very active organ, a fact attributed to the resident microbiota and its activities. Normally, the host organism lives in harmony with the complex microbiota of the intestine [23].

When in equilibrium, the intestinal microbiota prevents potentially pathogenic microorganisms from exerting their effects. On the other hand, the imbalance of this microbiota can result in the proliferation of pathogens, with consequent bacterial infection [23, 24]. The beneficial influence of probiotics on human intestinal microbiota includes factors such as antagonistic effects, competition against undesirable microorganisms, and immunological effects [25, 26].

The intestinal microbiota is also susceptible to contamination by passive pathogens, which alters the structure of normal colonization and can lead to acute and/or chronic intestinal disorders. Thus, the large intestine is the focus for functional foods, allowing the strengthening of the normal bowel functions and helping to prevent organic deficiencies. The colonization of the microbiota is determined by the susceptible action of certain dietary groups and mechanisms [27].

Hydrolysis and metabolism of carbohydrate in the large intestine are influenced by a variety of physical, chemical, biological, and environmental factors. The nature and quantity of available substrate have a greater influence, making diet the main and easiest mechanism by which the fermentation profile can be influenced. Other factors affecting the colonization and growth of bacteria in the gut are intestinal pH, production of inhibitory metabolites (acids and peroxides) and specific inhibitory substances (bacteriocins), bile salts, and immunological events [23].

The knowledge of the intestinal microbiota and its interactions led to the development of strategies aiming the maintenance and the stimulation of the resident bacteria. It is possible to increase the number of health-promoting microorganisms in the gastrointestinal tract by introducing probiotics included in food products or by consuming prebiotic food components, which will selectively modify the composition of the microbiota, providing the probiotic competitive advantage over other bacteria in the ecosystem [28].

The binding of probiotic bacteria to enterocyte cell surface receptors also initiates cascade reactions that result in the synthesis of cytokines. Experimental data indicate that several probiotics can modulate some characteristics of digestive physiology, such as mucosal immunity and intestinal permeability. One of the actions attributed to probiotics is the ability to adhere to certain types of receptors present in the intestinal mucosa, precluding their elimination by peristaltic movements and preventing pathogenic bacteria, such as *Salmonella typhimurium* and *Yersinia*

enterocolitica and pathogenic strains of *Escherichia coli* from exerting their enteropathogenic effects [29].

In the case of probiotics, consumption of adequate doses of *Lactobacillus* strains often results in a measurable increase in lactobacilli in feces, and in some cases, even undesirable organisms, such as *Staphylococci*, may be reduced. Bifidobacteria and lactobacilli in the colon ferment mainly the carbohydrates that escape the digestion in the upper gastrointestinal tract, resulting in a reduced pH in the colon. Bifidobacteria can ferment fructans because they have β-fructofuranosidase enzyme, which, in other bacteria, does not exist or has low activity. This confers competitive advantages to bifidobacteria when exposed to fructans in the human intestine.

Not all studies are conclusive regarding the administration of probiotics and alteration of the intestinal microbiota. Bartran et al. [30] argued that the fecal microbiota is relatively stable and generally unaffected by the use of probiotics. In an intervention study, 12 subjects consumed yogurt (500 mL) enriched with *Bifidobacterium longum*, and no significant difference was found for fecal weight, pH, fecal concentration of SCFA, bile acids, and neutral sterols after 3 weeks of intervention. Although there was an increase in fecal concentration of *B. longum*, the results suggest little or no modulation of the resident microbiota.

3.2 Improvement in Lactose Intolerance

Lactose intolerance is the condition in which there is partial or complete inability to digest the main milk sugar (lactose), because the body does not or insufficiently produce the digestive enzyme lactase, responsible for breaking down the lactose. Consequently, this substance reaches the large intestine unchanged and is accumulated and fermented by the resident bacteria, originating lactic acid and gases. These metabolites promote greater water retention and the appearance of diarrhea and abdominal discomfort. In addition to the clinical evaluation, the diagnosis of lactose intolerance can count on three specific tests: lactose intolerance test, hydrogen breath test, and fecal acid test.

There is evidence that probiotics may alleviate symptoms of lactose intolerance due to increased lactose hydrolysis in the dairy products and small intestine or through manipulation of colonic metabolism. Mechanisms of action may be related to increased activity of the enzyme lactase in bacterial preparations used in the manufacture of the products, by the decrease in lactose concentration in fermented products, and to the increased activity of the lactase enzyme that reaches the small intestine together with the fermented product or among the viable bacteria capable of surviving the acidity and the bile salts [31].

The alteration of the microbial metabolism by probiotics can occur through the increase or decrease of the enzymatic activity. A vital function of lactic bacteria in the intestinal microbiota is to produce the enzyme β-D-galactosidase (lactase), aiding the breakdown of lactose in the intestine. Several evidences have shown that the consumption of adequate amounts of appropriate strains of lactic acid bacteria

(including non-probiotic strains such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) can alleviate symptoms of lactose intolerance [32].

Studies have shown that people with lactose intolerance have higher tolerance to probiotic yogurt than to milk. It was assumed that the presence of lactase-producing bacteria in probiotic yogurt, especially *Lactobacillus acidophilus*, contributed to the digestion and absorption of lactose. In a study conducted with adolescents aged 12–14 years, a 200 mL of whole milk (Dancow®) was served twice a day for 7 days, and, in the sequence, 200 mL of whole milk (Dancow®) + two sachets of Lacto-B® probiotic (*Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Streptococcus faecium*) was served for 14 days twice a day. The results demonstrated that in the 2 weeks of probiotic therapy, there was an improvement in the symptoms of lactose intolerance, verified by breath hydrogen tests [33].

One specific strain that has been shown to be effective in lactose intolerance is *Lactobacillus rhamnosus*, a gram-positive anaerobic probiotic bacterium. When administered to lactose-intolerant infants, this bacterium could colonize the intestinal tract and produced positive changes within the intestinal microbiota [34].

3.3 Relief of Constipation

Constipation is one of the most common digestive complaints in the general population. It is not considered a disease but a symptom present in many diseases and can be defined as the condition of the digestive system where an individual has hard feces and difficulty to evacuate [35]. Symptoms of constipation are irregular bowel movements, hard stools, difficulty to evacuate, cramps, bloating, flatulence, and abdominal pain, which can affect patients' quality of life. In most cases, constipation is related to (1) inadequate eating habits, (2) sedentary lifestyle, (3) socioeconomic status, (4) psychological parameters, (5) medications, (6) age, and (7) gender.

One of the causes of constipation is intestinal dysbiosis, which is defined as an imbalance in the intestinal microbiota, with prevalence of pathogenic bacteria. Picard et al. [36] and Szajewska et al. [37] suggested that dysbiosis can be improved by the consumption of probiotics. Nutritional improvement with inclusion of probiotics in the diet represents an interesting non-pharmacological alternative to reduce the symptoms of constipation. In addition, bifidobacteria can lower the pH of the colon, because they produce lactic acid, acetic acid, and others. A lower pH tends to increase colonic peristalsis and, consequently, decreases the transit time of the colon, with a beneficial effect in the treatment of constipation symptoms. Since dietary modification is the main approach to improve constipation, probiotic or prebiotic therapy, in principle, fits well into the regimen.

The efficiency of probiotic cultures in the reduction of symptoms of constipation is associated with the strain used. Lactobacilli, bifidobacteria, and a combination of lactobacilli and propionibacteria have been clinically investigated in the treatment of patients. There is evidence that ingestion of probiotics may increase defecation frequency; however, these findings are not universal, with varying efficacy among strains of probiotic bacteria and among different studies. Two different strains *of*

Lactobacillus rhamnosus and *Propionibacterium freudenreichii* were used with constipated elderly, and a significant increase in defecation frequency was observed, whereas the use of a single strain did not affect the frequency of defecation [38]. Studies with constipated children showed that there were an increase in the frequency of defecation and a decrease of abdominal pain using *L. rhamnosus* as probiotic culture [39]. In contrast, another study conducted with *Lactobacillus rhamnosus* GG strain showed no significant effect on the symptoms of constipation when used as an adjunct in lactulose therapy [40].

A reduction in the symptoms and a better frequency of bowel movement and consistency of feces in constipated healthy individuals after consumption of a fermented milk containing *Lactobacillus casei* Shirota were observed. More recent studies have shown that the consumption of a fermented milk containing multiple probiotic strains such as *Streptococcus salivarius* subsp. *thermophilus*, *Enterococcus faecium*, *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Bifidobacterium* (*breve* and *animalis* subsp. *lactis*) and prebiotic fiber (fructooligosaccharides) was superior to placebo in improving constipation in patients with Parkinson disease [41].

Modifying the intestinal lumen with certain probiotic species and strains can affect motility and secretion in the intestine and thus provide benefits for patients with constipation. However, most of the current evidence is derived from animal studies, as its effect on humans is unclear because of lack of human studies. Additional high-quality methodological research is needed to fully establish the complex interactions of the luminal environment, immune system, and nervous system on intestinal motility and constipation and how different species and probiotic strains affect them. Further studies are needed to determine which species and strains of probiotics, dose, and duration of treatment are particularly effective in constipation, as well as to examine potential probiotic interactions and interindividual variability that may lead to differential probiotic responses [42].

3.4 Stimulation of the Immune System

The effect of probiotics on the immune response has been well studied. Much of the evidence from in vitro systems and animal and human models suggests that probiotics may stimulate both nonspecific and specific immune responses. Three possible mechanisms of action are attributed to probiotics: (1) the suppression of harmful microorganisms through the production of compounds with antimicrobial activity, competition for nutrients, and competition for adhesion sites; (2) the alteration of the microbial metabolism, through the increase or the decrease of the enzymatic activity; and (3) the stimulation of the host immunity by increasing antibody levels and macrophage activity [22].

Many researches have pointed out that certain strains of probiotic cultures are able to stimulate and regulate various aspects of natural and acquired immune responses. These include, for example, increased leukocyte phagocytic activity,

stimulation of nonspecific immunoglobulin A (IgA) and specific (antibody) response, and increased cytokine production in vivo. It is believed that these effects are mediated by macrophage activation, increases in cytokine levels, and increases in the activity of natural killer (NK) cells and/or immunoglobulin levels.

Olivares et al. [43] evaluated the effect of a fermented milk with two probiotic strains (*Lactobacillus gasseri* (CECT 5715) and *Lactobacillus coryniformis* (CECT 5711)) on the immune system of adult subjects for 4 weeks. It was used in a randomized, double-blind, placebo-controlled clinical trial. The probiotic fermented milk was compared to a standard fermented milk. Thirty healthy volunteers aged 23–43 years were recruited. Blood samples were collected before and after the experimental period, and stool samples were collected weekly. Consumption of the probiotic or the conventional products increased the proportion of phagocytic cells, including monocytes and neutrophils, as well as their phagocytic activity. However, the probiotic product containing the strains *L. gasseri* CECT 5714 and *L. coryniformis* CECT 5711 also induced an increase in the proportion of NK cells and in IgA concentrations. The effects were higher after 2 weeks of treatment than after 4 weeks, which suggests regulation of the immune system. In addition, the new product enhanced immunity in the participants to a greater extent than did the control standard fermented milk. The study, however, does not present the amount of fermented milk administered.

Strauss and Caly [44] point out that it is possible to optionally prevent some recurrent infectious processes, such as spontaneous bacterial peritonitis caused by cirrhosis or hepatic insufficiency, with probiotic cultures. These cultures can promote a decrease in the number of pathogenic bacteria and an increase in the intestinal immunity, when used with continuously used antibiotics, such as norfloxacin, which is already used in classical treatment. Therefore, probiotic cultures would be one more option to minimize recurrent infection. Surawicz et al. [45] treated adults with recurrent diarrhea due to *C. difficile* with high vancomycin dose and subsequently with *S. boulardii* (1 g/day, $n = 18$) or placebo ($n = 14$) for 28 days. They observed that the risk of recurrence was 16.7% for the group receiving the probiotic and 50% for the placebo group.

Considering the intestinal infections, a therapeutic value is attributed to probiotic bacteria, because in this specific area, the studies are already based on mechanisms of action well established and recognized by the scientific community. The intestinal epithelium plays a role as an immunological barrier, establishing the interface between the luminal content and the subepithelial immune cells. Any disturbance to this barrier, triggered by dietary antigens, pathogenic microorganisms, chemical agents, or radiation, leads to increased intestinal permeability and to structural changes in the epithelium, which can cause an increase in the flow of antigens and several types of inflammation.

Arvola et al. [46] performed a double-blind controlled trial with 119 children (mean age 4.5 years) who received antibiotics for respiratory infections along with *L. rhamnosus* GG or placebo capsules. Diarrhea was observed in 5% of the children who received the probiotic and in 16% of those who received the placebo ($p = 0.05$). However, in another study, the reduction of the risk of diarrhea with probiotic ingestion could not be observed ($n = 267$ hospitalized adults, 29.3% with

probiotic vs. 29.9% in placebo). In this way, probiotic effect may vary depending on the region or age [47].

Rembacken et al. [48] conducted a controlled study with 116 patients who had ulcerative colitis in order to evaluate the efficacy of a nonpathogenic *E. coli* strain Nissle 1917 versus Mesalazine® (considered an effective anti-inflammatory) to prevent such disease. The authors observed that the nonpathogenic probiotic *E. coli* obtained the same efficiency as the pharmacological drug in reducing ulcerative colitis.

In relation to children assisted by specialized treatment centers, Weizman et al. [49] investigated the effect of two different probiotic species on the prevention of gastrointestinal and respiratory infections. In a double-blind, placebo-controlled, randomized study, 201 full-term infants between 4 and 10 months of age were randomized into three groups receiving different treatments for 12 weeks: (1) fed with formulas containing *Bifidobacterium lactis* ($n = 73$, test 1) or *Lactobacillus reuteri* ($n = 68$, test2) and placebo ($n = 60$). Children who received supplementation (test 1 or 2) compared to placebo showed fewer episodes of fever and diarrhea, and these episodes were shorter. These effects were more prominent for test 2. Regarding the respiratory infection, no modification was observed between the treatments.

3.5 Anticarcinogenic Effect

According to the National Cancer Institute, the number of new cases of colon cancer will be higher than 108 thousand per year, thus making this type of cancer the second most diagnosed type in both men and women. Risk factors for colon and rectal cancer include both genetic and environmental factors, and many studies have suggested that interactions among dietary factors, colonic epithelium, and intestinal microbiota are central to the development of this type of cancer [50].

In 1962, a study reported that *L. bulgaricus* would be able to produce bioactive substances with antagonist effect on tumor development. Since then, a series of researches in the same area were developed, aiming to find explanations on the mechanisms involved. Today, the anticarcinogenic properties of probiotic bacteria are already known and divided into three categories: (1) inhibition of tumor cells; (2) suppression of enzyme-producing bacteria (β-glucosidases, β-glucuronidases, and azoreductases), responsible for the release of carcinogenic substances; and (3) destruction of carcinogenic substances, such as nitrosamines, or suppression of nitroreductase (enzyme involved in the synthesis of nitrosamines).

Several mechanisms of action are suggested involving the anticarcinogenic potential of probiotic bacteria [51, 52]:

- Stimulation of host immune response, by increasing phagocytic activity, IgA synthesis, and activation of T and B lymphocytes
- Binding and degradation of compounds with carcinogenic potential
- Qualitative and/or quantitative changes in the intestinal microbiota involved in the production of carcinogens and promoters of carcinogen (e.g., degradation of bile acids)

- Production of antitumorigenic or antimutagenic compounds in the colon (such as butyrate)
- Alteration of the metabolic activity of the intestinal microbiota
- Alteration of the physicochemical conditions of the colon, with decrease in the pH and effects on the physiology of the host
- Reduction of the inflammatory response (with decreased cytokines and hypersensitivity and increased phagocytic activity)

Bacterial β-glucuronidase appears to play an important role in the initiation of colon cancer due to its ability to hydrolyze several components and release carcinogenic aglycones into the intestinal lumen. The nitroreductases and azoreductases are related to the formation of aromatic amines that are harmful to the organism. Marteau et al. [53] verified the ability of fermented milk containing *L. acidophilus* A1, *B. bifidum* B1, *Streptococcus lactis*, and *Streptococcus cremoris* to modify the metabolic activity of the human colonic microbiota. Nine individuals who consumed 100 g of the product three times a day for 3 weeks showed reductions in fecal concentrations of nitroreductase, azoreductase, and β-glucuronidase compared to the control group. Haberer et al. [54] found that animals fed a diet based on meat and fat showed a reduction in the activity of β-glucuronidase and azoreductase after consumption of *Lactobacillus* probiotic strains (*L. johnsonii* and *L. reuteri*) for a period of 5 weeks. Rowland et al. [55] concluded that *B. longum*-treated rats reduced β-glucuronidase enzyme activity by 30%, while a greater reduction of about 55% was observed in those treated with probiotic *B. longum* combined with prebiotic inulin. In agreement, human studies showed that the consumption of *Lactobacillus casei* Shirota and *Lactobacillus acidophilus* also significantly reduced the activity of these enzymes [56]. Similar results were obtained by Lidbeck et al. [57], who supplemented the diet of 14 patients with colon cancer with *L. acidophilus* (approximately 3×10^{11} lactobacilli per day) for a period of 6 weeks and obtained a 14% reduction in β-glucuronidase enzyme.

Guérin-Danan et al. [58] evaluated the influence of supplementation with fermented milk containing *Lactobacillus casei* for a period of 1 month (125 g/day) on the metabolic indexes and the intestinal microbiota of healthy children aged 10–18 months. There was a significant increase in the percentage of children who had populations of lactobacilli above 6 log cfu g^{-1} in feces among those who received milk fermented with *Lactobacillus casei*. In parallel, the potentially harmful enzymatic activity of β-glucuronidase and β-glucosidase decreased significantly in these children. The authors concluded that such effects represent a healthy influence of *Lactobacillus casei* on the host.

Marinelli et al. [59] published a review paper about probiotics and their anticancer activity. The authors reported that the major studies used mice models, and, therefore, the results must be interpreted with caution. Furthermore, they stated that the health effect is restricted to each strain and the data obtained with different bacteria strains need to be integrated into a multidimensional view of tumor, considering tumor microenvironment and the host factors involved in therapeutic efficacy of drugs. Moreover, the dose, the frequency, and the timing of assumption

need to be assessed. Finally, the precise molecular mechanisms behind the probiotic action and potential side effects are required to delineate a safe and optimal use in humans. Summing up, soon, probiotic will be a new powerful ally in the demanding fight against cancer.

3.6 Cholesterol Metabolism

Cardiovascular diseases represent a major problem for public health, leading to mortality in Brazil and in the world. The increase in the life expectancy of a population is directly related to the prevention of cardiovascular diseases. Furthermore, hypercholesterolemia is closely linked with the complications of diseases such as heart attack and atherosclerosis. Better quality of life can be achieved by lowering blood cholesterol levels.

Much of the circulating cholesterol is synthesized in the body from fatty acids, and only about one third comes from the diet. The excess of low-density lipoprotein cholesterol (LDL-C) in the bloodstream is oxidized by free radicals and damages the vessels, facilitating the deposition of lipids in these channels and increasing the risk of cardiovascular diseases.

Liong and Shah [60] investigated the cholesterol removal abilities of 11 probiotic strains (*Lactobacillus acidophilus* and *L. casei*) to understand the possible mechanisms of action. They concluded that all 11 strains had varying abilities to remove cholesterol in vitro and proposed as possible mechanisms of action in the assimilation of cholesterol during growth, the incorporation of cholesterol into the cell membrane, and the binding of cholesterol to the surface of the cell. *L. casei* ASCC 292 and *L. acidophilus* 4962 were the ones that removed the most cholesterol, indicating that these strains may be promising candidates to be used as a dietary supplement to lower serum cholesterol in vivo.

Greany et al. [61] evaluated the effect of probiotics *Lactobacillus acidophilus* and *Bifidobacterium longum* in improving the effect of soy isoflavones in reducing serum levels of blood lipids. The study included 37 women who received different treatments: soy protein isolate (with isoflavone), milk protein isolate (without isoflavone), and soy protein isolate with probiotics (isoflavone + probiotics). The effect of isoflavone intake was evident, with a reduction of 2.2% ($p = 0.02$) and 3.5% ($p = 0.005$) in total cholesterol and LDL-C levels, respectively, and an increase of 4.2% ($p = 0.006$) in the high-density lipoprotein cholesterol (HDL-C) level. However, the soy protein isolate added with probiotics had no additional effect on total cholesterol levels and LDL and HDL fractions.

One of the properties of bifidobacteria is their influence on lipid metabolism, that is, the possibility of acting in dyslipidemic processes. Several clinical studies have shown a significant decrease in total cholesterol levels as a result of the use of probiotics because of the decrease in LDL cholesterol, while the HDL cholesterol levels increase slightly. The hypocholesterolemic effect of bifidobacteria results from decreased absorption and transport of dietary cholesterol to the liver via chylomicrons and, on the other hand, from the disjunction of bile salts with

lower cholesterol absorption by the intestine. Niacin formed by bifidobacteria decreases the flow of free fatty acids which, by decreasing the biosynthesis of very low-density lipoprotein (VLDL), contributes to the reduction of plasma levels of triglycerides [62].

Aydas and Aslim [63] published a review about the cholesterol-lowering effects of probiotic bacteria. The authors state that despite controversial or unclear results of previous studies on the effect of probiotics on cholesterol levels, recent research has favored these compounds for possible beneficial effects on hypercholesterolemia. It may nevertheless be possible that a cholesterol-lowering rate of 10% may also be secondary to typical cholesterol variation and measurement differences. Moreover, all clinical studies to date have been conducted in subjects with hypercholesterolemia. Probiotics should also be tested in normocholesterolemic individuals.

3.7 Health Benefits of Probiotic Minas Frescal Cheese Consumption

Health benefits of probiotic products are related to the strains used and to the food matrix; therefore, it will be presented studies with probiotic Minas Frescal cheese.

Minas Frescal cheese containing *Lactobacillus acidophilus* LA14 and *Bifidobacterium longum* BL05 (20 g/d) was fed for 2 weeks to adult Wistar rats, which then were brought to exhaustion on the treadmill [63]. Two hours after exhaustion, the rats were killed, and material was collected and analyzed. Exercise reduced lymphocyte counts, and the decrease was 22% in the group fed the probiotic cheese and 48% in the animals fed regular cheese. Monocyte counts were unaltered in the rats fed probiotic cheese compared with a significant decrease in the rats fed the regular cheese. Most importantly, ingestion of the probiotic cheese resulted in a >100% increase in serum HDL-C and a 50% decrease in triacylglycerols. The authors concluded that probiotic Minas Frescal cheese may be a viable alternative to enhance the immune system and could be used to prevent infections, particularly those related to the physical overexertion of athletes.

Favretto et al. [64] evaluated the effect of the consumption of Minas Frescal cheese enriched with *Bifidobacterium lactis* Bi-07 on the symptoms of constipated women ($n = 30$). The patients were randomized into two groups who received, for 30 days, 30 g of the probiotic cheese ($n = 15$) or the regular cheese ($n = 15$). Constipation symptoms were evaluated according to ROMA III consensus, before and after the nutritional intervention. Also, data of clinical and anthropometric characteristics of the individuals were collected. After 30 days, it was observed that the ingestion of the probiotic cheese promoted beneficial effects on the symptoms of strength to evacuate. Therefore, the consumption of 30 g/day of Minas Frescal cheese enriched with *Bifidobacterium lactis* Bi-07 has beneficial effects on constipation symptoms.

Lollo et al. [65] investigated the effects of ingestion of probiotic Minas Frescal cheese on hypertension parameters in spontaneously hypertensive rats. Twenty-eight animals were divided into four groups fed with different experimental diets: control

initial (CI), control final (CF), traditional Minas Frescal cheese (CMFC), and probiotic minas Frescal cheese. The latter two groups were fed with 20 g of cheese per day for 15 days. All groups were assessed for blood pressure and health parameters. The group fed with probiotic cheese exhibited significantly lower blood pressure when compared to the group fed with CMFC, CI, and CF. Regarding the other health parameters, an improvement in blood lipids (triglycerides and cholesterol) was observed for the group fed with probiotic cheese as compared with CMFC. No significant differences were observed in renal function parameters. The authors suggest that consumption of probiotic cheese can be potentially useful to improve the cardiovascular health parameters.

4 Technological Processing of Minas Frescal Cheese

Minas Frescal cheese is a typical Brazilian fresh cheese, which presents high water activity, pH above 5.0, low salt content (1.4–1.6%), and the absence of preservatives. It offers excellent conditions for survival and growth of probiotic strains and is obtained by enzymatic coagulation of the milk with rennet and/or other appropriate coagulant enzymes. The supplementation with specific lactic bacteria is facultative [9, 66].

The Minas Frescal cheese has some specific characteristics:

- Classified as a semi-fat cheese (25–44.9% fat in the dry matter)
- High moisture content (not less than 55%)
- Consumed fresh
- Soft consistency
- Presence of holes or not
- Mild to slightly acidic
- Presence or not of a thin crust
- Cylindrical shape
- Weigh of 0.3–5.0 kg

The processing technology of the Minas Frescal cheese involves pasteurization, enzymatic coagulation, addition or not of starter cultures, cutting, forming, wheying off, and salting. Figure 2 represents, schematically, the technological process of obtaining probiotic Minas Frescal cheese. In a general view, the basic difference is the addition of the probiotic culture.

The milk used in the preparation of Minas Frescal cheese must be of good quality and free from high levels of contamination by microorganisms, chemical agents, antibiotics, herbicides, or pesticides. These components are harmful to the health of the consumer and can inhibit the coagulation of the milk or alter the storage stability of the products.

Milk pasteurization for cheesemaking can be accomplished by the rapid process on plate heat exchangers (HTST, 72–75°C/15 s) or by the slow process (LTLT, 65°C/30 min). The aim of the pasteurization is to eliminate pathogenic

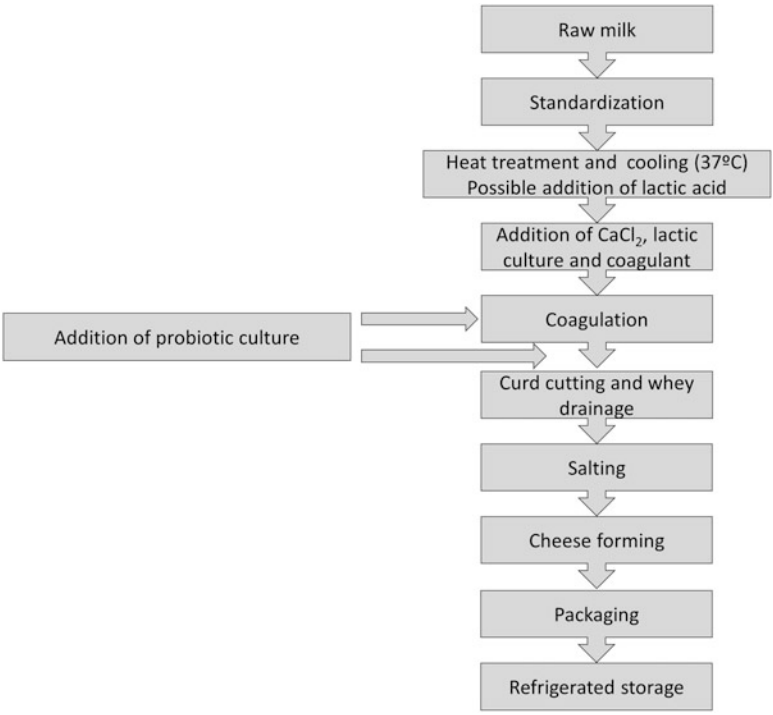


Fig. 2 Technological process of probiotic Minas Frescal cheese

bacteria and decrease the number of milk spoilage microorganism [67]. Pasteurization alters the microbiota of cheese, favoring the production of a more uniform cheese. However, it may impair the ability of the milk to coagulate, since it insolubilizes part of the soluble calcium, resulting in a weaker curd, which may increase the loss of whey from the curd. Thus, it is common to add calcium chloride (0.02%) to the pasteurized milk, aiming the replacement of the insolubilized calcium, increasing the firmness of the curd, and reducing the coagulation time [67].

The acidification of the product can be provided by the addition of lactic acid or starter culture, but this step is facultative. Lactic acid is added aiming to reduce the pH until 4.5 (isoelectric point of milk protein). At this pH, the casein micelles aggregate and precipitate, leaving the cheese with a firmer texture, besides a slightly acidic taste [68]. The direct acidification with lactic acid is commonly used by cheese industries, but it has the inconvenience that the final product has higher pH and moisture contents than the product made with starter cultures, making it more susceptible to contamination. Starter culture addition contributes to cheese aroma and flavor development during storage, by means of carbohydrate metabolism, proteolysis, and, to a lesser extent, lipolysis [16]. However, the presence of starter cultures in cheeses can influence the texture parameters, marked hardness, cohesiveness, chewiness, and gumminess, leading to more fragile cheeses, in addition to the

intense reduction of pH and increase of titratable acidity, due to the continuous production of lactic acid and other organic acids [9]. The addition of lactic acid or starter culture should be evaluated carefully, as this step is facultative. The higher acidity provided by both methodologies can increase the product shelf life, but it can impact negatively on the probiotic viability.

The process of cheese production requires the coagulation of the milk, in most cases, by the action of chymosin on k-casein, which is the layer that confers steric stability to the milk casein micelle. Rennet is, by definition, an extract from the stomach of ruminants and contains different amounts of chymosin and pepsin, depending on the age of the animal and its diet. However, to cope with the special needs of groups such as vegetarians and of certain religions, such as Muslims and Judaeans (kosher food), vegetable and microbial coagulants have been developed.

The coagulant is added to the milk, usually at 32–35°C and in sufficient quantity, to coagulate the milk within 30–40 min. Enzymatic coagulation occurs in two main phases. First, the addition of chymosin cleaves the link between amino acids Phe-105 and Met-106 of κ-casein, resulting in the destabilization of all casein micelles, which will subsequently aggregate to form the curd paste. After the hydrolysis of the peptide bonds of casein (with 60–85% of the total hydrolyzed casein), it becomes para-κ-casein, which precipitates in the presence of Ca^{2+} ions, forming the curd [68].

Milk coagulation for cheese production is influenced by temperature, pH, soluble calcium concentration, proteins, and enzymes. Some characteristics of the milk coagulation are:

- Coagulation does not occur at temperatures below 18°C and above 55–60°C.
- Temperatures near 40°C stimulate the action of the rennet and decrease the coagulation time.
- The closer to pH 6.0 (optimum enzyme action pH), the better the curd action, and the higher the curd strength.
- The greater the amount of soluble calcium present in the medium, the faster the formation of the curd, and the greater its firmness.
- The higher the percentage of milk proteins, the better the coagulation process.
- The higher the enzyme concentration, the shorter the milk coagulation time.

During cheese processing, probiotic cultures may be added prior to fermentation (along with the starter culture) or after this step. When added together with the starter culture, preliminary tests for knowledge of the concentrations of probiotic cells that are lost in the whey during the desorption process should be performed. This type of process has the advantage that the probiotic culture has time to adapt to the environment and participates from the decrease of the pH provided by the starter culture. The negative factor is that a higher concentration of probiotic should be used, as part of it is lost in whey. This contributes to the increase in the cost of the processing. If probiotic microorganisms are added after the addition of the starter culture, an immediate cooling (less than 8°C) should be performed, since the metabolic activity of the starter culture is drastically reduced at this temperature. When added after the addition of the starter culture, a resistant probiotic should be

selected, although cheese is not a harsh environment for its survival. Thus, the ideal amount of probiotic inoculum should be ascertained, according to the process to be employed [68].

When the end point in the tank is reached, i.e., the desired pH and moisture content is reached, the mass is cut, separated from the whey, and put into forms of specific size and shape so that whey drainage occurs. Salting can be performed after enzymatic coagulation of the milk through dry salting in the mass, dry salting on the surface, and salting in brine. Furthermore, it can be made in the milk. In addition to improving the sensory characteristics of the cheese, salt contributes to the occurrence of biochemical reactions during storage [68]. The characteristics of the salt addition are:

- Dry salting on the surface. It is the most archaic process, which consists of spraying refined salt on the two flat surfaces of the cheese after the forming process. The disadvantage of the dry-salting process is a possible irregularity in the salt distribution in the cheese.
- Salt in the mass. It is a practical process and consists of adding refined salt (which can be diluted in small amount of whey) to the mass of the cheese still in the manufacturing tank. The dosage is 1.2–1.5% refined salt calculated on the volume of milk to be manufactured.
- Salting in the milk. Some cheesemakers prefer the use of the salting in the milk before the addition of the ingredients by their practicality and final uniformity. Simply add about 2% refined salt to the milk. The disadvantage of the salting process in milk lies in the fact that more salt is expended (since some of it will be lost in the whey). Furthermore, the resulted whey will be salty and therefore unsuitable for use in other products.
- Salting in the brine. The cheese is immersed in brine (20% water and salt at a temperature of 8°C for a time of 60 min for pieces weighing 1 kg). The disadvantage of the brine salting process is a cheese with a less consistent characteristic, which can result in loss of moisture and loss of yield.

Whatever the choice of the salting process chosen, the curd should be shaped into its own forms as fast as possible, turning the cheese in 20 min after the deformation. Then, the cheeses are packaged and kept under refrigeration. The packaging aims to improve the stability and viability of the probiotic microorganisms that have been added in the cheese, which have anaerobic metabolism and, therefore, are sensitive to the presence of oxygen. Therefore, the choice of an appropriate packaging system, such as plastic films with low oxygen permeability and vacuum packaging, or the use of active packaging can be considered efficient alternatives to maintain the viability of probiotic bacteria [68].

4.1 Minas Frescal Cheese as a Probiotic Carrier

Fat content, concentration and type of proteins, sugar content, and pH are some factors that could affect probiotic growth and survival in food. Therefore,

product formulation can be manipulated to aid their efficacy. Dairy products are considered as an ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract.

Cheese is a dairy product with a strong potential for delivering probiotic microorganisms, because it presents a higher pH when compared to yogurts and fermented milks, making it a more stable matrix for the survival of probiotic microorganisms. In addition, cheeses, because they have a relatively high amount of fat, provide protection for probiotic bacteria during their passage through the gastrointestinal tract [66] and present lower titratable acidity, higher buffering capacity, more solid consistency, higher nutrient availability, and lower oxygen content than yogurts [69]. On the other hand, some cheeses have a very long maturation period, which hinders the survival of the probiotic microorganism. In the case of Minas Frescal cheese, because it is a fresh cheese with no maturation, it has a short shelf life (media of 20 days), making it a proper food for the incorporation of probiotic microorganisms [70]. In many parts of Brazil, Minas Frescal cheese is frequently consumed at least once a day, making it an excellent carrier for probiotic bacteria because of the high daily consumption.

Back et al. [66] evaluated the viability of probiotic microorganisms *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* sp. BB-12 in Minas Frescal cheese with reduced lactose content. The cheeses were submitted to microbiological counts of probiotic microorganisms weekly during the storage period (21 days), as well as physical and chemical analyses of fat, protein, moisture, ash, fat in the dry matter, titratable acidity, and pH. *Lactobacillus acidophilus* counts (4.38 + 0.01 to 7.88 + 0.07 log cfu g^{-1}) did not reach the minimum values of 10^6 CFU.g^{-1} on all days of cheese storage, while the population of *Bifidobacterium* sp. (6.05 ± 0.08 and 7.90 ± 0.04 log cfu g^{-1}) remained above 6 log cfu g^{-1} in all formulations developed, thus conferring probiotic characteristics to the cheeses. The results demonstrate the possibility of the incorporation of probiotic bacteria in cheeses with reduced lactose content, thus being an innovative alternative to the market of intolerant to this carbohydrate.

Alves et al. [71] produced two different Minas Frescal cheeses: (1) with the addition of lactic acid culture and (2) with *Lactobacillus acidophilus*. The *L. acidophilus* count was above 10^8 cfu g^{-1}, considered a sufficient population to classify the cheese as a probiotic food. The Minas Frescal cheese was suitable for incorporation of probiotic and the use of *L. acidophilus.*

Buriti et al. [9] investigated the effect of a probiotic *Lactobacillus paracasei* culture on sensory performance and on the behavior of instrumental texture profile and related properties of Minas fresh cheese during storage at 5°C for 21 days. Viable counts of *L. paracasei* in probiotic cheeses started above 10^6–10^7 cfu g^{-1} and increased during storage. The authors concluded that Minas Frescal cheese with *L. paracasei* showed a great potential as a functional food.

Souza and Saad [16] examined the viability of a probiotic *L. acidophilus* culture added only in coculture with *S. thermophilus* during the manufacture of Minas Frescal cheese. The authors observed that Minas Frescal cheese showed efficacy as a vehicle for delivery of the probiotic culture and the counts of *L. acidophilus*

remained with populations above 6 log cfu g^{-1} during the whole storage period (21 days). The supplementation of cheeses with a starter culture of *S. thermophilus* resulted in more acid cheeses, but these results had no influence on the viability of the probiotic culture.

There are some technological hurdles to maintain the viability of the probiotic cultures on Minas Frescal cheese. Therefore, it is suggested to:

- Select strains that are resistant to oxygen, acid, and bile salts.
- Evaluate the compatibility of the probiotic and lactic cultures, if a starter is used in the process.
- Standardize the salt content considering the salt taste but also the probiotic survival.
- Select a plastic packaging made with a material with low oxygen permeability, or use vacuum package.
- Control the storage temperature at refrigerated conditions.

4.2 Effect of Probiotic Addition on the Physicochemical and Sensory Properties of Minas Frescal Cheese

The addition of probiotic cultures can alter the physicochemical and sensory characteristics of Minas Frescal cheese. Table 2 presents the studies that evaluated the impact of probiotic cultures on the physicochemical characteristics of the products. The addition of probiotic cultures has no effect on the yield and composition of Minas Frescal cheese [13, 16, 72]. Probiotic cultures do not have significant activity on the main components of the cheeses, and, therefore, their concentration could not affect the cheese composition [70].

The effect of probiotic addition on pH and titratable acidity is dependent on the strain and type of milk used, and different results were observed. No influence of probiotic culture addition (*Bifidobacterium* Bb-12, *Lactobacillus acidophilus* (*La-5*), *Lactobacillus paracasei*, or *Bifidobacterium animalis* subsp. *lactis)* on the pH and/or titratable acidity of Minas Frescal cheese made with buffalo or cow milk was observed [9, 13, 16, 72]. In other study [12], a higher pH was observed in probiotic Minas Frescal cheese (*L. acidophilus*) when compared to the conventional product. The authors stated that this result is possibly due to production of an increased amount of NH_3 among the products of proteolysis. Finally, Gomes et al. [17] reported that the probiotic cheeses were more acidic (lower pH and higher titratable acidity) than the conventional products. The acidification of the products was a consequence of the post-acidification, with consumption of the lactose by the probiotic cultures and production of organic acids, mainly lactic acid. The addition of higher quantities of probiotic cultures resulted in a more acid product, and acetic acid was also produced. The authors stated that *Lactobacillus* strains are predominantly homofermentative, but they also present a heterofermentative pathway, fermenting glucose in equimolar amounts of lactic acid, CO_2, and ethanol or acetic acid. More acid probiotic products were also observed by Dantas et al. [2] when using *L. casei* Zhang as probiotic culture.

Table 2 Studies involving probiotic Minas Frescal cheeses

Probiotic culture	Objective	Effect of probiotic addition	Reference
Lactobacillus acidophilus	The effect of *Lactobacillus acidophilus* on instrumental texture profile and related properties of Minas fresh cheese during storage at 5° C and on sensory performance was investigated	Probiotic cheeses behaved very similarly to their controls in relation to evolution of texture and physicochemical parameters during refrigerated storage of the product Probiotic cheeses presented a pH slightly higher, when compared to their controls, possibly due to production of an increased amount of NH_3 among the products of proteolysis	[12]
Lactobacillus paracasei	The effect of a probiotic *Lactobacillus paracasei* culture on sensory performance and on the behavior of instrumental texture profile and related properties of Minas fresh cheese during storage at 5° C was investigated	Probiotic cheeses were not significantly different from their controls in relation to general instrumental texture attributes The addition of *Lactobacillus paracasei* to Minas fresh cheese resulted in a product with great potential as a functional food, with instrumental texture and sensory features similar to control cheeses, and the best overall results were in cheeses prepared through direct acidification with lactic acid	[9]
Lactobacillus acidophilus La-5 and *Bifidobacterium animalis* Bb-12	The effect of a mixed probiotic culture on instrumental texture and on sensorial and related properties of Minas fresh cheese during refrigerated storage was investigated	Texture profile of Minas fresh cheeses manufactured with the addition of a probiotic culture revealed to have a greater stability in relation to the different texture parameters evaluated, during refrigerated storage for up to 21 days, when compared to texture profile of Minas fresh cheeses processed according to the traditional dairy technologies, involving addition of the type O lactic culture Cheeses supplemented with the probiotic culture, as well as those made adding lactic acid only, showed to be less brittle	[10]

(*continued*)

Table 2 (continued)

Probiotic culture	Objective	Effect of probiotic addition	Reference
Lactobacillus acidophilus (*La-5*)	The effect of a probiotic culture of *Lactobacillus acidophilus* (La-5), added solely or in coculture with a starter culture of *Streptococcus thermophilus*, on texture, proteolysis, and related properties during storage at 5° C for 21 days	No effect of probiotic addition on composition Probiotic addition resulted in a more acid product (lower pH and higher titratable acidity)	[16]
Lactobacillus acidophilus	Probiotic Brazilian Minas Frescal cheese was produced by ultrafiltration. Cheese was produced from retentates obtained from ultrafiltration of milk with a volumetric concentration factor of 5:1. The pasteurized retentates were inoculated with 10^6, 10^7, and 10^8 cfu.mL^{-1} of *Lactobacillus acidophilus*. The viability of probiotic, protein breakdown, and pH changes were monitored during cheese storage at 5° C for 1, 7, 14, 21, and 28 days	*L. acidophilus* did not significantly change cheese composition, pH, and proteolysis	[70]
Bifidobacterium Bb-12	The effects of a probiotic bacterium (*Bifidobacterium* Bb-12) and lactic acid on the microbiological, physicochemical, rheological, and microstructural properties of Minas Frescal cheese were evaluated after 1 day and after 28 days of storage (5 ± 1 C)	The addition of bifidobacteria to the cheese did not influence its yield, protein, or lipid levels 1 day after production The addition of *Bifidobacterium* Bb-12 to Minas Frescal cheese resulted in a product with great potential as a functional food, and the best overall results were in the cheese formulations prepared through direct acidification with lactic acid	[15]
Lactobacillus acidophilus	Evaluate the effect of the supplementation of increasing amounts of *Lactobacillus acidophilus* (0, 0.4, or 0.8 g/L of milk) on the physicochemical parameters and sensory	Lower pH values, increased proteolysis, and greater production of organic acids were found in the probiotic cheeses inoculated with increasing concentrations of *L. acidophilus* and in the	[17]

(*continued*)

Table 2 (continued)

Probiotic culture	Objective	Effect of probiotic addition	Reference
	acceptance of Minas Frescal cheese	commercial cheese supplemented with *B. animalis*	
Bifidobacterium animalis subsp. *lactis*	Physicochemical characteristics of a Brazilian fresh cheese samples with *Bifidobacterium animalis* subsp. *lactis* as well as samples with this probiotic and polydextrose, a prebiotic ingredient, were evaluated	No effect of probiotic addition on moisture, ashes, total protein, dry matter, salt, and pH	[72]
Bifidobacterium Bb-12	Buffalo Minas Frescal cheese produced with a probiotic culture was evaluated in relation to the viability of *Bifidobacterium* Bb-12, physicochemical, color, rheological, and microstructural properties during 30 days of storage	No effect of probiotic addition on yield, physicochemical composition, pH, syneresis, and rheological properties The probiotic cheese showed a greenish-white color	[13]
Lactobacillus casei Zhang	The addition of *Lactobacillus casei* Zhang in the manufacture of Minas Frescal cheese was investigated	The addition of probiotic resulted in low pH values and high proteolysis indexes during storage The addition of *L. casei* Zhang led to higher lactic acid and acetic acid levels The addition of probiotic culture decreases the rigidity, resulting in softer and less elastic cheeses when compared to cheese produced by direct acidification Probiotic cheeses were harder and stiffer than control cheeses manufactured by enzymatic coagulation	[2]

Therefore, there are conflicting results about pH and titratable acidity parameters. The alterations on the acidity (pH and titratable acidity) of the Minas Frescal cheese with probiotic addition have positive and negative impacts. The maintenance of these parameters suggests that the probiotic product keeps the conventional characteristics, as pH and acidity can alter the flavor and texture characteristics of the products. An acidification promoted by probiotic addition has the advantage to

possibly provide an increase in the shelf life of the products, which is important, considering that this product has short shelf life (20–30 days). However, it can impact on the acceptance, as Minas Frescal cheese has slightly acid taste.

The utilization of a starter culture with the probiotic culture is related to an increase in the acidity of the products. Souza and Saad [16] reported that the supplementation of the probiotic product with *S. thermophilus* resulted in the highest acidity, because this microorganism produces small amounts of CO_2 and formic acid from lactose. Therefore, the utilization of starter cultures together with the probiotic cultures must be previously evaluated, so that a product with low acidification can be obtained.

Probiotic and conventional Minas Frescal cheeses have similar behavior during storage, considering the decrease in the moisture content and increase in syneresis and firmness. According to Souza and Saad [16], the rate of syneresis is directly related to the acidity and therefore is inversely related to pH. As the concentration of hydrogen ions increases during acidification, the repulsive forces decrease, and the casein micelles begin to aggregate.

Minas Frescal cheese is a fresh cheese; therefore, it is not submitted to a ripening process. However, during refrigerated storage, events such proteolysis can occur, mainly primary proteolysis, promoted by the coagulant agents and other components (plasmin and microbial enzymes). According to Cruz et al. [73], probiotic cultures have influence only on the secondary proteolysis, with increases in the total free amino acid content, and consequent formation of aroma and flavor compounds. As secondary proteolysis occurs with frequency in ripened cheeses, the probiotic addition frequently does not alter the proteolytic profile of Minas Frescal cheese, that is, a fresh cheese. However, Dantas et al. [2] observed higher proteolytic activity in probiotic cheeses and stated that the probiotic culture *L. casei* Zhang has an enzymatic system with sufficient activity for the growth in milk.

The effect of the probiotic culture *L. casei* Zhang on the texture parameters of Minas Frescal cheese was studied by Dantas et al. [2]. The authors observed a decrease in the rigidity of the products with probiotic addition, resulting in a softer and less elastic product when compared to the products produced by direct acidification. However, probiotic cheeses were harder and stiffer than control cheeses manufactured by enzymatic coagulation. Consumers of Minas Frescal cheese expect products with soft texture, and alterations on the texture parameters, mainly increased firmness, have negative impact on the acceptance of the products. At the same time, too soft products or products with brittle texture are rejected by consumers [16]. It is suggested to evaluate the impact of the probiotic strain on the texture parameters of the products and its impact on the acceptance of the products by consumers.

Table 3 presents the studies that evaluated the impact of probiotic cultures on the sensory characteristic of the products. The effect of the addition of probiotic cultures on the acceptance of Minas Frescal cheese is related to the strain, quantity of probiotic culture, presence of starter culture, and technology used by cheese processor, among others.

Table 3 Studies with sensory properties of probiotic Minas Frescal cheese

Probiotic culture	Objective	Effect of probiotic addition	Reference
Lactobacillus acidophilus La-5 and *Bifidobacterium animalis* Bb-12	The effect of a mixed probiotic culture on instrumental texture and on sensorial and related properties of Minas fresh cheese during refrigerated storage was investigated	Cheeses supplemented with the probiotic culture, as well as those made adding lactic acid only, showed more favorable sensorial features (particularly expressed by means of higher pH values) for acceptance by consumers	[10]
Lactobacillus acidophilus	Sensory acceptance of formulations of probiotic Minas Frescal cheese was investigated. Cheeses were prepared and supplemented with *Lactobacillus acidophilus* (T1 probiotic) and *Lactobacillus acidophilus* + *Streptococcus thermophilus* (T2 – probiotic + starter) or produced with no addition of cultures (T3 – control). Sensory acceptance tests were performed after 7 and 14 days of storage at 5° C, using a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely)	The addition of the probiotic *L. acidophilus* La-5 culture conferred sensory stability during storage of the product for up to 14 days, particularly in cheese produced with the addition of the probiotic in coculture with *S. thermophilus* All the three cheeses studied presented good acceptance in both sampling periods of sensory evaluation (after 7 and 14 days of storage) Control cheese T3, produced with no addition of cultures, was more susceptible to sensory alterations, reflected by a reduction of its acceptance between 7 and 14 days of storage	[74]
Lactobacillus acidophilus	Evaluate the effect of the supplementation of increasing amounts of *Lactobacillus acidophilus* (0, 0.4, or 0.8 g/L of milk) on the physicochemical parameters and sensory acceptance of Minas Frescal cheese. The sensory acceptance of probiotic cheeses was assessed using a consumer test and compared with commercial cheeses (conventional and probiotic)	The probiotic cheeses presented lower scores for appearance, aroma, and texture compared with conventional cheeses The use of 0.4% did not result in significant sensory differences, and a suitable mean value for acceptance was obtained The addition of 0.8% resulted in rejection by consumers with respect to taste and texture Internal preference mapping demonstrated the greater preference for the	[17]

(*continued*)

Table 3 (continued)

Probiotic culture	Objective	Effect of probiotic addition	Reference
		conventional samples The findings indicated that some negative sensory effects could occur when high level of supplementation with *L. acidophilus* is used in probiotic cheese processing	
Bifidobacterium animalis subsp. *lactis*	Sensorial and microbiological characteristics of a Brazilian fresh cheese samples with *Bifidobacterium animalis* subsp. *lactis* as well as samples with this probiotic and polydextrose, a prebiotic ingredient, were evaluated	No influence of probiotic addition on acceptance of the products	[72]
Lactobacillus casei Zhang	The addition of *Lactobacillus casei* Zhang in the manufacture of Minas Frescal cheese was investigated	Increased consumer acceptance was observed for the control sample produced by direct acidification (7.8), whereas the cheeses containing *L. casei* Zhang presented lower values for all sensory attributes, especially flavor and overall liking (5.37 and 4.61 for enzymatic coagulation and 5.57 and 4.72 for direct acidification, respectively) The optimization of *L. casei* Zhang dosage during the manufacturing of probiotic Minas Frescal cheese should be performed	[2]

The quantity of probiotic culture was evaluated in some studies [17, 73]. Gomes et al. [17] evaluated the effect of the supplementation of increasing amounts of *Lactobacillus acidophilus* (0, 0.4, or 0.8 g/L of milk) on the physicochemical parameters and sensory acceptance of Minas Frescal cheese. The sensory acceptance of probiotic cheeses was assessed using a consumer test and compared with commercial cheeses (conventional and probiotic). The authors reported that the amount of probiotic influenced the sensory acceptance of the products. The use of 0.4% did not result in significant sensory differences, and a suitable mean value for acceptance was obtained. The addition of 0.8% resulted in rejection by consumers with respect

to taste and texture. Ribeiro et al. [70] produced Brazilian Minas Frescal cheese using retentates obtained from ultrafiltration of milk with a volumetric concentration factor of 5:1. The authors evaluated the addition of 10^6, 10^7, or 10^8 cfu mL^{-1} of *Lactobacillus acidophilus*. The sensorial analysis showed a nonsignificant preference among the three types of cheese. Therefore, it seems that probiotic cultures in suitable quantities do not have a negative impact on the sensory acceptance, but, in excessive amounts, a decrease in the acceptance scores is observed.

The type of microorganism used as probiotic culture has importance on the acceptance of the products. Gomes et al. [11] observed that, among the commercial brands, supplementation with *B. animalis* significantly reduced ($P < 0.05$) the acceptance of probiotic Minas fresh cheese. A possible explanation was the interaction between the pH values and acetic acid concentration. The lower pH value allows a greater fraction of the acetic acid in an undissociated form, increasing the vinegar perception and resulting in an increased acetic taste. Dantas et al. [2] reported a reduction in the acceptance of probiotic products (*L. casei* Zhang) when compared to the conventional products. The intensified acidity of the products, the altered texture parameters, and the presence of off-flavors were the main factors associated with the negative impact. The impact of probiotic bacteria on the flavor profile of cheese is dependent on the species and strains added and their metabolic activity during cheese processing and storage. The metabolites produced by probiotic strains may enhance the bitter taste and negatively affect the cheese texture, depreciating the product's flavor. On the other hand, no negative impact of probiotic addition on acceptance was observed in other studies [72, 74].

There are some suggestions when considering the development of probiotic Minas Frescal cheeses, such as:

- Select a probiotic culture with low capacity of acidifying the product.
- Select a probiotic culture with low proteolytic capacity.
- Select a starter culture compatible with the probiotic culture and without negative impact on the sensory properties.
- Define the quantity of probiotic culture necessary to maintain the viability at suitable levels ($>10^6$ cfu/g) and not alter the acceptance of the products.
- Control the storage temperature.

5 Conclusion

The use of Minas Frescal cheeses as a food matrix for the administration of probiotic cultures has potential advantages, in addition to be an important alternative for the dairy industry. Minas Frescal cheese is one of the most popular cheeses consumed in Brazil, has mild and pleasant flavor and aroma, is slightly acidic and whitish in color, and presents soft texture. It has characteristics that allow the incorporation of probiotic cultures and maintenance of the viability until the end of the shelf life, such as the presence of nutrients that can be assimilated by the probiotic cultures, high pH (in the range of 5–6), high moisture content, low salt content (1.4–1.6%),

medium fat content, capability of protecting the microorganisms from the adverse conditions during the digestion process, refrigerated storage, and short shelf life. However, the development of the probiotic products on an industrial scale requires knowledge of all the technological steps involved in the traditional process of cheese production, as well as possible additional steps to adapt the protocol already used. Therefore, some suggestions are made aiming to obtain probiotic Minas Frescal cheeses with suitable counts of probiotic cultures and no negative impact on the physicochemical and sensory characteristics: (1) select strains that are resistant to oxygen, acid, and bile salts; (2) select probiotic strains with low capacity of acidifying the product and low proteolytic capacity; (3) evaluate the compatibility of the probiotic and lactic cultures, if a starter is used in the process; (4) define the quantity of probiotic culture necessary to maintain the viability at suitable levels ($>10^6$ cfu/g) and not alter the acceptance of the products; (5) standardize the salt content considering the salt taste intensity but also the probiotic survival; (6) select a plastic packaging made with a material with low oxygen permeability, or use vacuum package; and (6) control the storage temperature at refrigerated conditions.

References

1. FAO/WHO (2001) Evaluation of health and nutritional properties of powder milk with live lactic acid bacteria. Córdoba, FAO/WHO Expert Consultation
2. Dantas AB, Jesus VF, Silva R, Almada CN, Esmerino EA, Cappato LP, Silva MC, Raices RSL, Cavalcanti RL, Carvalho CC, Sant'Ana AS, Bolini HMA, Freitas MQ, Cruz AG (2016) Manufacture of probiotic Minas Frescal cheese with *Lactobacillus casei* Zhang. J Dairy Sci 99:18–30
3. Oliveira EW, Esmerino EA, Carr BT, Pinto LPF, Silva HLA, Pimentel TC, Bolini HMA, Cruz AG, Freitas MQ (2017) Reformulating Minas Frescal cheese using consumers' perceptions: insights from intensity scales and check-all-that-apply questionnaires. J Dairy Sci 100:1–14
4. Brazil. Ministry of Agriculture, Livestock and Supply. Ordinance No. 352 of September 4, 1997. Approves the technical regulation on identity and quality of Minas Frescal cheese. Official Union Journal. 08 Sep 1997; Section 1: 19684
5. Brazil. Ministry of Agriculture, Livestock and Supply. Ordinance No. 146 of March 7, 1996. Approves the technical regulations on the identity and quality of dairy products. Official Union Journal. 11 Mar 1996; Section 1: 3977
6. Vinderola G, Gueimonde M, Gomez-Gallego C, Delfederico L, Salminen S (2017) Correlation between in vitro and in vivo assays in selection of probiotics from traditional species of bacteria. Trends Food Sci Technol 68:83–90
7. Isepon JS, Oliveira AJ (1993) Influência das culturas lácticas no índice de proteólise do queijo Minas frescal. Ciênc Agr 50:451–454
8. Nascimento M, Moreno I, Kuaye A (2008) Applicability bacteriocin-producing *Lactobacillus plantarum*, *Enterococcus faecium* and *Lactococcus lactis* spp *lactis* as adjunct starter in Minas Frescal cheesemaking. Dairy Technol 61:352–357
9. Buriti FCA, Rocha JS, Assis EG, Saad SMI (2005) Potential probiotic of Minas fresh cheese prepared with the addition of *Lactobacillus paracasei*. LWT Food Sci Technol 38:173–180
10. Buriti FCA, Okazaki TY, Alegro JHA, Saad SMI (2007) Effect of a probiotic mixed culture on texture profile and sensory performance of Minas fresh cheese in comparison with the traditional products. Arch Latinoam Nutr 57:179–185

11. Alegro JHA (2003) Development of probiotic Minas frescal cheese with '*Lactobacillus acidophilus*' and '*Bifidobacterium lactis*' isolated and in co-culture. Master dissertation, University of São Paulo – USP – Faculty of Pharmaceutical Sciences
12. Buriti FCA, Rocha JS, Saad SMI (2005) Incorporation of *Lactobacillus acidophilus* in Minas fresh cheese and its implications for textural and sensorial properties during storage. Int Dairy J 15:1279–1288
13. Verruck S, Prudencio ES, Müller CMO, Fritzen-Freire CB, Amboni RDMC (2015) Influence of *Bifidobacterium Bb*-12 on the physicochemical and rheological properties of buffalo Minas Frescal cheese during cold storage. J Food Eng 151:34–42
14. Verruck S, Vieira CRW, Amante ER, Amboni RDMC (2015) The buffalo Minas Frescal cheese as a protective matrix of *Bifidobacterium* BB-12 under in vitro simulated gastrointestinal conditions. LWT Food Sci Technol 63:1179–1183
15. Fritzen-Freire CB, Müller CMO, Laurindo JB, Prudêncio ES (2010) The influence of *Bifidobacterium* Bb-12 and lactic acid incorporation on the properties of Minas Frescal cheese. J Food Eng 96:621–627
16. Souza CHB, Saad SMI (2009) Viability of *Lactobacillus acidophilus* La-5 added solely or in co-culture with a yoghurt starter culture and implications on physico-chemical and related properties of Minas fresh cheese during storage. LWT Food Sci Technol 42:633–640
17. Gomes AA, Braga SP, Cruz AG, Cadena RS, Lollo PCB, Carvalho C, Amaya-Farfán J (2011) Effect of the inoculation level of *Lactobacillus acidophilus* in probiotic cheese on the physico-chemical features and sensory performance compared with commercial cheeses. J Dairy Sci 94:4777–4786
18. Felicio TL, Esmerino EA, Vidal VAS, Cappato LP, Garcia RKA, Cavalcanti RN, Freitas MQ, Conte Junior CA, Padilha MC, Silva MC, Raices RSL, Arellano DB, Bollini HMA, Pollonio MAR, Cruz AG (2016) Physico-chemical changes during storage and sensory acceptance of low sodium probiotic Minas cheese added with arginine. Food Chem 196:628–637
19. Talwalkar A, Miller CW, Kailasapathy KN, Guyen MH (2004) Effect of packaging materials and dissolved oxygen on the survival of probiotic bacteria in yoghurt. Int J Food Sci Technol 39:605–611
20. Anvisa National Health Surveillance Agency (2016) Food with claims of functional and/or health properties: list of approved functional property claims. Updated Dec 2016
21. Chua JK, Kwok WC, Aggarwal N, Sun T, Chang MW (2017) Designer probiotics for the prevention and treatment of human diseases. Curr Opin Chem Biol 40:8–16
22. Saad SMI (2006) Probióticos e prebióticos: o estado da arte. Braz J Pharm Sci 42:1–16
23. Gibson GR, Fuller R (2000) Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. J Nutr 130:391–394
24. Ziemer CJ, Gibson GR (1998) An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. Int Dairy J 8:473–479
25. Isolauri E, Salminen S, Ouwehand AC (2004) Probiotics. Best Pract Res Clin Gastroenterol 18:299–313
26. Puupponen-Pimiä R, Aura AM, Oksmancaldentey KM, Myllärinen P, Saarela M, Mattila-Sanholm T, Poutanen K (2002) Development of functional ingredients for gut health. Trends Food Sci Technol 13:3–11
27. Salminen S, Boukley C, Boutron-Ruault MC, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau MC, Roberfroid M, Rowland IR (1998) Functional food science and gastrointestinal physiology and function. Br J Nutr 80:147–171
28. Crittenden RG (1999) Prebiotics. In: Tannock GW (ed) Probiotics: a critical review. Horizon Scientific Press, Norfolk, pp 141–156
29. Stürmer E, Casasola S, Gall M, Gall M (2012) A importância dos probióticos na microbiota intestinal humana. Rev Bras Nutr Clin 27:264–272
30. Bartran HP, Sheppach W, Schmid H, Hofmann A, Dusel G, Richter F, Richter A, Kasper H (1993) Proliferation of human colonic as an intermediate biomarker of carcinogenesis: effects of butyrate, deoxycholate, calcium, ammonia, and pH. Cancer Res 53:3283–3288

31. Salminen S, Playne M, Lee YK (2004) Successful probiotic lactobacilli: human studies on probiotic efficacy. In: Shortt C, O'Brien J (eds) Handbook of functional dairy products. CRC Press, Boca Raton, pp 13–69
32. Lourens-Hattingh A, Viljoen BC (2001) Yogurt as probiotic carrier food. Int Dairy J 11:1–17
33. Arifin Z, Boediarso A, Tambunan T (2006) Probiotic treatment in children with lactose intolerance – an open labeled the one group pre-test post-test experimental study. Paedr Indon 46:139–146
34. Canani RB, Sangwan N, Stefka AT, Nocerino R, Paparo L, Aitoro R, Calignano A, Khan AA, Gilbert JA, Nagler CR (2016) *Lactobacillus rhamnosus* GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. ISME J 10:742–750
35. Nordqvist C (2017) Constipation: causes, symptoms, and treatments**,** Medicalnewstoday. https://www.medicalnewstoday.com/articles/150322.php. Accessed 27 Oct 2017
36. Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C (2005) Bifidobacteria as probiotic agents – physiological effects and clinical benefits. Aliment Pharmacol Therap 22:495–512
37. Szajewska H, Setty M, Mrukowicz J, Guandalini S (2006) Probiotics in gastrointestinal diseases in children: hard and not-so-hard evidence of efficacy. J Pediatr Gastroenterol Nutr 42:454–475
38. Ouwehand AC, Lagström H, Suomalainen T, Salminen S (2002) Effect of probiotics on constipation, fecal azoreductase activity and fecal mucin content in the elderly. Ann Nutr Metab 46:3–4
39. Bu LN, Chang MH, Ni YH, Chen HL, Cheng CC (2007) *Lactobacillus casei rhamnosus Lcr35* in children with chronic constipation. Pediatr Int 49:485–490
40. Banaszkiewicz A, Szajewska H (2005) Ineffectiveness of *Lactobacillus GG* as an adjunct to lactulose for the treatment of constipation in children: a double-blind, placebo-controlled randomized trial. J Pediatr 146:364–369
41. Barichella M, Pacchetti C, Bolliri C, Cassani E, Iorio L, Pusani C, Pinelli G, Privitera G, Cesari I, Faierman SA, Caccialanza R, Pezzoli G, Cereda E (2016) Probiotics and prebiotic fiber for constipation associated with Parkinson disease. Neurology 87:1274–1280
42. Dimidi E, Christodoulides S, Scott M, Whelan K (2017) Mechanisms of action of probiotics and the gastrointestinal microbiota on gut motility and constipation. Adv Nutr 8:484–494
43. Olivares M, Díaz-Ropero MP, Gómez N, Lara-Villoslada F, Sierra S, Maldonado JA, Martín R, Rodríguez JM, Xaus J (2006) The consumption of two new probiotic strains, *Lactobacillus gasseri* CECT 5714 and *Lactobacillus coryniformis* CECT 5711, boosts the immune system of healthy humans. Int Microbiol 9:47–52
44. Strauss E, Caly WR (2003) Peritonite bacteriana espontânea. Rev Soc Bras Med Trop 36:711–717
45. Surawicz CM, Mcfarland LV, Greenberg RN, Rubin M, Fekety R, Mulligan ME, Garcia RJ, Brandmarker S, Bowen K, Borjal D, Elmer GW (2000) The search for a better treatment for recurrent clostridium difficile disease: use of high-dose vancomycin combined with *Saccharomyces boulardii*. Clin Infect Dis 31:1012–1017
46. Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, Maunula L, Isolauri E (1999) Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics 104:1121–1122
47. Santos MS, Ferreira CLLF, Gomes PC, Santos JL, Pozza PC, Teshima E (2003) Influência do fornecimento de probiótico à base de *Lactobacillus* sp. sobre a microbiota intestinal de leitões. Ciênc Agrotec 27:1395–1400
48. Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT (1999) Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet 354:635–639
49. Weizman Z, Asli G, Alsheikh A (2005) Effect of a probiotic infant formula on infections in child care centres: comparison of two probiotic agents. Pediatrics 115:5–9
50. Adolfsson O, Meydani SN, Russel RM (2004) Yogurt and gut function. Am J Clin Nutr 80:245–256

51. Rafter J (2003) Probiotics and colon cancer. Best Pract Res Clin Gastroenterol 17:49–59
52. Gaudier E, Michel C, Segain JP, Cherbut C, Hoebler C (2005) The VSL#3 probiotic mixture modifies microflora but does not heal chronic dextran-sodium sulfate-induced colitis or reinforce the mucus barrier in mice. J Nutr 27:53–61
53. Marteau PR, de Vrese M, Cellier CJ, Scherezenmeir J (2001) Protection from gastrointestinal diseases with the use of probiotics. Am J Clin Nutr 73:430–436
54. Haberer P, Tait M, Dicks LMT, Ahrens F, Halzapfel WH (2003) Effect of potentially probiotic lactobacilli on faecal enzyme activity in minipigs on high-fat, high-cholesterol diet a preliminary in vivo trial. Int J Food Microbiol 87:287–291
55. Rowland I, Rumney C, Counts J, Lievense L (1998) Effects of *Bifidobacterium longum* and inulin on gut bacterium metabolism and carcinogen induced aberrant crypt foci in rats. Carcinogenics 19:281–285
56. Spanhaak S, Havenaar R, Schaafsma G (1998) The effect of consumption of milk fermented by *Lactobacillus casei* strain Shirota on the intestinal microflora and immune parameters in humans. Eur J Clin Nutr 52:899–907
57. Lidbeck A, Nord CE, Gustafsson JA, Rafter JJ (1992) Lactobacilli anticarcinogenic activities and human intestinal microflora. Eur J Cancer Prev 3:41–53
58. Guerin-Danan C, Chabanet C, Pedone C, Popot F, Vaissade P, Bouley C, Szyitt O, Andrieux C (1998) Milk fermented with yogurt cultures and *Lactobacillus casei* compared with yogurt and gelled milk: influence on intestinal microflora in healthy infants. Am J Clin Nutr 67:111–117
59. Marinelli L, Tenore GC, Novellino E (2017) Probiotic species in the modulation of the anticancer immune response. Semin Cancer Biol 46:182–190
60. Liong MT, Shah NP (2005) Optimization of cholesterol removal by probiotics in the presence of prebiotics by using a response surface method. Appl Environ Microbiol 71:1745–1753
61. Greany KA, Nettleton JA, Wangen KE, Thomas W, Kurzer MS (2004) Probiotic consumption does not enhance the cholesterol-lowering effect of soy in postmenopausal women. J Nutr 134:3277–3283
62. Aydas SB, Aslim B (2016) The cholesterol-lowering effects of probiotic bacteria on lipid metabolism. In: Probiotics, prebiotics and synbiotics, London: Academic Press, pp 699–722
63. Lollo PCB, Cruz AG, Morato PN, Moura CS, Carvalho-Silva LB, Oliveira CAF, Faria JAF, Amaya-Farfan J (2011) Probiotic cheese attenuates exercise-induced immune suppression in Wistar rats. J Dairy Sci 95:3549–3558
64. Favretto DC, Pontin B, Moreira TR (2013) Effect of the consumption of a cheese enriched with probiotic organisms (*Bifidobacterium lactis Bi-07*) in improving symptoms of constipation. Arq Gastroenterol 50:196–201
65. Lollo PCB, Morato PN, Moura CS, Almada CN, Felicio TL, Esmerino EA, Barros ME, Amaya-Farfan J, Sant'Ana AS, Raices RRS, Silva MC, Cruz AG (2015) Hypertension parameters are attenuated by the continuous consumption of probiotic Minas cheese. Food Res Int 76:611–617
66. Back DMP, Andrade D, Simões G, Richards N (2013) Probiotic viability of Minas Fresh cheeses with reduced lactose content. Rev Inst Lat Când Tostes 68:27–35
67. Paula JCJ, Carvalho AF, Furtado MM (2009) Princípios Básicos de Fabricação de queijo: Do Histórico à Salga. Rev Inst Lat Când Tostes 367(368):19–25
68. Cruz AG, Buriti FCA, Souza CHB, Faria JAF, Saad SMI (2011) Queijos probióticos e prebióticos. In: Saad SMI, Cruz AG, Faria JAF (eds) Probióticos e prebióticos em alimentos. Fundamentos e aplicações tecnológicas, 1st edn. Livraria Varela Ltda, São Paulo, pp 85–104
69. Araujo EA, Pires ACS, Pinto MS, Jan G, Carvalho AF (2012) Probiotics in dairy fermented products. Intech, Rijeja, pp 129–148
70. Ribeiro EP, Simões LG, Jurkiewicz CH (2009) Desenvolvimento de queijo minas frescal adicionado de *Lactobacillus acidophilus* produzido a partir de retentados de ultrafiltração. Ciênc Tecnol Alim 29:19–23
71. Alves CCC, Gemal NDH, Cortez MAS, Franco RM, Mano SB (2011) *Lactobacillus acidophilus* and of direct acidification to Minas Frescal cheese production. Arq Bras Med Vet Zoot 63:1559–1566

72. Azambuja NC, Zacarchenco PB, Fleuri LF, Andrade JC, Moreno I, Van Dender AGF, Gallina DA (2013) Characterization of fresh cheese with addition of probiotics and prebiotics. J Life Sci 7:189–195
73. Cruz AG, Buriti FCA, Souza CHB, Faria JAF, Saad SMI (2009) Probiotic cheese: health benefits, technological and stability aspects. Trends Food Sci Technol 20:344–354
74. Souza CHB, Buriti FCA, Behrens JH, Saad SMI (2008) Sensory evaluation of probiotic Minas fresh cheese with *Lactobacillus acidophilus* added solely or in co-culture with a thermophilic starter culture. Int J Food Sci Technol 43:871–877

Part IX

Methods of Food Analysis and Quality Control

Dietary Phenolic Compounds in Biological Samples: Current Challenges in Analytical Chemistry

66

Maike Passon

Contents

Abstract

Phenolic compounds are bioactive molecules relevant in plant-derived foods and are associated with beneficial health effects in humans. The metabolism of these phytonutrients comprises of the absorption, distribution, metabolism, and excretion and is investigated in in vivo intervention or in vitro cell culture studies. Blood and urine samples are collected during animal or human trials, and matrix effects caused by high protein and salt concentration are a major challenge during analysis. This chapter describes the context between the matrix effects which arise during phenolic compound analysis from biological samples and possible analytical techniques to handle these challenges. Difficulties arise from interfering matrix compounds, low concentrations of chemically heterogeneous

M. Passon (✉)
Institute of Nutritional and Food Sciences, Molecular Food Technology, University of Bonn, Bonn, Germany
e-mail: passon@uni-bonn.de

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_19

metabolites, and the lack of reference compounds. Therefore, interactions of phenolic compounds with plasma proteins are reviewed, as well as ion suppression as one of the most common matrix effects during LC-MS analysis. Frequently used analytical techniques for sample preparation, compound synthesis, separation, and detection are described in this chapter.

Keywords

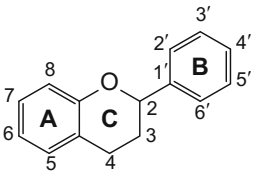
Polyphenols · Biological samples · Serum albumin interactions · Matrix effect · SPE · LC-MS

Abbreviations

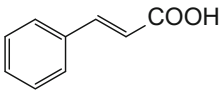
ADME	Absorption, distribution, metabolism, excretion
APCI	Atmospheric-pressure chemical ionization
BSA	Bovine serum albumin
CCS	Collision cross-section
CE	Capillary electrophoresis
CEAD	Coulometric electrode-array detection
COMT	Catechol-*O*-methyltransferase
DMF	Dimethylformamide
EC	Epicatechin
ECG	Epicatechin-3-gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin-3-gallate
ESI	Electrospray ionization
HILIC	Hydrophilic interaction liquid chromatography
HSA	Human serum albumin
IT	Ion trap
LC	Liquid chromatography
m/z	Mass-to-charge ratio
MALDI	Matrix-assisted laser desorption ionization
MRM	Multiple reaction monitoring
MRP	Multidrug resistance protein family
MS	Mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
NP	Normal-phase
QqQ	Triple quadrupole
RP	Reversed-phase
SIM	Selected ion monitoring
SPE	Solid-phase extraction
SULT	Sulfotransferase
TFA	Trifluoroacetic acid
ToF	Time-of-flight
UGT	Uridine-5′-diphosphate glucuronosyltransferases
(U)HPLC	(Ultra)-High-performance liquid chromatography

1 Introduction

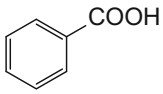
Phenolic compounds are secondary plant metabolites which strongly differ in their structure and physicochemical behavior. They can broadly be classified into flavonoids, including anthocyanins, hydroxycinnamic and hydroxybenzoic acids, stilbenes, and xanthones. Flavonoids (1), hydroxycinnamic acids (2), and hydroxybenzoic acids (3) consist of a C6-C3-C6, C6-C3, and C6-C1 backbone, respectively. A definition of "polyphenols" is still discussed in the literature and until today the term is inconsistently used [1]. In the following, the term "polyphenol" is used to subsume simple phenolic acids as well as flavonoids and oligomers.



1



2



3

Polyphenols are found in almost all plant-derived foods such as vegetables, fruits, cereals, legumes, spices, and herbs and are therefore an integral part of the human diet [2]. As part of the plant defense system, polyphenols show UV-protecting activities and serve as growth regulators or colorants, to name just a few [2, 3]. Due to solubility reasons, polyphenols are generally present as glycosides with the exception of, for example, flavanols in tea, where the aglycones are predominantly synthesized [4]. In addition, tannins are more complex polyphenols with intermediate to high molecular weight, which can be further classified into hydrolysable (e.g., gallotannins) and condensed (proanthocyanidins) tannins [2].

In former times, polyphenols have been considered antinutritive because of their bitterness, astringency, or browning reactions. Today, phenolic compounds are associated with a reduced risk of cardiovascular and neurodegenerative diseases, due to their antioxidative, antimicrobial, or, for example, antiproliferative effects [5]. However, our knowledge concerning the metabolic fate and the modes of action of dietary polyphenols is limited. As secondary plant compounds, phenolics are usually synthesized in small quantities so that the intake generally comprises 500–1700 mg per day [6]. After release from the food matrix, they undergo extensive metabolism in the small intestine, liver, or by the colonic microbiota, and as result, various metabolites of different chemical structures appear in very low concentrations (rarely exceeding nmol l^{-1} levels) in biological fluids like blood or urine.

Polyphenol metabolism in vivo roughly comprises of the absorption, distribution, metabolism, and excretion (ADME) [7]. Depending on a number of factors, for example, the food matrix, concentration, or individual compound structure, these molecules are subject to structural modifications due to the activity of hydrolytic,

phase II, and efflux transporting enzymes [8]. Metabolism starts when polyphenols enter the mouth and interactions with enzymes and proteins of the saliva take place [8, 9]. Then the compounds reach the acidic environment of the stomach, which affects the compound stability, especially in the case of anthocyanins. The structure of anthocyanins, or rather anthocyanidins, depends on the pH value and a comprehensive review about their bioavailability is given by Fernandes et al. [10].

In order to reach target cells, polyphenols must pass the epithelium via active, passive, or facilitated transport [11]. The phases of metabolic breakdown are roughly divided into three steps of detoxification. In phase I, the hydrophilicity is increased by oxidative or reductive reactions and hydrolysis through different types of enzymes. During phase II reactions, further functional groups are conjugated catalyzed by uridine-5′-diphosphate glucuronosyltransferases (UGT), sulfotransferases (SULT), and catechol-*O*-methyltransferases (COMT) [7]. When sulfation or glucuronidation is the preferred reaction, depending on the dose, a positive discrimination of sulfation at lower concentrations is possible [12]. Methylation reactions decrease the hydrophilicity, with the two exceptions of *N*- and *S*-methylation, and in general it is a minor pathway of polyphenol transformation. Nevertheless, methylated conjugates were found during in vitro and in vivo studies [13, 14]. To facilitate excretion and efflux into the small intestine, further structural modifications take place during phase III, indicating that transporters of the multidrug resistance protein family (MRP) are involved [15]. Phase I and phase II reactions occur in the enterocytes, but with the highest activity in the liver [16, 17]. In comparison to the phase II reactions, the CYP450-mediated metabolic pathway (phase I) of polyphenols has less frequently been investigated, for example, by Camilo Melo-Filho et al. [18].

Polyphenols which cannot be absorbed in the small intestine or which enter the enterohepatic circulation reach the colon and are metabolized by the microbiota enzymes. Small molecules including, for example, hydroxyphenylacetic acids, hydroxybenzoic acids, or γ-valerolactones were released as a result of ring cleavage of the flavonoid backbone [19]. The bidirectional relationship between polyphenols and the gut microbiome is one of the major topics in polyphenol research in recent years [7, 20]. Two comprehensive overviews about the whole metabolism and the bio availability of polyphenols are given by Manach and Williamson, who reviewed more than 90 intervention studies [21, 22].

The different transport forms and phase II reaction types, like glucuronidation, sulfation, or methylation, and the colon derived phenolic acids result in a complex profile of structurally diverse metabolites. The lack of knowledge about polyphenol metabolism and the growing interest in new analytical techniques are the result of the low concentration of numerous metabolites in a complex matrix consisting of proteins and salts [23]. Liquid chromatography (LC), and especiallyultra-high-performance liquid chromatography (UHPLC), coupled with mass spectrometric detection is the technique of choice to analyze polyphenolic metabolites in biological samples. Mass spectrometry (MS) is a powerful tool to identify and quantify the compounds of interest, but it is a misconception that due to the high selectivity and specificity, the chromatographic separation and extensive sample preparation can be minimized

or eliminated [24]. A prerequisite to develop efficient sample preparation methods is the knowledge about the interactions of the analyte (polyphenol) and the interfering matrix molecules (proteins, salts). Furthermore, implications of the sample preparation on the detections system must be monitored, because even highly sophisticated detection techniques can suffer from adverse effects of endogenous and exogenous matrix compounds.

To shade more light on polyphenol metabolism, targeted and untargeted studies were carried out to establish accurate methods for identification and quantification. In this chapter, the challenges of polyphenol analytical chemistry in biological samples will be emphasized.

2 Primary Considerations and Challenges

Biological fluids, like blood and urine, are valuable samples, which are collected during animal or human intervention trials. Especially in the case of blood samples, the amount of liquid is limited, due to justified ethical reasons. Therefore, primary considerations about the scientific outcome and the need of biological samples are necessary before the design of an intervention trial.

Intervention trials can be designed to investigate the metabolism (identification of unknown metabolites) or the bioavailability (quantification of metabolites) of phenolic compounds. Where asmetabolism studies often include the identification and characterization of unknown metabolites, bioavailability studies are focusing on the quantitative aspects of ADME of known metabolites. The former studies include the analysis of the intact metabolite profile, which is accompanied by low concentrations and numerous metabolites of different physicochemical nature. Methods for the latter type of studies often include enzymatic hydrolysis to release the phase II metabolites. It is quite evident that the enzymatic release entails the loss of biological information, but allows for increased concentration of the metabolites of interest [25].

In the case of bio availability studies, correct sampling is a crucial step as realistic plasma kinetics are only available when the whole metabolic processes from absorption to complete excretion are considered. To estimate the correct time point for blood sampling, it seems evident that all metabolites of interest must be known. Maximal plasma concentrations are reached between 0.5 and 7.5 h and depend on the chemical nature of the polyphenol, the food matrix, and the concentration [21]. In addition, it should be taken into account which type of metabolite is to be expected. Colon-derived metabolites may be reabsorbed and occur as free or conjugated forms in plasma at later time points in comparison to polyphenols which are absorbed in the small intestinal tract [15].

As it was mentioned above, the bidirectional relationship of polyphenols and the gut microbiota is a promising research area and by now feces samples are an integral part of long-term intervention studies. Monagas et al. [19] reviewed the metabolism and microbial biotransformation of flavan-3-ols and approximately 20 catabolites belonging to the subclasses of hydroxyphenylvalerolactones, valeric acids, hydroxyphenylpropionic, and hydroxybenzoic acids. Identification of polyphenol

catabolites is carried out by the analysis of feces samples from intervention trials or by in vitro fermentation studies [26, 27]. In vitro fermentation can be challenging, as an anaerobic environment should be present at all times directly after the sampling to simulate the conditions in the lower intestine. Human feces is comprised of various facultative anaerobic or obligate anaerobic microbial types, like *Lactobacillus* or *Clostridium*, respectively, and the presence of molecular oxygen may alter the composition of living microbes and thus the metabolic fate of polyphenols [28].

The extensive metabolism and catabolism of polyphenols depends on various factors, which lead to another challenging aspect. Among others, host genetics and thus the composition of the human gut microbiota differs decisively from person to person, and as a consequence, the results for polyphenol metabolites come along with high interindividual variations [20, 29].

At this point it is worth mentioning that the analysis of the mode of action of polyphenols in the human body is a prerequisite to understand the potential beneficial health effects of these phytonutrients. For this reason, bioavailability studies often include parameters like blood pressure or inflammation biomarkers to demonstrate the health effects. The mode of action is studied on the molecular level with in vitro studies including human intestinal epithelial caco-2-cells or brain endothelial cells forming the blood-brain barrier [30, 31]. In vitro studies are an integral part of the whole research area and were used to study the bio activity of polyphenols. However, implementation and interpretation of the results must be done very carefully, because among others the cellular metabolism, the concentration, and structure of the applied polyphenols may alter the metabolic fate and thus the bio activity [32]. For this reason, Aragonés and coworkers [32] proposed an experimental design which mainly includes the analysis of the culture media, culture media and cells, culture media and compounds, as well as culture media, cells, and the compounds at two different time points, respectively. With this approach, confounding reactions with, for example, the media are monitored and the identification of the bioactive metabolites is facilitated.

The accurate quantification is one of the most challenging aspects in the analysis of polyphenol metabolites in biological samples. In the case of enzymatic hydrolysis, phase II metabolites are released to their corresponding parent compounds, the aglycones, which are often commercially available. The use of reference compounds facilitates accurate polyphenol quantification. Accurate quantification of phase II metabolites is very difficult, because the availability of reference substance is highly decreased [23]. For example, anthocyanin glucuronides and phenolic sulfates need to be synthesized or isolated.

Furthermore, the complex matrix, including, for example, protein and salt, directly affects the sample preparation and detection of metabolites and consequently the quantification. The small concentrations of the chemically heterogeneous metabolites contribute to the complexity of sample preparation as well as the detection and should not be underestimated. Liquid extraction and/or solid-phase extraction (SPE) in combination with LC-MS is widely used for identification and quantification. Even though nuclear magnetic resonance spectroscopy

(NMR) is the technique of choice for identification, the application for the analysis of polyphenol metabolites is very limited. Lehtonen et al. [33] used an NMR-based metabolic fingerprinting of polyphenol urine metabolites. NMR analysis of synthesized reference compounds, used for quantification and identification reasons, is imperative.

The characterization of the physicochemical properties of both the analytes and the interfering compounds in the matrix to be extracted should be the first step. The former point comprises the different types of polyphenol metabolites, namely, phase II, glycosides, aglycones, and colonic-derived phenolic acids. These metabolites are very divergent in their physicochemical behavior, which must be considered by the simultaneous extraction of, for example, aglycones and polar metabolites like sulfated phenolics. Liquid extraction and/or SPE with or without hydrolysis are predominantly used as extraction methods and have extensively been reviewed by Day and Morgan [25].

Samples regardless of their nature, that is, plasma or urine, should immediately be stabilized with an antioxidant solution and frozen at −20 °C or −80 °C. However, degradation processes may take place, as it was shown by Felgines et al. [34] for anthocyaninmetabolites from strawberry. As a result, quantification of metabolites may be falsified and the best way to handle this issue is analyzing them immediately after sampling.

Sample amount and interindividual variationsare immutable, but current challenges in polyphenol analytical chemistry are:

- Interfering matrix compounds
- Low analyte concentration
- Chemical heterogeneity of the analytes
- Stability of analytes in biological samples
- Lack of reference compounds

3 Polyphenols and Interfering Compounds

The interaction of matrix compounds with the analyte and with the instrument is a yet neglected fact in polyphenol metabolite determination and can be the Achilles heel in quantitative analysis, as reported by Taylor [35] in the context of LC-MS analysis. The interfering compounds differ decisively in their nature and composition and therefore a short summary about the biological samples, namely, blood and urine, are given before interferences will be discussed in more detail.

3.1 Blood and Urine

Krebs [36] already described the chemical composition of blood plasma and serum in 1950. In general, blood is composed of 50% plasma, which is a yellowish fluid, and 50% formed elements, including erythrocytes, leukocytes, and platelets. Besides

water, the main compounds of plasma are proteins, enzymes, nutrients, waste, hormones, and gases. One of the main functions of blood is the transport of gases, mainly O_2 and CO_2, nutrients, waste products to be detoxified, hormones, and heat to skin. Besides the leukocytes, platelets and antibodies serve as protection agents in the case of, for example, inflammation. The regulation of pH value and water balance is the third main function of the whole blood.

Serum albumins are the major plasma proteins and serve both as solubiliziers of small molecules like amino acids, fatty acids, or hormones and as a transport "vehicle" [36]. Therefore, it seems obvious that plasma is the preferred matrix to study the metabolism of polyphenols. To reduce the risk of cardiovascular or neurodegenerative diseases, polyphenols must be able to reach the target tissue via the circulation system. In this context, Andres-Lacueva and coworkers [37] showed that anthocyanins in aged blueberry-fed rats are able to cross the blood-brain barrier and metabolites are localized in various brain regions. The blood-brain barrier is a highly selective, semipermeable membrane, which separates the blood from the brain and the extracellular fluid of the central nervous system. The interaction of proteins with polyphenols is a pivotal point to study both the mode of action of the beneficial health effects of polyphenols and the analytical chemistry of sample preparation and analysis.

Proteins, and more specific albumins, globulins, and fibrinogens, are responsible for clotting, defense, and transport [36, 38]. Globulins mainly transport lipids like cholesterol either as high density lipoprotein (HDL) or low density lipoprotein (LDL). Fibrinogens play a key role in coagulation of blood and are therefore responsible for clotting processes after vascular injury. Due to the transport of electrolytes and mainly of sodium ions, plasma proteins keep up the colloid osmotic pressure [38]. Albumins are the major plasma proteins and are responsible for the transport of micronutrients. Interactions of albumin from different origins with polyphenols will be reviewed in more detail under Sect. 3.2.

Besides human plasma, urine is one of the most analyzed biological fluids in case of polyphenol bioavailability studies, because it is easy to collect and high sample amounts are available. However, the large volume causes the dilution of the analyte to be detected so that selective sample preparation is needed.

The analysis of urinary excretion of phenolic compounds is often used to quantify a high number of metabolites. This is important for epidemiological studies, where the polyphenols from urine serve as biomarkers for food intake [39, 40]. Urine is a yellow biological fluid produced by the kidneys to remove water-soluble waste products from the organism and pH value depends on factors such as diet, drugs, or urinary disorders, but normally ranges from 5.5 to 7 [38]. It contains nitrogen end products like urea, uric acid, and creatinine and other compounds with variable chemical composition. Besides hormones, fatty acids, and pigments, the inorganic ions such as the cations sodium, potassium, magnesium, ammonium as well as the anions chloride, sulfates, phosphates, and hydrogen carbonate are compounds which can affect the analysis of polyphenols and their metabolites. In contrast to blood, high amounts of proteins and glucose are not part of urine from a healthy person.

3.2 Interactions of Bovine and Human Serum Albumin with Polyphenols

The constitution of serum albumins and the ligand binding was extensively reviewed by Kragh-Hansen [41] as early as 1981. Although bovine serum albumin (BSA) and human serum albumin (HSA) differ in their amino acid sequence, the structural organization of each protein is very similar. The primary structure of BSA is a sequence of 582 amino acid residues, while HSA consists of 585 amino acid residues. Both serum albumins share a high sequence homology and differences are due to the exchange of hydrophobic amino acids by other amino acids with comparable polarities. Furthermore, BSA contains two tryptophan residues, one in position 134 in the first and another in position 212 in the second domain as well as one single and eight pairs of disulfide bonds. In contrast, HSA contains only one tryptophan residue in position 214 and 17 disulfide bonds. Tryptophan residues are located in different positions of serum albumins, Trp-212 of BSA, for example, is located in the hydrophobic binding pocket, and Trp-134 is located on the protein surface.

The three dimensional structure of the serum albumins is influenced by the presence of cysteinyl residues in the primary sequence and, as a result, by the distribution of the disulfide bridges. BSA, for example, contains three domains and can be further subdivided into two subdomains with long loop and an intradomanial hinge region. Furthermore, subdomains are composed of three different helices. A comprehensive review about the structure of BSA and HSA as well as the molecular aspects of binding is given elsewhere [41].

Serum albumins possess different regions with high or weak affinity binding sites to interact with ligands. The association constant, or binding constant, describes the binding and unbinding reaction of, for example, serum albumin and its ligand and differs due to the chemical structure of the ligand. Several researchers conducted studies to investigate the different binding regions of serum albumins and binding specificities towards drugs, nutrients, and also polyphenols [42–46].

To determine the binding affinities of ligands to serum albumins, fluorescence quenching is the technique of choice. Recently, several studies using fluorescence quenching to investigate the interactions of polyphenols with BSA or HSA were published [47–50]. The principles of quenching fluorescence are given by Lakowicz [51]. The decrease in the quantum yield of fluorescence from a fluorophore, which is induced by a variety of molecular interactions with the quencher molecule, is called fluorescence quenching. The reduction of the fluorescence intensity includes several types of reactions, but the basic principles are either that the fluorophore returns to the ground state without emission of a photon or rather the transition of a photon to the excited state is disabled. Interactions that result in quenching comprise excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching [51]. Both the ground-state complex formation and the collisional quenching require molecular contact between the fluorophore and quencher and have been studied as a source of information about the interactions of serum albumins and, for example, resveratrol, genistein, ellagitannins, and epigallocatechin-3-gallate (EGCG) [50, 52, 53].

Collisional quenching, resulting from collisional encounters between the fluorophore and the quencher, is also called dynamic quenching. The ground-state complex between serum albumin and polyphenol is the so-called static quenching. Fluorescence quenching is described by the Stern-Volmer equation,and the Stern-Volmer plot can be linear in the case of a single class of fluorophores or the plot deviate from the linearity toward the x-axis in the case if two fluorophores are present, but one is not accessible to the quencher. For a detailed explanation of the Stern-Volmer equation and plot, see [51]. Especially the latter case is important for the studies of BSA with polyphenols. BSA contains two tryptophan residues located at different regions of the molecule. The accessibility of the Trp-212 is impeded as it is located in the hydrophobic binding pocket, so probably only Trp-134, which is located on the protein surface, will be quenched.

The binding affinity between fluorophore and quencher is determined by measuring the quenching constants using the Stern-Volmer equation. Temperature and viscosity are influencing factors of fluorescence quenching [51]. Higher temperatures facilitate the amounts of collisional quenching by faster diffusion. Furthermore, the dissociation of weakly bound complexes is increased, which results in a smaller amount of static quenching. In contrast, Liu and coworkers [54] investigated the interactions of different flavonoids with BSA at 25 °C and 36 °C, showing that the quenching constants are inversely correlated with temperature, and therefore, the quenching mechanism should follow the static quenching. In addition, more factors like the concentration of the quencher and the pH value should be considered by the interpretation of the data and make the comparison of results quite challenging. Another important fact is the composition of the serum albumin preparation, as the fatty acid content may influence the binding affinity, because these compounds can bound to albumin [41].

The differences of fluorescence intensity can be explained by both dynamic and static quenching. To distinguish the two types of quenching is rather difficult but can be done by the measurement of the fluorescence lifetimes and the examination of the absorption spectra of the fluorophores [51]. During static quenching, the number of fluorophores to be activated is merely reduced. In contrast, during dynamic quenching, the lifetime of the activated fluorophore is reduced. Therefore, the ratio of the lifetimes in the absence and presence of a quencher is constant during static quenching, whereas an important characteristic of dynamic quenching is the equivalent decrease in fluorescence intensity and lifetime. As mentioned above, the tryptophan residues of BSA are located in two separate domains of the protein and both tryptophan residues are differently accessible to the quencher. Due to the selective quenching, a spectral shift of the emission spectra is expected.

The quenching of the intrinsic fluorescence of the BSA tryptophan has been used by Papadopoulou et al. [47] to study the interaction of four different flavonoids. Two flavanols, catechin and epicatechin (EC), and two flavonols, quercetin and its rutinoside rutin, were used in this study. Both types of quenching, static and dynamic, were observed at high flavonoid concentrations. Quercetin and rutin had a higher binding affinity than catechin and EC. Differences in compound structure and concentration resulted in a lower molar ratio of quercetin (10:1) to cause total

quenching than for rutin (25:1). In addition, only the binding affinity of quercetin was significantly affected by changes of the pH value from 6.8 to 7.4. It was not surprising that binding constant of catechin, $K_{sv} = 12.5 \pm 2.8 \times 10^3\ M^{-1}$ (pH 7.4), and EC, $K_{sv} = 13.8 \pm 1.9 \times 10^3\ M^{-1}$ (pH 7.4), did not differ significantly, as these compounds are enantiomers. In contrast, binding constants of quercetin and rutin at the same pH value were $530 \pm 72 \times 10^3\ M^{-1}$ and $192 \pm 6 \times 10^3\ M^{-1}$, respectively. Similar quenching constants for quercetin were determined by other researchers, e. g., Skrt et al. [48] measured $400 \pm 1 \times 10^3\ M^{-1}$ at a pH value of 7.5. Both the exposed (Trp-134) and the buried (Trp-212) tryptophan residue were quenched with similar affinities in the study of Papadopoulou et al. [47]. As no significant shift in the emission spectra of BSA occurred in the presence of the flavonoids, the authors concluded that the molecular conformation of the protein was not affected. Other researchers also used the circular dichroism experiment to proof the fact that polyphenols do not alter the secondary structure of BSA [48, 55].

The polarity of the ligand plays an important role in the interaction of BSA and polyphenols, which was shown in the study conducted by Papadopoulou et al. [47], since different binding affinities were observed for the hydrophobic aglycone quercetin and the more polar glycoside rutin, respectively. Another reason for the lower affinity of rutin may be the steric hindrance of the sugar moiety [54]. Also Liu et al. [54] confirmed that the hydrophobic interactions are the major driving force. The authors studied the structural binding reactions of methoxylated phenolics (formononetin glucoside, calycosin, and its glucoside) and hydroxylated phenolics (quercetin and its rutinoside) with BSA. The quenching constant ranked in the order quercetin > rutin > calycosin > calycosin glucoside ≈ formononetin glucoside.

Besides fluorescence quenching, also ultracentrifugation was used to determine the equilibrium association for binding of [^{14}C]-quercetin to HSA [46]. The authors concluded that the primary binding site of quercetin to HSA was the subdomain IIA.

Another study, published by Skrt et al. [48], also investigated the interaction of BSA with different flavonoids and confirmed the influence of polyphenol structure on the binding affinity towards BSA. The authors concluded that the binding affinity is decreased with glycosylation and reduced numbers of hydroxyl groups of the B-ring. In addition, Xiao et al. [56] analyzed the degree of B-ring hydroxylation on the fluorescence quenching and also verified the increased binding affinity with higher number of hydroxyl groups. Glycosylation and the degree of hydroxylation of the B-ring directly affect the polarity of the flavonoids, which should be considered in the context of sample preparation. Within the group of the flavonols, differences occur between quercetin and quercetin glycosides and also between quercetin and myricetin [47, 56]. It remains to be clarified whether the differences in polarity are sufficient to impair the selectivity of sample preparation.

Seven flavonoids – EGCG, epigallocatechin (EGC), epicatechin-3-gallate (ECG), kaempferol, kaempferol-3-glucoside, quercetin, and naringenin – as well as three hydroxycinnamic acids – rosmarinic, caffeic, and *p*-coumaric acids – were deployed in the study conducted by Skrt et al. [48]. The authors observed a bathochromic shift (shift towards higher wavelengths) and a hypochromic shift (shift towards lower wavelengths) of the emission spectra as a function of the added polyphenols to BSA,

which is indicative of changes in polarity of the surrounding tryptophan residues. For EGCG and ECG, a red shift of 23 nm was observed, and therefore interactions with Trp-212 are assumed by the authors. Due to the interactions of quercetin and kaempferol, the environment of the tryptophan residues became less polar and as a result a blue shift was observed. The authors concluded that direct and indirect interactions of the polyphenols with Trp-212 are probable, but such shifts can also be caused by, for example, solvent relaxation or by intrinsic fluorescence, as shown previously for EC [47, 51].

In contrast to Papadopoulou et al. [47], Skrt et al. [48] assumed that the main driving forces of the binding affinity were the hydrogen bonds and electrostatic interactions instead of hydrophobic actions. Furthermore, the authors studied the potential binding sites for ECG with a blind-docking experiment. The results indicated two preferred binding sites of ECG to BSA and both were closed to the position of Trp-212, which is located in the hydrophobic binding pocket of BSA.

Soares and coworkers [49] investigated the interactions of different subclasses of polyphenols with BSA. Besides catechin, EC and ECG, malvidin-3-glucoside from the subclass of the anthocyanins, tannic acid (a hydrolysable gallotannin), procyanidin B4, procyanidin B2 gallate, and procyanidin oligomers from the subclass of condensed tannins were investigated. Soares et al. [49] corroborated the results of previous studies [57] that compounds with a high number of galloyl groups have a high binding affinity towards proteins. Tannic acid is a hydrolysable tannin rich in galloyl groups. Each galloyl residue comprises of three hydroxyl groups and a benzene ring, which increases the binding affinity to BSA due to hydrogen or rather hydrophobic bonds [49]. The interaction of five ellagitannins with BSA was determined by Dobreva et al. [53], showing a dependence of binding affinity towards tannin concentration and flexibility, as well as the presence of free galloyl groups. Furthermore, Minoda et al. [58] analyzed the binding affinities of EC, EGC, ECG, and EGCG to HSA and confirmed that the galloyl moiety favored the interactions, as ECG and EGCG showed the highest binding affinities to HSA.

The interaction of EGCG with HSA under physiological conditions was investigated by Maiti et al. [50]. As shown before for BSA, EGCG is also a strong quencher of fluorescence for the tryptophan residue 214 of HSA. However, this study indicated an alteration in the secondary structure of HSA. Docking studies showed that EGCG is within hydrogen bonding distance of Trp-214 and located within the binding pocket of subdomains IIa and IIIa.

Especially for anthocyanins, pH value is a crucial factor influencing the structure of anthocyanins. At pH below 2 anthocyanins exist predominantly as the positively charged flavylium ion and the color ranked as a function of B-ring substitution from magenta and red to purple. At pH values from 3 to 6, which was used in the aforementioned study (pH values 4.0 and 5.0), the flavylium cation is hydrated to the colorless carbinol pseudobase [49, 59]. This aspect should be mentioned, when studying the interaction of anthocyanins with serum albumins, as the interaction presumably takes place between BSA and the carbinol pseudobases of malvidin-3-glucoside. Nevertheless, the study showed that malvidin-3-glucoside affected the

tryptophan residues at both the interior hydrophobic pocket and the hydrophilic surface of BSA [49]. Cahyana and Gordon [60] investigated the interactions of different anthocyanins with HSA and concluded that the pH values affect the binding affinity due to the pH-dependent structure differences of the anthocyanins and because of the change of state of HSA. At pH 4, hydrophobic interactions are the major driving force of interactions, which confirmed the results from Soares et al. [49, 60]. Although for the aglycones electrostatic interactions and hydrogen bonding (hydrophilic) play a major role at pH 7.4, and binding affinity increased with the number of hydroxyl groups. In more detail, methoxylated anthocyanins like malvidin had a less strong binding affinity than delphinidin with three hydroxyl groups in the B-ring [60].

It should be noted that the comparison of studies on the interactions of serum albumins with polyphenols is quite challenging as the fluorescence quenching and thus the use of the Stern-Volmer equation depends on various factors. Concentration of serum albumins and quencher, the pH value, the temperature, and the viscosity are important influencing factors. The structure of the phenolic compound and serum albumins may be altered due to the different experiment conditions. The different quenching constants caused, for example, by the polarity and steric hindrance should be considered during extraction of polyphenols from plasma. Differences in the strength of interaction of polyphenols with BSA or HSA may influence the selectivity, not only during transport in the human body but also during liquid/liquid or solid-phase extraction. Recovery and accuracy of sample preparation may be influenced by the different binding affinities of polyphenols to serum albumins. However, it remains unclear if the different binding affinities are high enough to cause discrimination, for example, during SPE.

3.3 Matrix Effects During MS Analysis of Polyphenols in Biological Samples

Matrix effects, in particular ion suppression, in mass spectrometry are a major concern in the trace analysis of biological samples as shown by several review articles [24, 35, 61]. The advantage of tandem MS or multiple MS is the high specificity, but ion suppression occurs in the previous stage, that is, the ionization step. Therefore, single and tandem MS are equally susceptible to ion suppression. Matrix effects can be caused by endogenous compounds from the sample itself and furthermore, by exogenous molecules from contamination during sample preparation [62]. Both types of interference influence the extent of ionization, however, as a function of ionization technique [24]. Annesley [24] stated that electrospray ionization (ESI) is more prone to ion suppression than atmospheric-pressure chemical ionization (APCI), whereas Antignac et al. [63] pointed out that both techniques are equally affected since the composition of the mobile phase is known to impact the matrix effects to a much higher extent than the ionization technique. In general, matrix compounds cause a loss of analyte response.

Protein, salts, and ion-pairing agents like trifluoroacetic acid (TFA) are not or less volatile compounds, and during ESI, the efficiency of droplet formation and evaporation is affected, but required for gas phase ions to be emitted. Furthermore, the influence of commonly used anticoagulants like EDTA and heparin on the ion suppression needs to be evaluated [24]. As a matter of fact, ion suppression can lead to falsified results, both false negative and positive results are possible. The amount of endogenous interfering molecules varies from sample to sample and results in more or less pronounced matrix effects during analysis, which makes it even more important to determine and monitor the dimension of ion suppression [64]. For this reason, the monitoring of matrix effects on, among others, ion suppression is included in the guidance for bioanalytical method validation, provided by the US Department of Health and Human Services Food and Drug Administration (FDA, Guidance for Industry Bioanalytical Method Validation).

Handling ion suppression can be quite challenging, although different strategies including sample preparation, calibration techniques, and others exist. In general, the negative ionization mode is less subjected to ion suppression as the majority of molecules prefer positive ionization. Unfortunately, polyphenols and their metabolites are very good candidates for negative ionization, with the exception of the positively charged anthocyanins [65]. Alternative ionization techniques, which are less susceptible to ion suppression, and an efficient UHPLC to remove coeluting interfering molecules, may be a good way to reduce ion suppression [35, 63]. Over time, advanced source geometries have been developed and MS suppliers promise a decrease in ion suppression using different types of interface. For polyphenol analysis from biological samples, the influence of Z-spray, orthogonal spray, or linear spray geometry on matrix effects still needs to be examined.

Another more general strategy to reduce interfering compounds is the dilution of the sample amount to be injected. However, polyphenol metabolites occur in trace amounts and therefore concentration steps rather than dilution are recommended, depending on the sensitivity of the mass analyzer. One of the most efficient strategies is sample preparation, for example, SPE or protein precipitation. Bylda et al. [62] gave an overview about novel sample preparation techniques to overcome matrix effects during LC-MS analysis. However, their applicability in polyphenol analysis needs to be evaluated.

In case that matrix effects cannot be overcome by general strategies or sample preparation, special calibration techniques, for example, using internal isotopically labeled standard substances, are required [61]. Gosetti and coworkers described further calibration techniques including the echo-peak technique, external matrix-matched calibration (e.g., polyphenol-free plasma and urine), or the well-known standard addition [61].

Studies investigating the effects of matrix compounds on the analysis of trace amounts are often focusing on compounds with toxicological or environmental relevance, like drugs or pesticides, but investigations determining the matrix effect on polyphenol metabolite analysis are still missing.

4 Analysis of Polyphenols in Biological Samples

In the past decade, a vast number of manuscripts were published dealing with the metabolism and the bioavailability of polyphenols, but only few publications addressed the challenges of method developmentin biological samples [66–68]. Different separation and detection techniques allow the sensitive and selective detection, and therefore, targeted and untargeted metabolomic studies have gained increasing attention [69, 70]. In general, methods used for the analysis of polyphenol metabolites in biological samples include a sample preparation step as well as the separation and detection to ensure highly selective, sensitive, reliable, and accurate analysis [23]. A comprehensive validation comprised of specificity, linearity, accuracy, precision (repeatability and reproducibility), range, limit of detection, and limit of quantification needs to be an integral part of polyphenol analysis (U.S. Department of Health and Human Services Food and Drug Administration, Guidance for Industry Bioanalytical Method Validation).

4.1 Sample Preparation

Sample preparation commonly includes the steps sampling and extraction of the analyte of interest. During a human intervention trial, blood sampling is conducted by a physician and urine samples are collected by the subjects themselves, both in accordance with the specifications of the ethics proposal. Whole blood is taken from an antecubital vein, directly centrifuged to obtain the plasma and stabilized with an antioxidant solution, normally containing a buffer, ascorbic acid, and a chelating agent like EDTA or heparin. The objectives of the extraction process are to release the analyte from the matrix and to concentrate it prior to further separation and detection.

Removal of interfering compounds depends on the mode of interaction and the kind of linkage between the polyphenol and the matrix molecules. Primary chemical bonds include the ionic and metallic bonding as well as covalent bonds. More interesting in the case of polyphenols, however, are the secondary types of chemical bonds comprising of dipole-dipole interactions, especially hydrogen bonds, and van der Waals forces. The binding affinities of polyphenols and the tryptophan residues of serum albumin include different driving forces. As mentioned above, hydrophobic actions and hydrogen bonds are the main driving forces, and their intensity depends, among others, on polyphenol structure. The bonding between inorganic ions, e.g., calcium ions, to be transported by serum albumin is reversible and the binding affinity to serum albumins is very high [41, 71]. It seems promising to take advantage of these interactions to facilitate the release of polyphenols from HSA and increase the extraction efficiency.

Different extraction techniques are available to remove proteins, salts, and other matrix compounds from the sample. Extraction with solvents of different polarity as well as SPE with or without prior precipitation is the predominantly used techniques.

The solvent extraction of polyphenols from biological fluids and tissues is extensively reviewed by Day and Morgan [25] which is not covered in this chapter.

The application of SPE, including pretreatments like protein precipitation, and enzymatic hydrolysis to release phase II metabolites, will be reviewed in more detail.

4.1.1 Solid-Phase Extraction (SPE)

SPE has become an indispensable part of the sample preparation procedure and represents a reliable and selective approach in trace analysis. It can be performed off-line or on-line by direct connection to further analytical systems like LC-MS. SPE is a powerful chromatographic technique, which includes the separation of the analytes from interfering matrix compounds and a concentration step. The samples can be used directly for SPE or after solvent extraction. According to their physicochemical nature, polyphenols from a plasma or urine extract retain on the solid phase and interfering matrix compounds are removed. The stationary phase is rinsed with appropriate solvents to elute the polyphenols. By selecting the sorbent type and the elution solvent as well as the solvents for washing, improved analysis for polyphenols from biological samples can be accomplished [66, 67, 72]. Furthermore, optimization of an SPE method includes the amount of the stationary phase (as a function of analyte concentration), particle size, volume, and diameter of the cartridge. A comprehensive, not compound-specific review about the SPE technique is given by Hennion [73].

Polyphenol metabolites in plasma and even more in urine are polar analytes in an aqueous matrix, and therefore, hydrophobic so-called reversed stationary phases (RP) are commonly used.Reversed phases used for polyphenol metabolite analysis are silica-based or polymeric-based stationary phases with different surface functionalization. Until today, a high number of various functionalized sorbents, developed by different suppliers, are available with relevance to biological samples, for example, hydrophilic–lipophilic polymers, mixed-mode, or molecularly imprinted polymers [73]. The sorbents for SPE should be selected due to the chemistry of both the analyte which is supposed to be retained and the interfering compound of the matrix. Day and Morgan reviewed the use of SPE for biological samples and provide a good overview about the stationary phases used [25]. Furthermore, the authors emphasized the particular problem of the determination of sulfates. As a common phase II metabolite, polyphenols conjugated to sulfate have a high polarity and it seems evident that a special extraction procedure, including solvent and solid phases, is needed [25].

Although polyphenol metabolites differ in their polarity, high-throughput SPE methods were developed to analyze the structurally diverse metabolites in one run. Gasperotti and coworkers [66] investigated the application of different sorbents for SPE for the quantification of 23 colon-derived metabolites with spiked samples of animal liver, kidney, heart, brain, blood, and urine. The authors used a silica-based C18 and two polymeric-based stationary phases, one with a hyper crosslinked hydroxylated polystyrene-divinylbenzene copolymer and another polymer that contains *N*-vinylpyrrolidone. The three stationary phases were tested using an aqueous solution with H_2SO_4 (0.01 N), which was spiked with the polyphenol mixed

standard. The silica-based C18 and the hydroxylated polystyrene-divinylbenzene copolymer showed the same affinity towards the analytes, and recoveries were in the range of 70–120%. The copolymer was used for the validation of the sample preparation and LC-MS detection of the 23 metabolites in the above-mentioned biological samples. The results illustrate the challenges associated with such kind of metabolomic studies, as only 58% of the metabolites were within the acceptable range of 70–120%. The complexity arises on the one hand from the structurally heterogeneous metabolites and on the other hand from the matrix. Urolithin B showed recoveries from 1% to 2% in heart and kidneys, while in the other matrices, recoveries from 29% to 38% were achieved, which is neither in the acceptable range.

An advancement is the use of micro-SPE, a technique which was applied for phenolic compounds in food and in biological samples [72, 74]. Recently, Feliciano et al. [72] published a method for the use of micro-SPE for the identification and quantification of 67 phenolic metabolites in human plasma and urine. The study was performed using 2 mg of a polymeric sorbent with a semi-automated device and needed only 600 μL of plasma or rather urine. Method validation was performed with spiked plasma and urine showed recovery rates in a range of 70–110%. Several metabolites from the subclasses of benzoic acids, phenylacetic acids, phenylpropionic acids, benzaldehydes, pyrogallols, hippuric acids, cinnamic acids, valerolactones, and flavonoids like quercetin and epicatechin were analyzed. As part of the validation parameters, Feliciano et al. [72] also analyzed the matrix effects and identified 56 polyphenol metabolites in plasma and 58 in urine at baseline, respectively. Besides being a new, fast, and reliable methodology, the results of this study showed the difficulties of such metabolomic approaches, because more than half of the analyzed metabolites were detected at baseline. It seems obvious that a 72 h low-polyphenol diet is insufficient to reach a baseline without any polyphenol metabolites. The baseline issue can be overcome by the use of, for example, isotopically labeled polyphenols, as the different mass-to-charge ratio (*m/z*) allows the distinction of polyphenols originating from the diet (not labeled) and those given with a bolus ingestion (labeled) [75].

Furthermore, the results of this study demonstrated the above-mentioned issue of the simultaneous analysis of sulfated metabolites beside others, as the recovery of sulfates of methylpyrogallols and dihydrocinnamic acids was less than 20% in urine. Nevertheless, methods for phenolic metabolite fingerprinting are needed for sample analysis from epidemiological cohort studies, and the repeatability of 1.7–9.2% in plasma and 2.2–10.4% in urine allows the comparison of plasma and urine samples of different individuals [72].

Although SPE combines the advantages of cleanup, trace concentration, metabolite fractionation, and the possibility of automatization, the methodology is not without challenges [73]. Frequently occurring problems are the poor batch-to-batch reproducibility or the leaching of solid-phase material that impact MS response. High concentrations of proteins may lead to plugging of the stationary phase. For this reason, a previous precipitation step may be included in sample preparation. However, it needs to be noted that due to the high molecular weight and the resulting voluminous precipitate, co-precipitation (entrapment or adsorption) of polyphenol

metabolites may cause high losses and poor recovery [76]. Protein precipitation is often used due to its simplicity, but results in analyte losses and incomplete precipitation. Plugging of the LC column and decreased ionization efficiency are the consequences. For precipitation, solvents like dimethylformamide (DMF), acetonitrile, methanol or acids like formic acid are used and should be given priority, as the commonly employed TFA negatively impacts the spray stability during ionization, reduces signal response, and causes severe memory effects in MS [24, 77].

Quercetin is extensively bound to human serum albumin and the extent of binding differs significantly between individuals [46]. Due to the right selection of a sorbent, the binding affinity of quercetin to the stationary phase may exceed the affinity to HSA, but as it was shown for EGCG, polyphenols may interact with the Trp-214 residue of HSA, which is located within the binding pocket of subdomains IIa and IIIa [50]. HSA is a high molecular weight protein and poor retention of polyphenols on the stationary phase may occur due to steric hindrance of the polyphenol-HSA complex. A new approach to reduce the interactions of polyphenols and HSA to increase the analytical recovery may include the step of protein degradation by proteases. Proteases release shorter fragments by cleaving the peptide bonds and it is a widely used technique for the identification of proteins via peptide mass fingerprinting [78]. Decreased binding affinities of polyphenols towards the shorter protein fragments and the lower steric hindrance may facilitate the extraction efficiency and result in higher recoveries. However, side activities of proteases and reactions with their cofactors need to be monitored. It is well known that L-cysteine, the cofactor of the endopeptidase papain, may interact with polyphenols under specific oxidative conditions, which would result in a loss of analyte [79].

4.1.2 Enzymatic Hydrolysis to Release Phase II Metabolites

Enzymatic hydrolysis is used to cleave the glucuronate and sulfate moieties and release the parent compounds, which were conjugated during phase II metabolism. Researchers employed this approach to facilitate metabolite analysis, because after enzymatic hydrolysis, the complex metabolite profile is broken down to the aglycones. Higher concentrations and quantification with commercially available reference compounds are the main advantages, but enzymatic hydrolysis has to be used with care [25]. Although this approach is not suitable to remove interfering matrix compounds, it is often employed prior to SPE and therefore it is part of the sample preparation.

The biological source, specific enzyme activities and side activities, as well as the purity of the enzyme preparation need to be monitored. Even if highly selective-multiple reaction monitoring (MRM) is employed during MS detection, the results will be falsified if the biological source of the enzymes is contaminated with polyphenols or substances of similar molecular weight. Nakamura and coworkers [80] observed contamination of enzymes from *Helix pomatia* and abalone entrails (*Haliotis rufescens*) with different flavanols. Catechin and EC were detected in the preparation of β-glucuronidase derived from *Helix pomatia*, and in the preparation of sulfatase derived from abalone entrails even 7 flavanols were detected. Flavanol concentrations ranged between 0.5 and 380 fmol unit^{-1} with highest levels for

catechin, EGCand EGCG. In contrast, the biological sources of the enzymes derived from *Escherichia coli* and *Aerobacter aerogenes* were free from flavonols [80]. *Helix pomatia* (Roman snail) and abalone entrails are living organisms and subsist mainly on plants, which makes a contamination with secondary plant compounds much more likely than in the case of bacteria-derived enzyme preparations.

At this point it should be mentioned that the enzymatic release of phase II metabolites comes along with the loss of biological information. The aglycones derived only from the cleaved glucuronide and sulfate metabolites, but, for example, methylated metabolites need to be detected separately. However, it was shown for quercetin that different isomers of quercetin conjugated to glucuronic acid alone or in combination with sulfate are present in human plasma and urine [13].

4.2 Synthesis of Reference Compounds

Authentic reference compounds are a prerequisite for the accurate quantification not only of polyphenols in biological samples. The FDA recommend reference standards of known identity and purity to perform the quantification during bioanalytical methods (U.S. Department of Health and Human Services Food and Drug Administration, Guidance for Industry Bioanalytical Method Validation). Ideally, an identical isotopically labeled reference standard is used. Unfortunately, phenolic phase II metabolites are neither available as certified nor as commercially-supplied substances. Custom-synthesized or isolated compounds are the only choice. Not only for quantification purposes, but also for the application in in vitro assays to investigate the mode of action and the bioactivity of polyphenols on a molecular level, reference compounds are mandatory [81].

Polyphenols occur mainly as glycosides in plants and the isolation of, for example, glucuronides is limited due to the small quantities of the raw material [4]. Nevertheless, isolation of precursor molecules is commonly the first step of compound synthesis. In general, glucuronated, sulfated, or methylated (other than the naturally occurring compounds) polyphenols can be synthesized using a chemical or enzymatic approach.

4.2.1 Chemical Synthesis

The chemical synthesis of polyphenols is not new. As early as 1987, Barron and Ibrahim [82] published an article describing the dicyclohexylcarbodiimide-mediated esterification of flavones and flavonols with tetrabutylammonium hydrogen sulfate, which was used by other authors to characterize quercetin metabolites in human plasma [83]. The sulfate was predominantly linked at positions 7, 4′, and 3 of the polyphenol and beside mono-, also di- and tri-esters could be confirmed by NMR [82]. Jones et al. [84] synthesized quercetin sulfate analogues including mono- and disulfates. In comparison the purchased sulfates, which provide a purity of over 98%, the synthesized sulfates were 90% pure as determined by HPLC. The yield of monosulfates ranked between 1% and 16% and disulfates were synthesized to a much lesser extent, namely, 0.8–3.8%.

A lot of research was conducted on the stereoselective synthesis of EGCG, one of the most investigated flavanols in tea, and is reviewed by Nagle et al. [85]. Sulfates, glucuronides, and methyl ethers of catechin were synthesized by González-Manzano et al. [86] and more than 10 polyphenols of metabolic interest were prepared. The Koenigs-Knorr reaction was used to synthesize five monoglucuronide analogues, including A- and B-ring substitution at positions 5, 7, 3 and 4′, 3′ respectively, with highest reactivity at the B-ring. In addition, monosulfates and methyl ethers of catechin were obtained.

Recently, Zhang, and coworkers [87] focused on metabolites resulting from structural modifications of the B-ring of different flavonoids during phase II metabolismas well as during colonic degradation and synthesized several compounds, among others glucuronated, methylated, and sulfated conjugates of 4-hydroxybenzoic acid, protocatechuic acid, and vanillic acid.

Especially the synthesis of anthocyanin phase II metabolites is challenging, as the stability and structure of anthocyanins depends on the pH value of the solution [10]. Cruz et al. [88] described the chemical synthesis of an in vivo occurring anthocyanin metabolite, namely, cyanidin-4′-*O*-methyl-3-glucoside. The synthesis comprises several steps beginning with the generation of the A-ring (2,4-diacetyl-6-hydroxybenzaldehyde, "Western") and the B-ring (2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-3′-benzyloxy-4′-methoxyacetophenon "Eastern"), respectively. The C-ring was obtained by an aldol-type condensation between the "Western" and the "Eastern" part. After the removal of the protection group and further purification steps via reversed phase, cyanidin-4′-*O*-methyl-3-glucoside was synthesized with a purity of 99.9% (HPLC) and confirmed by NMR. The anthocyanin was synthesized with a yield of 18%, thus provides the opportunity to enhance identification and accurate quantification of anthocyanin-derived metabolites.

4.2.2 Enzymatic Synthesis

Besides chemical synthesis, enzymatic approaches for the synthesis with higher specificity are used by some authors. Blount et al. [89] published the enzymatic synthesis of conjugated EC as well as 3′-*O*-methyl epicatechin to generate metabolites occurring in human and rat plasma, for example, epicatechin 3′-*O*-glucuronide or 3′-*O*-methyl-epicatechin 5-*O*-glucuronide. The authors tested 12 enzymes belonging to the human glucuronosyl transferases of the UGT1A and UGT2B families, a mouse liver microsomal preparation, and the human COMT. The glucuronosyl transferases have different activities towards EC and UGT1A9 produced two possible epicatechin glucuronides, epicatechin 3′-*O*-glucuronide, which was identified by NMR analysis, and epicatechin 5-*O*-glucuronide tentatively identified via LC-MS. In addition, the authors showed that the yield was influenced by various factors like temperature, pH, reaction time, and concentration of enzyme and cofactors. Using UGT1A9, 3′-*O*-methyl-epicatechin-5-*O*-glucuronide was synthesized and produced approximately 50% of substance, whereas for UGT1A8, only a yield of 15% was obtained, based on 50 mM EC [89].

In addition to the application of pure enzymes, the use of animal liver enzyme preparations is a promising approach to synthesize polyphenol metabolites. S9 and

microsome fractions from liver are employed in clinical trials to simulate the metabolism of liver derived enzymes in vitro and commercially available from mice, rats, and humans [90]. The enzymatic synthesis of reference compounds using liver preparations was shown before by Crespy and coworkers [91], who successfully used hepatic, jejunal, and ileal microsomes from rats for the glucuronidation of quercetin, ECG, and EGCG. Highest rates for glucuronidation were found in the liver for quercetin with 96.3 ± 0.1%, ECG with 12.2 ± 0.2%, and EGCG with 7.5 ± 0.2% (approximately 300 μL L^{-1} quercetin, ECG, and EGCG, respectively). The authors stated that the structural difference of the flavonoid, especially the galloyl moiety, influenced the binding affinity towards UGT, resulting in the low glucuronidation rates for ECG and EGCG.

The use of chemically or enzymatically synthesized polyphenol metabolites will facilitate the quantification, if these substances are used in bioavailability studies. In addition, new potential metabolites may be synthesized and then be identified in biological samples to shade more light on the metabolic fate of polyphenols.

4.3 Separation and Detection of Polyphenol Metabolites

In recent years, several articles were published dealing with the polyphenol metabolome [92]. Metabolomics is gaining more and more popularity, and with this approach, researchers attempted to analyze the overall systematic fingerprint of metabolites in an organism [93]. This "omics" approach is implemented as targeted and untargeted metabolomics to characterize as many polyphenol derived metabolites as possible in biological samples to identify potential biomarkers of food intake [66, 70, 94]. To identify and quantify polyphenol metabolites, efficient sample preparation and powerful separation and detection techniques are needed. Several analytical instruments are available, and besides capillary electrophoresis (CE), LC-MS is indisputably the most widespread and important method. CE is a powerful tool, for example, to separate chiral flavan-3-ols in biological samples, but coupling to MS is much more challenging due to high salt concentrations in the mobile phase [95].

4.3.1 Separation

HPLC, or more recently UHPLC, is the most widely used separation technique, and improved chromatographic conditions were shown to be very effective to reduce matrix effects and enhance metabolite analysis [61, 96]. Chromatographic efficiency in UHPLC is increased using short columns with small internal diameter and particle sizes below 2 μm, resulting in very high operating pressures. This column technology, if correctly applied, raises sensitivity and resolution, which is indispensable in high-throughput trace analysis. The stationary phases available for metabolite separation with UHPLC comprise, among others, RPs, normal phases (NP), and an alternative stationary phase based on hydrophilic interaction liquid chromatography (HILIC) [96]. In addition, chiral stationary phases are used to separate flavanol enantiomers, as shown by Ritter et al. [97]. Numerous different stationary phases

with advanced configurations and chemistry, like core shell silica particles or surface modifications, are introduced by the suppliers, promising good separation of a wide range of analytes. The selection of the stationary phase depends on the chemical nature of the metabolite of interest. For example, very polar sulfated metabolites will less interact with RP, in contrast to moderately and nonpolar analytes like polyphenol aglycones. Nevertheless, modern RPs allow the separation of a wide range of polyphenolic metabolites and are applied during most of the studies [66, 70, 72].

4.3.2 Detection

After chromatographic separation, different types of detectors are coupled to identify and quantify polyphenol metabolites in biological samples. In case of previous enzymatic release of phase II metabolites, coulometric electrode-array detection (CEAD) is used more frequently than diode array detection (DAD) [97, 98]. While for plant polyphenols the application of a DAD can be useful to differentiate between the polyphenol subclasses, in biological samples coelution of matrix compounds makes the use of UV spectra unfeasible. Moreover, it is state of the art to use MS to simultaneously identify and quantify polyphenol metabolites with enhanced sensitivity, resolution, and selectivity.

The principle of MS is comprehensively described by Gross [99]. Briefly, the basic steps of MS include the generation, separation, and detection of ions. As mentioned above, two main ionization techniques, able to operate in both negative and positive ionization mode, exist to analyze polyphenol metabolites in biological samples, namely, ESI and APCI. Nevertheless, for solid biological materials like tissues, matrix-assisted laser desorption ionization (MALDI) is widely applied for drugs [100]. Mass analyzers including triple quadrupole (QqQ), ion traps (IT), and time-of-flight (ToF) are predominantly used in polyphenol metabolite analysis [37, 72, 101]. The ion separation of these analyzers is based on different physical principles. Specifically with QqQ and IT, ions are filtered in an alternating electrical field, and with ToF analyzers, the separation of ions takes place in a field-free region after acceleration through a fixed accelerating potential [99]. Recently, Orbitrap instruments, which are based on the same separation technique like QqQs and ITs, have been employed for the metabolite analysis and provide accurate mass and the calculation of the chemical formula through multiple stage fragmentation [102].

Multiple steps of mass spectrometric selection and fragmentation are realized with the so-called tandem MS, which can be divided in the tandem-in-space MS (ToF, QqQ) and the tandem-in-time MS. Tandem-in-time MS are trapping instruments like the linear IT (LIT). LIT, or ITs in general, are powerful techniques to elucidate the tentative structure of unknown polyphenol metabolites, by the acquisition of spectra of product ions after multiple steps of analysis (MS^n). In contrast, QqQs are scanning instruments with a high potential for quantification via selected ion monitoring (SIM) or MRM mode. In the past, ToF MS analyzers were only used with MADLI interface, but due to improvements, the combination of continuous ionization sources, like ESI, is possible with orthogonal acceleration ToF analyzers. With ToFs, the measurement of accurate mass and the calculation of the derived

molecular formula are possible, and this technique is characterized by high resolution power and sensitivity [99].

A yet neglected fact is the integration of ion mobility, a technique to calculate the compound-specific collision cross-section (CCS). The determination of the CCS values may provide more confidence for the identification, as the separation of isobaric metabolites, for example, glucuronide isomers is possible. The combination of CCS value, *m/z*, fragmentation pattern and accurate mass seems to be promising to enhance metabolite analysis. Recently, Chalet et al. [103] published results for phase II metabolites of flavonoids, revealing the potential of ion mobility in combination with MS and the measurement of CCS values.

5 Conclusions

Polyphenols are secondary plant compounds ubiquitously found in plant-derived foods and are associated with several beneficial health effects [2, 5]. These phytonutrients are associated with various biological activities, for example, the antioxidative, anti-inflammatory, or antiproliferative effects may be responsible for the reduced risk to suffer from cardiovascular and neurodegenerative diseases [5]. The bioavailability and thus the ADME of polyphenols in the human body were extensively investigated with in vivo and in vitro approaches in the recent decades. Intervention studies were conducted to identify polyphenol metabolites with certain biological activity, as well as to quantify the analytes in biological samples like blood and urine, and both are subject to the limitations of analytical chemistry. Current challenges in polyphenol analytical chemistry in biological samples include the interfering matrix compounds (protein, salt), low analyte concentrations, chemical heterogeneity of the analytes, as well as the lack of reference compounds [23]. In addition, confounding reactions during cell metabolism should be monitored in the case of in vitro studies [32].

Efficient analysis of polyphenol metabolites requires in depth knowledge about the interference which can occur during extraction of the analyte from the matrix or through detection for example with MS. Therefore, the binding affinities of polyphenols were determined via fluorescence quenching, circular dichroism, or docking experiments with BSA or HSA. Flavonols, like quercetin and rutin, as well as flavanols, like catechin and ECG, are predominantly used to describe binding affinities to serum albumins. Main driving forces for the bonding are hydrophobic interactions and to some extent hydrogen bonds and electrostatic interactions [47, 48, 54]. The binding affinity depends, among others, on the polyphenol structure and is decreased by glycosylation but increased with high numbers of hydroxyl groups of the B-ring [48, 56]. Due to the different mechanism of static and dynamic quenching during fluorescence quenching, further parameters like quencher concentration, pH value, and temperature should be carefully monitored and considered during interpretation [51].

The binding affinity of polyphenol to serum albumins varies with compound polarity and therefore, the efficiency of metabolite extraction from plasma may be

affected. Techniques to remove proteins and other interfering compounds should be free of discrimination and can be handled during effective sample preparation. The deployment of different sample preparation methods, like solvent extraction, SPE, or protein precipitation, is necessary, but matrix effects should be determined and monitored. Ion suppression is a major problem during analysis of biological samples, as the method of choice for trace analysis in this area is the LC coupled with MS [35]. A number of strategies exist to handle matrix effects, but extensive sample preparation in combination with LC seems to be most promising [63]. Source geometry, ionization technique (ESI, APCI), and ionization mode (positive, negative) are limited to the instrument configuration and analyte structure, respectively. Furthermore, a number of calibration techniques, like matrix matched, echo-peak technique, or the use of an isotopically labeled internal standard, are available, but should not be used to compensate for poor sample preparation.

Solvent extraction, SPE, and protein extraction, occasionally also in combination, are the preferred techniques for sample cleanup and analyte concentration. SPE with silica-based or polymeric-based stationary phases with different surface functionalization is widely used. The simultaneous extraction of several metabolites with one stationary phase is quite challenging due to the different polarities of, for example, phase II metabolites (especially sulfates) and colonic derived phenolic acids. However, promising SPE and micro-SPE approaches were established with recoveries ranging between 70% and 120% [66, 67, 72]. In the case of pretreatment with glucuronidases and sulfatases, it is highly recommended to monitor and determine the enzyme activity and possible contamination, especially in the case of enzymes derived from *Helix pomatia* and abalone entrails [25, 80].

Syntheses of reference compounds for identification and quantification purposes were established during the last years and chemical and enzymatic approaches are described, especially for flavanols, flavonols, and anthocyanins [84, 88, 89, 91]. In combination with highly sensitive LC-MS techniques like UHPLC-ESI-TOF-MS, the use of enzymatic hydrolysis to facilitate analysis decreased in importance. The integration of ion mobility to high resolution MS allows the determination of isobaric polyphenol metabolites via the CCS values, and ion mobility is a promising technique to expanded the knowledge of polyphenol metabolism [103].

The use of polyphenol metabolites as biomarkers for food intake is quite challenging, due to analytical and physiological aspects. The accuracy of the results depends on the chemical nature of the metabolite and is directly correlated with the method used and is further complicated by the interindividual variability of polyphenol metabolism and occurring metabolites. Approaches for the simultaneous analysis of numerous polyphenol metabolites in biological samples are often required in large intervention or cohort studies. In case that the used methodology provides good repeatability of polyphenol metabolites, there is nothing wrong with it. Regrettably, the occurrence of metabolites at baseline, which makes them less suitable as biomarkers, is an often neglected fact. Nevertheless, phenolic compounds are bioactive molecules in food and therefore, an integral part of the human diet. The fate of these phytonutrients during the human body is extensively investigated by researchers of multidisciplinary nature and scientists working in the field of

analytical chemistry, nutrition and health sciences, microbiology, and epidemiology. Future studies should include in vivo, in vitro, and observational studies, as well as further investigations which address the analysis of phenolic compounds in biological samples.

References

1. Quideau S, Deffieux D, Douat-Casassus C, Pouységu L (2011) Plant polyphenols: chemical properties, biological activities, and synthesis. Angew Chem Int Ed 50:586–621. https://doi.org/10.1002/anie.201000044
2. Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56:317–333. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x
3. Watzl B, Leitzmann C (2005) Bioaktive Substanzen in Lebensmitteln, 3. unveränderte. Hippokrates Verlag, Stuttgart
4. Manach C, Scalbert A, Morand C et al (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–747
5. Hollman PCH (2014) Unravelling of the health effects of polyphenols is a complex puzzle complicated by metabolism. Arch Biochem Biophys 559:100–105. https://doi.org/10.1016/j.abb.2014.04.013
6. Zamora-Ros R, Knaze V, Rothwell JA et al (2016) Dietary polyphenol intake in Europe: the European prospective investigation into cancer and nutrition (EPIC) study. Eur J Nutr 55:1359–1375. https://doi.org/10.1007/s00394-015-0950-x
7. Cassidy A, Minihane A-M (2017) The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. Am J Clin Nutr 105:10–22. https://doi.org/10.3945/ajcn.116.136051
8. Gormaz JG, Valls N, Sotomayor C et al (2016) Potential role of polyphenols in the prevention of cardiovascular diseases: molecular bases. Curr Med Chem 23:115–128
9. Bennick A (2002) Interaction of plant polyphenols with salivary proteins. Crit Rev Oral Biol Med 13:184–196. https://doi.org/10.1177/154411130201300208
10. Fernandes I, Faria A, Calhau C et al (2014) Bioavailability of anthocyanins and derivatives. J Funct Foods 7:54–66. https://doi.org/10.1016/j.jff.2013.05.010
11. Bohn T, McDougall GJ, Alegría A et al (2015) Mind the gap-deficits in our knowledge of aspects impacting the bioavailability of phytochemicals and their metabolites-a position paper focusing on carotenoids and polyphenols. Mol Nutr Food Res 59:1307–1323. https://doi.org/10.1002/mnfr.201400745
12. Koster H, Halsema I, Scholtens E et al (1981) Dose-dependent shifts in the sulfation and glucuronidation of phenolic compounds in the rat in vivo and in isolated hepatocytes. Biochem Pharmacol 30:2569–2575. https://doi.org/10.1016/0006-2952(81)90584-0
13. Mullen W, Edwards CA, Crozier A (2006) Absorption, excretion and metabolite profiling of methyl-, glucuronyl-, glucosyl- and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions. Br J Nutr 96:107–116. https://doi.org/10.1079/BJN20061809
14. O'Leary KA, Day AJ, Needs PW et al (2003) Metabolism of quercetin-7- and quercetin-3-glucuronides by an in vitro hepatic model: the role of human beta-glucuronidase, sulfotransferase, catechol-*O*-methyltransferase and multi-resistant protein 2 (MRP2) in flavonoid metabolism. Biochem Pharmacol 65:479–491
15. Crozier A, Del Rio D, Clifford MN (2010) Bioavailability of dietary flavonoids and phenolic compounds. Mol Asp Med 31:446–467. https://doi.org/10.1016/j.mam.2010.09.007
16. Boersma MG, van der Woude H, Bogaards J et al (2002) Regioselectivity of phase II metabolism of Luteolin and quercetin by UDP-Glucuronosyl transferases. Chem Res Toxicol 15:662–670. https://doi.org/10.1021/tx0101705

17. Spencer JP, Chowrimootoo G, Choudhury R et al (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett 458:224–230. https://doi.org/10.1016/S0014-5793(99)01160-6
18. Melo-Filho CC, Braga RC, Andrade CH (2014) Advances in methods for predicting phase I metabolism of polyphenols. Curr Drug Metab 15:120–126
19. Monagas M, Urpi-Sarda M, Sánchez-Patán F et al (2010) Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. Food Funct 1:233–253. https://doi.org/10.1039/c0fo00132e
20. Tomás-Barberán FA, Selma MV, Espín JC (2016) Interactions of gut microbiota with dietary polyphenols and consequences to human health. Curr Opin Clin Nutr Metab Care 19:471–476. https://doi.org/10.1097/MCO.0000000000000314
21. Manach C, Williamson G, Morand C et al (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S–242S
22. Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies1–4. Am J Clin Nutr 81:243S–255S
23. Gleichenhagen M, Schieber A (2016) Current challenges in polyphenol analytical chemistry. Curr Opin Food Sci 7:43–49. https://doi.org/10.1016/j.cofs.2015.10.004
24. Annesley TM (2003) Ion suppression in mass spectrometry. Clin Chem 49:1041–1044. https://doi.org/10.1373/49.7.1041
25. Day AJ, Morgan MR (2003) Methods of polpyhenol extraction from biological fluids and tissues. In: Santos-Buelga C, Williamson G (eds) Methods in polyphenol analysis. Royal Society of Chemistry, Cambridge, pp 17–47
26. Gonzalez-Barrio R, Edwards CA, Crozier A (2011) Colonic catabolism of ellagitannins, ellagic acid, and raspberry anthocyanins: in vivo and in vitro studies. Drug Metab Dispos 39:1680–1688. https://doi.org/10.1124/dmd.111.039651
27. Dall'Asta M, Calani L, Tedeschi M et al (2012) Identification of microbial metabolites derived from in vitro fecal fermentation of different polyphenolic food sources. Nutrition 28:197–203. https://doi.org/10.1016/j.nut.2011.06.005
28. Savage DC (1977) Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol 31:107–133. https://doi.org/10.1146/annurev.mi.31.100177.000543
29. Qin J, Li R, Raes J et al (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65. https://doi.org/10.1038/nature08821
30. Hu M (2003) Metabolism of flavonoids via enteric recycling: mechanistic studies of disposition of Apigenin in the Caco-2 cell culture model. J Pharmacol Exp Ther 307:314–321. https://doi.org/10.1124/jpet.103.053496
31. Youdim KA, Dobbie MS, Kuhnle G et al (2003) Interaction between flavonoids and the blood-brain barrier: in vitro studies: interaction between flavonoids and the blood-brain barrier. J Neurochem 85:180–192. https://doi.org/10.1046/j.1471-4159.2003.01652.x
32. Aragonès G, Danesi F, Del Rio D, Mena P (2017) The importance of studying cell metabolism when testing the bioactivity of phenolic compounds. Trends Food Sci Technol 69:230–242. https://doi.org/10.1016/j.tifs.2017.02.001
33. Lehtonen H-M, Lindstedt A, Järvinen R et al (2013) 1H NMR-based metabolic fingerprinting of urine metabolites after consumption of lingonberries (*Vaccinium vitis-idaea*) with a high-fat meal. Food Chem 138:982–990. https://doi.org/10.1016/j.foodchem.2012.10.081
34. Felgines C, Talavéra S, Gonthier M-P et al (2003) Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates in humans. J Nutr 133:1296–1301
35. Taylor PJ (2005) Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography–electrospray–tandem mass spectrometry. Clin Biochem 38:328–334. https://doi.org/10.1016/j.clinbiochem.2004.11.007
36. Krebs HA (1950) Chemical composition of blood plasma and serum. Annu Rev Biochem 19:409–430. https://doi.org/10.1146/annurev.bi.19.070150.002205
37. Andres-Lacueva C, Shukitt-Hale B, Galli RL et al (2005) Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. Nutr Neurosci 8:111–120. https://doi.org/10.1080/10284150500078117

38. Hall JE (2016) Pocket companion to Guyton and hall textbook of medical physiology, 13th edn. Elsevier, Philadelphia
39. Ito H, Gonthier M-P, Manach C et al (2005) Polyphenol levels in human urine after intake of six different polyphenol-rich beverages. Br J Nutr 94:500–509. https://doi.org/10.1079/BJN20051522
40. Edmands WM, Ferrari P, Rothwell JA et al (2015) Polyphenol metabolome in human urine and its association with intake of polyphenol-rich foods across European countries. Am J Clin Nutr 102:905–913. https://doi.org/10.3945/ajcn.114.101881
41. Kragh-Hansen U (1981) Molecular aspects of ligand binding to serum albumin. Pharmacol Rev 33:17–53
42. Sudlow G, Birkett DJ, Wade DN (1976) Further characterization of specific drug binding sites on human serum albumin. Mol Pharmacol 12:1052–1061
43. Fehske KJ, Müller WE, Wollert U (1981) The location of drug binding sites in human serum albumin. Biochem Pharmacol 30:687–692. https://doi.org/10.1016/0006-2952(81)90151-9
44. Ghuman J, Zunszain PA, Petitpas I et al (2005) Structural basis of the drug-binding specificity of human serum albumin. J Mol Biol 353:38–52. https://doi.org/10.1016/j.jmb.2005.07.075
45. Dufour C, Dangles O (2005) Flavonoid–serum albumin complexation: determination of binding constants and binding sites by fluorescence spectroscopy. Biochim Biophys Acta 1721:164–173. https://doi.org/10.1016/j.bbagen.2004.10.013
46. Boulton DW, Walle UK, Walle T (1998) Extensive binding of the bioflavonoid quercetin to human plasma proteins. J Pharm Pharmacol 50:243–249. https://doi.org/10.1111/j.2042-7158.1998.tb06183.x
47. Papadopoulou A, Green RJ, Frazier RA (2005) Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. J Agric Food Chem 53:158–163. https://doi.org/10.1021/jf048693g
48. Skrt M, Benedik E, Podlipnik Č, Ulrih NP (2012) Interactions of different polyphenols with bovine serum albumin using fluorescence quenching and molecular docking. Food Chem 135:2418–2424. https://doi.org/10.1016/j.foodchem.2012.06.114
49. Soares S, Mateus N, de Freitas V (2007) Interaction of different polyphenols with bovine serum albumin (BSA) and human salivary α-amylase (HSA) by fluorescence quenching. J Agric Food Chem 55:6726–6735. https://doi.org/10.1021/jf070905x
50. Maiti TK, Ghosh KS, Dasgupta S (2006) Interaction of (−)-epigallocatechin-3-gallate with human serum albumin: fluorescence, fourier transform infrared, circular dichroism, and docking studies. Proteins Struct Funct Bioinf 64:355–362. https://doi.org/10.1002/prot.20995
51. Lakowicz JR (2006) Quenching of fluorescence. In: Principles of fluorescence spectroscopy. Springer, Boston, pp 277–330
52. Bourassa P, Kanakis CD, Tarantilis P et al (2010) Resveratrol, genistein, and curcumin bind bovine serum albumin. J Phys Chem B 114:3348–3354. https://doi.org/10.1021/jp9115996
53. Dobreva MA, Green RJ, Mueller-Harvey I et al (2014) Size and molecular flexibility affect the binding of ellagitannins to bovine serum albumin. J Agric Food Chem 62:9186–9194. https://doi.org/10.1021/jf502174r
54. Liu E-H, Qi L-W, Li P (2010) Structural relationship and binding mechanisms of five flavonoids with bovine serum albumin. Molecules 15:9092–9103. https://doi.org/10.3390/molecules15129092
55. Wu Y, Cheng H, Chen Y et al (2017) Formation of a multiligand complex of bovine serum albumin with retinol, resveratrol, and (−)-epigallocatechin-3-gallate for the protection of bioactive components. J Agric Food Chem 65:3019–3030. https://doi.org/10.1021/acs.jafc.7b00326
56. Xiao J, Suzuki M, Jiang X et al (2008) Influence of B-ring hydroxylation on interactions of flavonols with bovine serum albumin. J Agric Food Chem 56:2350–2356. https://doi.org/10.1021/jf7037295
57. de Freitas V, Mateus N (2001) Structural features of Procyanidin interactions with salivary proteins. J Agric Food Chem 49:940–945. https://doi.org/10.1021/jf000981z

58. Minoda K, Ichikawa T, Katsumata T et al (2010) Influence of the Galloyl moiety in tea Catechins on binding affinity for human serum albumin. J Nutr Sci Vitaminol (Tokyo) 56:331–334. https://doi.org/10.3177/jnsv.56.331
59. He J, Giusti MM (2010) Anthocyanins: natural colorants with health-promoting properties. Annu Rev Food Sci Technol 1:163–187. https://doi.org/10.1146/annurev.food.080708.100754
60. Cahyana Y, Gordon MH (2013) Interaction of anthocyanins with human serum albumin: influence of pH and chemical structure on binding. Food Chem 141:2278–2285. https://doi.org/10.1016/j.foodchem.2013.05.026
61. Gosetti F, Mazzucco E, Zampieri D, Gennaro MC (2010) Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. J Chromatogr A 1217:3929–3937. https://doi.org/10.1016/j.chroma.2009.11.060
62. Bylda C, Thiele R, Kobold U, Volmer DA (2014) Recent advances in sample preparation techniques to overcome difficulties encountered during quantitative analysis of small molecules from biofluids using LC-MS/MS. Analyst 139:2265–2276. https://doi.org/10.1039/c4an00094c
63. Antignac J-P, de Wasch K, Monteau F et al (2005) The ion suppression phenomenon in liquid chromatography–mass spectrometry and its consequences in the field of residue analysis. Anal Chim Acta 529:129–136. https://doi.org/10.1016/j.aca.2004.08.055
64. Matuszewski BK, Constanzer ML, Chavez-Eng CM (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC−MS/MS. Anal Chem 75:3019–3030. https://doi.org/10.1021/ac020361s
65. Cuyckens F, Claeys M (2004) Mass spectrometry in the structural analysis of flavonoids. J Mass Spectrom 39:1–15. https://doi.org/10.1002/jms.585
66. Gasperotti M, Masuero D, Guella G et al (2014) Development of a targeted method for twenty-three metabolites related to polyphenol gut microbial metabolism in biological samples, using SPE and UHPLC–ESI-MS/MS. Talanta 128:221–230. https://doi.org/10.1016/j.talanta.2014.04.058
67. Mülek M, Högger P (2015) Highly sensitive analysis of polyphenols and their metabolites in human blood cells using dispersive SPE extraction and LC-MS/MS. Anal Bioanal Chem 407:1885–1899. https://doi.org/10.1007/s00216-014-8451-y
68. de Ferrars RM, Czank C, Saha S et al (2014) Methods for isolating, identifying, and quantifying anthocyanin metabolites in clinical samples. Anal Chem 86:10052–10058. https://doi.org/10.1021/ac500565a
69. Bagchi D, Swaroop A, Bagchi M (2015) Genomics, proteomics and metabolomics in nutraceuticals and functional foods, 2nd edn. Wiley, Chichester/Hoboken
70. Urpi-Sarda M, Boto-Ordóñez M, Queipo-Ortuño MI et al (2015) Phenolic and microbial-targeted metabolomics to discovering and evaluating wine intake biomarkers in human urine and plasma: general. Electrophoresis 36:2259–2268. https://doi.org/10.1002/elps.201400506
71. Katz S, Klotz IM (1953) Interactions of calcium with serum albumin. Arch Biochem Biophys 44:351–361. https://doi.org/10.1016/0003-9861(53)90054-X
72. Feliciano RP, Mecha E, Bronze MR, Rodriguez-Mateos A (2016) Development and validation of a high-throughput micro solid-phase extraction method coupled with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry for rapid identification and quantification of phenolic metabolites in human plasma and urine. J Chromatogr A 1464:21–31. https://doi.org/10.1016/j.chroma.2016.08.027
73. Hennion M-C (1999) Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography. J Chromatogr A 856:3–54. https://doi.org/10.1016/S0021-9673(99)00832-8
74. Suárez M, Romero M-P, Macià A et al (2009) Improved method for identifying and quantifying olive oil phenolic compounds and their metabolites in human plasma by microelution solid-phase extraction plate and liquid chromatography–tandem mass spectrometry. J Chromatogr B 877:4097–4106. https://doi.org/10.1016/j.jchromb.2009.10.025
75. Gleichenhagen M, Zimmermann BF, Herzig B et al (2013) Intrinsic isotopic 13C labelling of polyphenols. Food Chem 141:2582–2590. https://doi.org/10.1016/j.foodchem.2013.05.070

76. Souverain S, Rudaz S, Veuthey J-L (2004) Protein precipitation for the analysis of a drug cocktail in plasma by LC–ESI–MS. J Pharm Biomed Anal 35:913–920. https://doi.org/10.1016/j.jpba.2004.03.005
77. Kuhlmann FE, Apffel A, Fischer SM et al (1995) Signal enhancement for gradient reverse-phase high-performance liquid chromatography-electrospray ionization mass spectrometry analysis with trifluoroacetic and other strong acid modifiers by postcolumn addition of propionic acid and isopropanol. J Am Soc Mass Spectrom 6:1221–1225. https://doi.org/10.1016/1044-0305(95)00571-4
78. Cottrell JS (1994) Protein identification by peptide mass fingerprinting. Pept Res 7:115–124
79. Makris DP, Rossiter JT (2002) Effect of natural antioxidants on heat-induced, copper(II)-catalysed, oxidative degradation of quercetin and rutin (quercetin 3-*O*-rutinoside) in aqueous model systems. J Sci Food Agric 82:1147–1153. https://doi.org/10.1002/jsfa.1159
80. Nakamura T, Tanaka R, Ashida H (2011) Possible evidence of contamination by Catechins in deconjugation enzymes from *Helix pomatia* and *Abalone entrails*. Biosci Biotechnol Biochem 75:1506–1510. https://doi.org/10.1271/bbb.110210
81. Kroon PA, Clifford MN, Crozier A et al (2004) How should we assess the effects of exposure to dietary polyphenols in vitro? Am J Clin Nutr 80:15–21
82. Barron D, Ibrahim RK (1987) Synthesis of flavonoid sulfates: 1. Stepwise sulfation of positions 3, 7, and 4 using *N*,*N*′-dicyclohexylcarbodiimide and tetrabutylammonium hydrogen sulfate. Tetrahedron 43:5197–5202. https://doi.org/10.1016/S0040-4020(01)87695-X
83. Day AJ, Mellon F, Barron D et al (2001) Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. Free Radic Res 35:941–952. https://doi.org/10.1080/10715760100301441
84. Jones DJL, Jukes-Jones R, Verschoyle RD et al (2005) A synthetic approach to the generation of quercetin sulfates and the detection of quercetin 3′-*O*-sulfate as a urinary metabolite in the rat. Bioorg Med Chem 13:6727–6731. https://doi.org/10.1016/j.bmc.2005.07.021
85. Nagle DG, Ferreira D, Zhou Y-D (2006) Epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives. Phytochemistry 67:1849–1855. https://doi.org/10.1016/j.phytochem.2006.06.020
86. González-Manzano S, González-Paramás A, Santos-Buelga C, Dueñas M (2009) Preparation and characterization of Catechin sulfates, glucuronides, and methylethers with metabolic interest. J Agric Food Chem 57:1231–1238. https://doi.org/10.1021/jf803140h
87. Zhang Q, Raheem KS, Botting NP et al (2012) Flavonoid metabolism: the synthesis of phenolic glucuronides and sulfates as candidate metabolites for bioactivity studies of dietary flavonoids. Tetrahedron 68:4194–4201. https://doi.org/10.1016/j.tet.2012.03.100
88. Cruz L, Mateus N, de Freitas V (2013) First chemical synthesis report of an anthocyanin metabolite with in vivo occurrence: cyanidin-4′-*O*-methyl-3-glucoside. Tetrahedron Lett 54:2865–2869. https://doi.org/10.1016/j.tetlet.2013.03.100
89. Blount JW, Ferruzzi M, Raftery D et al (2012) Enzymatic synthesis of substituted epicatechins for bioactivity studies in neurological disorders. Biochem Biophys Res Commun 417:457–461. https://doi.org/10.1016/j.bbrc.2011.11.139
90. Wrighton SA, Vandenbranden M, Stevens JC et al (1993) In vitro methods for assessing human hepatic drug metabolism: their use in drug development. Drug Metab Rev 25:453–484. https://doi.org/10.3109/03602539308993982
91. Crespy V, Nancoz N, Oliveira M et al (2004) Glucuronidation of the green tea catechins, (−)-epigallocatechin-3-gallate and (−)-epicatechin-3-gallate, by rat hepatic and intestinal microsomes. Free Radic Res 38:1025–1031. https://doi.org/10.1080/10715760410001728424
92. Rothwell JA, Urpi-Sarda M, Boto-Ordoñez M et al (2016) Systematic analysis of the polyphenol metabolome using the phenol-explorer database. Mol Nutr Food Res 60:203–211. https://doi.org/10.1002/mnfr.201500435
93. Kim S, Kim J, Yun EJ, Kim KH (2016) Food metabolomics: from farm to human. Curr Opin Biotechnol 37:16–23. https://doi.org/10.1016/j.copbio.2015.09.004
94. Mülek M, Fekete A, Wiest J et al (2015) Profiling a gut microbiota-generated catechin metabolite's fate in human blood cells using a metabolomic approach. J Pharm Biomed Anal 114:71–81. https://doi.org/10.1016/j.jpba.2015.04.042

95. Ramautar R, Somsen GW, de Jong GJ (2015) CE-MS for metabolomics: developments and applications in the period 2012-2014: CE and CEC. Electrophoresis 36:212–224. https://doi.org/10.1002/elps.201400388
96. Gika HG, Theodoridis GA, Plumb RS, Wilson ID (2014) Current practice of liquid chromatography–mass spectrometry in metabolomics and metabonomics. J Pharm Biomed Anal 87:12–25. https://doi.org/10.1016/j.jpba.2013.06.032
97. Ritter C, Zimmermann BF, Galensa R (2010) Chiral separation of (+)/(−)-catechin from sulfated and glucuronidated metabolites in human plasma after cocoa consumption. Anal Bioanal Chem 397:723–730. https://doi.org/10.1007/s00216-010-3542-x
98. Egert S, Tereszczuk J, Wein S et al (2013) Simultaneous ingestion of dietary proteins reduces the bioavailability of galloylated catechins from green tea in humans. Eur J Nutr 52:281–288. https://doi.org/10.1007/s00394-012-0330-8
99. Gross JH (2013) Massenspektrometrie: ein Lehrbuch. Springer Spektrum, Berlin/Heidelberg
100. Turker SD, Dunn WB, Wilkie J (2017) MALDI-MS of drugs: profiling, imaging, and steps towards quantitative analysis. Appl Spectrosc Rev 52:73–99. https://doi.org/10.1080/05704928.2016.1207659
101. Orrego-Lagarón N, Vallverdú-Queralt A, Martínez-Huélamo M et al (2016) Metabolic profile of naringenin in the stomach and colon using liquid chromatography/electrospray ionization linear ion trap quadrupole-Orbitrap-mass spectrometry (LC-ESI-LTQ-Orbitrap-MS) and LC-ESI-MS/MS. J Pharm Biomed Anal 120:38–45. https://doi.org/10.1016/j.jpba.2015.10.040
102. Sasot G, Martínez-Huélamo M, Vallverdú-Queralt A et al (2017) Identification of phenolic metabolites in human urine after the intake of a functional food made from grape extract by a high resolution LTQ-Orbitrap-MS approach. Food Res Int 100:435–444. https://doi.org/10.1016/j.foodres.2017.01.020
103. Chalet C, Hollebrands B, Janssen H-G et al (2018) Identification of phase-II metabolites of flavonoids by liquid chromatography–ion-mobility spectrometry–mass spectrometry. Anal Bioanal Chem 410:471–482. https://doi.org/10.1007/s00216-017-0737-4

Rheology of Food Gum

67

Seyed Mohammad Ali Razavi and Mahdi Irani

Contents

S. M. A. Razavi (✉)
Food Hydrocolloids Research Centre, Department of Food Science and Technology, Ferdowsi University of Mashhad (FUM), Mashhad, Iran
e-mail: s.razavi@um.ac.ir; sma_razavi@yahoo.com

M. Irani
Gum Group Knowledge Base Company, Technology Development Center, Ferdowsi University of Mashhad, Mashhad, Iran
e-mail: ask4irani@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_20

Abstract

This introductory chapter provides an overview of three main rheological properties of food gums including dilute solution, steady shear, and viscoelastic rheological properties. Examples of a number of different food gums are given, and a brief introduction to the rheological properties of them at three mentioned rheological aspects is provided. Different circumstances such as temperature, concentration, presence of ions and sugars, which affect the rheological behavior of food gums, are summarized. Dilute regime properties determine the molecular role of gums in food products. Steady shear rheological properties of food gum manifest useful data to evaluate the flow behavior, quality control, and processing/storage stability of food products consisting food gums. Dynamic rheological characteristics of food gums illustrate the viscoelasticity, stability, and textural features of final formulated foods. To sum up, the results showed that various food gums, with variety of structure and nature at different situations, exhibit different rheological behavior.

Keywords

Biopolymer · Dilute regime · Concentration dependency · Steady shear · Temperature dependency · Time dependency · Viscoelasticity

List of Abbreviations

[η]	Intrinsic viscosity
BSG	Basil seed gum
CSG	Cress seed gum
GG	Guar gum
LBG	Locust bean gum
LPSG	*Lepidium perfoliatum* seed gum
LVE	Linear viscoelastic region
M_w	Molecular weight
SSG	Sage seed gum
XG	Xanthan gum

1 Introduction

The word gum is widely used to depict a range of polysaccharides, which have the ability of causing a large increase in a solution viscosity, even at small concentration. In food industry, commercial gums are usually utilized as thickener, stabilizer, fat-replacer, and gelling agents. Most of them are water-soluble macromolecules and then are used in the water-continuous systems. In some sectors of the food industry like dairy, having products with a special texture and mouthfeel is crucial, in which gums play an important role to provide those characters. Generally, gums are used in relatively small portion to expose proper rheological or textural properties of food products.

In many studies, the effect of food elements (certain sugars and ions) or systems (temperature and pH) on rheological performance of gums were determined. In particular, measuring rheological responses of food gums at macroscopic level are related to the changes and properties of them at microscopic level [1]. Thus, rheological properties of food gum dispersions can provide useful information about their structures and behaviors at different conditions used in food processing [2]. This chapter describes some rheological properties of a couple of food gums including dilute solution, steady shear, and dynamic rheological properties.

2 Dilute Solution Properties

2.1 Introduction

In dilute solution, each macromolecules' coils are separated from each other and freely moved. In this situation, measurement of the viscosity can provide some extremely useful information about molecular properties, biopolymer behavior, and its interaction with solvents or copolymers. The intrinsic viscosity demonstrates the hydrodynamic volume occupied by each unit of macromolecule which is related to the molecular size, conformation, molecular weight, and solvent quality [3]. Hence, intrinsic viscosity determination can supply deep understanding of molecular properties of macromolecules in solution [4]. Intrinsic viscosity, [η], is defined as the ratio of the specific viscosity (η_{sp}) or natural logarithm of relative viscosity (η_{sp}) to the mass concentration of the polymer in the limit of "infinite dilution" or zero concentration:

$$[\eta] = \lim_{c\to\infty}\left(\frac{\eta_{sp}}{c}\right) = \lim_{c\to\infty}\left(\frac{ln\eta_{rel}}{c}\right) \tag{1}$$

where, $\eta_{rel} = \eta/\eta_s$ and $\eta_{sp} = (\eta - \eta_s)/\eta_s$. The parameters of η_s and η are the solvent viscosity and solution viscosity, respectively. There are different equations to determine [η], including intercept-based models (Huggins and Kraemer models) and slope-based models (Tanglertpaibul-Rao and Higiro models) which are represented as Eqs. 2, 3, 4, 5, and 6:

Huggins' equation [5]:

$$\eta_{sp}/C = [\eta] + K_H[\eta]^2 C \tag{2}$$

Kraemer's equation [6]:

$$ln\eta_{rel}/C = [\eta] + K_K[\eta]^2 C \tag{3}$$

Tanglertpaibul-Rao's equation [7]:

$$\eta_{rel} = 1 + [\eta]C \tag{4}$$

Higiro's equations [8]:

$$\eta_{rel} = e^{[\eta]C} \tag{5}$$

$$\eta_{rel} = 1/(1 - [\eta]C) \tag{6}$$

where, K_H, K_K, and C are Huggins constant, Kraemer constant, and solute concentration, respectively.

2.2 Intrinsic Viscosity

The intrinsic viscosity and molecular weight values of some commercial and novel food gums are shown in Table 1. It is noticeable that differences of intrinsic viscosity magnitudes are dependent on structural features, like different degree of substitution and molecular mass [9]. As seen in Table 1, xanthan gum represents the highest [η] among reported gums (155.7 dl.g^{-1}), which is related to the higher molecular weight (M_w) and stronger chain stiffness of it [10]. In contrast, starch samples have high M_w (24–360 × 10^6 Da) and then should be resulted in higher [η], but they have lowest amount of [η] between selected gums (Table 1). The low intrinsic viscosity of starches rather than other gums such as xanthan is related to high portion of amylopectin with low volume in starches. Actually, amylopectin is a highly branched polysaccharide with high M_w and low molecular extension, then in dilute regime, the volume of amylopectin molecules is less than linear polysaccharide with the same Mw [11].

The [η] of biopolymers can be affected by different situations, like changing temperature and pH, presence of salts and sugars, etc. As seen in Table 1, with increasing temperature, the intrinsic viscosity of some gums increase, some decrease, and some of them roughly change. For example, [η] of xanthan gum increase from 155 to 173 (dl.g^{-1}) by increasing temperature from 25 °C to 80 °C (Table 1) which is related to increased chain dimension of it at higher temperatures [10]. On the other hand, the intrinsic viscosity of some gums like Balangu seed gum, sage seed gum, and basil seed gum decline with increasing temperature (Table 1). For instance, [η] of Balangu seed gum goes down from 72 to 50 (dl.g^{-1}) by changing temperature from 20 °C to 50 °C, which is related to increasing kinetic energy, phase separation, decreasing solvent quality, and debranching of the gum [3].

Both increasing and decreasing of [η] have reported for food gums in the presence of sugars. As shown in Table 1, [η] of cress seed gum increased from 2.4 to 2.8 and 2.9 (dl.g^{-1}) with adding lactose (up to 15% w/v) and sucrose (up to 40% w/v). Decreasing solvent dielectric constant, sugar dehydration, and aggregation formation of gum molecules were reported to explain this phenomena [19]. In contrast, the intrinsic viscosity of Balangu seed and basil seed gums decreased from 72.4 and 11.4 (dl.g^{-1}) to 12 and 8.8 (dl.g^{-1}), respectively, with adding 15% lactose to the solution (Table 1). To elucidate this trend, the literatures used the explanation: the competition between sugar and gum molecules for water and likely less free water molecules

Table 1 Dilute solution properties of some food gums

Gum	Solution	M_w (Da)	[η] (dl/g)	K_H	Used model	Reference
Guar gum	Distilled water (25 °C)	1.45×10^6	9.25	–	Huggins and Kraemer	[10, 12–14]
	Distilled water (25 °C)		12.0	–		
	Distilled water (80 °C)		11.5	–		
	Distilled water (25 °C)		11.0	0.215		
Locust bean gum	Distilled water (25 °C)	8.12×10^5	13.8		Huggins and Kraemer	[12, 15]
	Distilled water (25 °C)		12.49	0.008		
	NaCl (50 mM, 25 °C)		10.75	0.031		
	KCl (50 mM, 25 °C)		11.25	0.059		
	$CaCl_2$ (50 mM, 25 °C)		13.21	0.029		
Xanthan	Distilled water (25 °C)	4.05×10^6	155.7	–	Huggins and Kraemer	[10, 15, 16]
	Distilled water (80 °C)		173.0	–		
	Distilled water (25 °C)		214.2	0.001		
	NaCl (50 mM, 25 °C)		68.72	0.016		
	KCl (50 mM, 25 °C)		73.82	0.009		
	$CaCl_2$ (50 mM, 25 °C)		59.77	0.011		
Balangu seed gum	Distilled water (20 °C)	3.6×10^6	72.36	0.333	Huggins and Kraemer	[17]
	Distilled water (50 °C)		50.04	0.077		
	NaCl (0.5 M, 20 °C)		7.15	1.412		
	$CaCl_2$ (0.5 M, 20 °C)		3.94	1.045		
	Lactose (15% w/v, 20 °C)		11.99	0.533		

(*continued*)

Table 1 (continued)

Gum	Solution	M_w (Da)	[η] (dl/g)	K_H	Used model	Reference
Sage seed gum	Distilled water (25 °C)	1.5×10^6	10.11	–	Higiro 1	[18]
	Distilled water (65 °C)		2.96	–		
	NaCl (200 mM, 25 °C)		3.87	–		
	$CaCl_2$ (200 mM, 25 °C)		2.48	–		
	$MgCl_2$ (200 mM, 25 °C)		2.09	–		
	KCl (200 mM, 25 °C)		4.86	–		
Cress seed gum	Distilled water (25 °C)	5.4×10^5	2.42	–	Higiro 1	[19, 20]
	NaCl (100 mM, 25 °C)		1.88	–		
	$CaCl_2$ (15 mM, 25 °C)		1.54	–		
	Lactose (15% w/v, 25 °C)		2.83	–		
	Sucrose (40% w/v, 25 °C)		2.89	–		
Basil seed gum	Distilled water (25 °C)	1.73×10^6	11.38	–	Higiro 2	[21]
	Distilled water (65 °C)		8.76	–		
	Lactose (15% w/v, 25 °C)		8.74	–		
	Sucrose (40% w/v, 25 °C)		6.79	–		
Waxy corn starch	90%DMSO-10%water (25 °C)	$37–360 \times 10^6$	1.26	1.22	Huggins and Kraemer	[11, 22]
Rice starch	90%DMSO-10%water (25 °C)	$59–88 \times 10^6$	1.04	1.89	Huggins and Kraemer	[23, 24]
Canary seed starch	90%DMSO-10%water (30 °C)	24.6×10^6	1.17	1.32	Higiro 1	[25]

to interact with gum molecules causing more intermolecular association in gum molecules and result in less intrinsic viscosity of them [3, 19, 21].

With a glance to the Table 1, it is easy to notice that by adding salts to the solution (regardless of the ion type), the intrinsic viscosity of all gums decreased except locust bean gum. For instance, [η] of xanthan gum decreased from 214 to 68, 74, and 60 ($dl.g^{-1}$) by adding NaCl, KCl, and $CaCl_2$ (at 50 mM), respectively. This behavior has been regarded to the shielding effect of charges (Na^+ & Ca^{2+}) on macromolecular chains. In other words, with adding salts to the solution and the effect of shielding of charges, the molecular repulsion is going to be reduced and cause more aggregation between molecular gums and finally result in reducing intrinsic viscosity [3, 26]. In contrast, unchanging or low changing intrinsic viscosity of locust bean gum in presence of salts is related to non-polyelectrolyte nature of this gum, whose salts have little effect on the hydrodynamic volume [11]. Again, we should repeat this sentence according to the information provided above that intrinsic viscosity supplies deep understanding of molecular properties of macromolecules in solution and consequently the role of gums in final food products in real systems.

2.3 Huggins' Constant

The Huggins' constant (K_H) is a key parameter of Huggins equation, which differs from solvent to solvent for a special polymer [11]. The values of K_H can be affected by molecular architecture and extent of coil expansion of the polymer coil [12, 27]. K_H generally is the range of 0.3–1, which at good solvent it would be from 0.3 to 0.4 for flexible macromolecules with extended shape [4, 12, 28]. However, the K_H more than 1 would endorse the aggregate formation of the polymer [29]. The K_H value of some food gums is shown in Table 1. Like intrinsic viscosity, the K_H can be impacted by different circumstances such as different temperatures, presence of salts and sugars and various solvents. As seen in Table 1, with increasing temperature from 25 °C to 50 °C, the K_H of Balangu seed gum decreases from 0.333 (value in good solvent condition) to 0.077, which shows temperature makes the solvent quality worse and molecular association happen [30]. A decline in space prohibition among adjacent molecules via debranching that allows them to associate easier than branched form was stated to elucidate this behavior [3]. The presence of sugar, as seen in Table 1, changes the K_H from 0.33, related to the good solvent, to 0.53. It was stated that an increase in the interaction between Balangu seed gum molecules may be the cause, and they have less tendency to interact with solvent [3]. The influence of increasing salts (ionic strength), in contrast to the temperature, increased the K_H of LBG, xanthan, and Balangu seed gum (Table 1). For LBG and xanthan gum, a reduced tendency to aggregate was suggested [15]. However, as the K_H approached to unity by increasing ionic strength, the aggregation form was proposed for Balangu seed gum (Table 1). As shown in Table 1, all selected starches have K_H values more than unity, indicating the presence of aggregates. The compact structures for the branched molecules of starches that

major fraction of them is amylopectin was illustrated to clarify this behavior [25]. Also, it was mentioned that amylopectin would behave in 90% DMSO-10% water like an aggregate of linear polymer [11].

3 Steady Shear Rheological Properties

3.1 Introduction

Determination of steady shear rheological properties of food gums is needed to design the fluid transportation and pumping calculations, quality control, and processing/storage stability evaluation of food products. Thickening and stabilizing roles of gums are depended on shear rate, time of shearing, concentration, and temperature. Generally, to describe or predict the rheological behavior of gums, numerous mathematical models can be used. For example in shear rate dependency, the power-law, Herschel–Bulkley, Bingham plastic, Casson, and Mizrahi–Berk models, and for time dependency, the Weltman model, first-order stress decay models, and structural kinetic models are applied. To elucidate concentration dependency, the concentration-sensitive models like linear, power-low, exponential, and polynomial models were encountered. Also, to depict the temperature dependency, the Arrhenius-type model is usually applied.

3.2 Shear Rate Dependency

Most of hydrocolloids are non-Newtonian materials which usually show shear-thinning behavior. Pseudoplastic is the popular name of shear-thinning fluids; however, the expression of shear-thinning is preferred. During shearing, the hydrodynamic forces generate and the structural units of gums start to breakdown which assume as shear-thinning behavior [9].

Figure 1 demonstrates the shear-thinning behavior of some selected hydrocolloids. Marcotte et al. [31] reported the same trend, e.g., shear-thinning behavior, at other concentrations and temperatures. They used the power-law model (Eq. 7) and Herschel-Bulkley model (Eq. 8) to describe the shear-thinning behavior of starch and pectin, and carrageenan and xanthan, respectively.

$$\tau = \tau_{0H} + k_H \dot{\gamma}^{n_H} \tag{7}$$

where τ_{0H} is the yield stress (Pa), k_H is the consistency coefficient (Pa s^n), and n_H is flow behavior index for Herschel-Bulkley model.

$$\tau = k_P \dot{\gamma}^{n_P} \tag{8}$$

where k_p is the power-law consistency coefficient (Pa s^n) and n_p is the power-law flow behavior index.

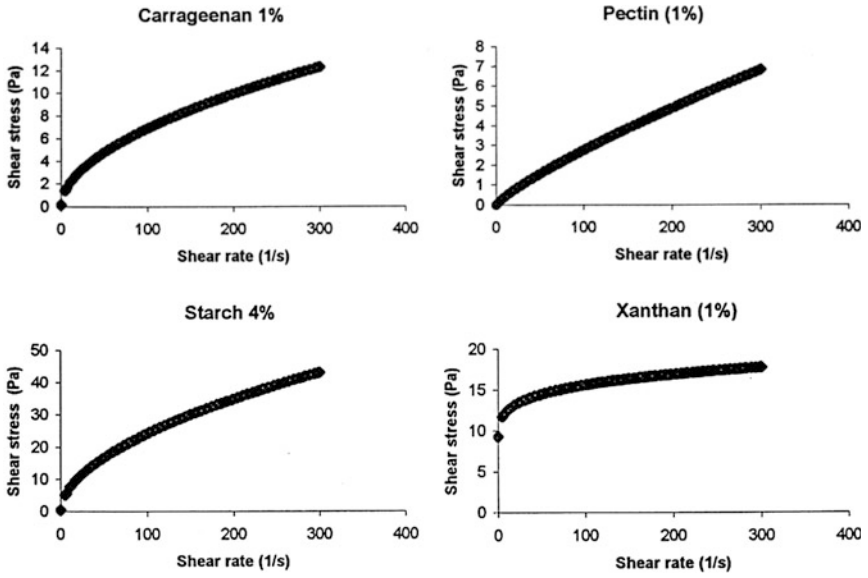


Fig. 1 Typical flow curves for some selected hydrocolloids at 20 °C [34]

Table 2 The steady shear rheological properties of some gums represented by the power-law model

Gum	k_p (Pa.s^n)	n_p	γ (s^{-1})	Reference
Xanthan (1% w/w)	14.43	0.22	0.01–300	[33]
Guar (1% w/w)	4.13	0.76	0.01–316	[34]
Sage seed (1% w/w)	8.72	0.28	0.01–300	[33]
Balangu seed (1% w/w)	0.22	0.42	6.12–245	[35]
Cress seed (1% w/w)	0.65	0.44	10–500	[36]
Sweet potato starch (gel 5% w/w)	20.4	0.38	0.4–1000	[37]
Canary seed starch (gel 6% w/w)	22.5	0.30	0.1–400	[38]

k_p, the consistency coefficient; n_p, flow behavior index

Among selected hydrocolloids, xanthan and guar gum showed yield stress along with shear-thinning behavior. The yield stress of selected gums increased with increasing concentration and decreasing temperature [31]. One of the useful properties of food gums is yield stress which can help to keep ingredients of food formulation in place. For instance, high yield stress of food gums is useful as stabilizer in products like mayonnaise and salad dressings [32].

The power-law model parameters of some hydrocolloids, one of the common models which is used to describe rheological properties of food gums, are shown in Table 2. All the selected hydrocolloids showed n_p value less than 1. Comparatively,

the magnitude of flow behavior index of xanthan (0.22) and sage seed gum (0.24) were the lowest and guar gum (0.76) was the highest. Starches, cress seed, and Balangu seed gum had intermediate values (0.30–0.44). Food gums with high pseudoplasticity expose the slimy feeling in the mouth, then for food products with high viscosity and good mouth feeling, the food gums with lower n_p values are the option.

3.3 Concentration Dependency

It was reported that the values and changes of flow behavior index (n_p) with concentration are efficiently dependent on the molecular size [39, 40]. Table 3 illustrates the model parameters *a* and *b* for power-law (Eq. 9), exponential (Eq. 10), and polynomial (Eq. 11) models of some selected hydrocolloids at different temperatures.

$$\eta = aC^b \tag{9}$$

$$\eta = a \exp(bC) \tag{10}$$

$$\eta = 1 + aC + bC^2 \tag{11}$$

where, *a* and *b* are models' constants and *C* is the concentration of gum.

As seen in Table 3, based on R^2 (coefficient of determination) values (0.78–1.00), all the three models properly described concentration dependency of selected hydrocolloids. Although in some cases just one of the models was selected to describe concentration dependency, for example, for basil seed gum [41] and sage seed gum [42], the power and exponential models were chosen. Marcotte et al. [31] related to power-law model, reported the decrease of *a* value with increasing temperature for carrageenan, pectin, starch, and xanthan gums (Table 3). Also, they informed the decrease of *b* value for carrageenan and pectin, but for xanthan and starch, the related changes were very small. Moreover, they depicted that polynomial model described concentration dependency of selected gums well. For all selected gums, except xanthan, parameters *a* and *b* decreased at higher temperatures. Marcotte et al. [31] concluded that there is a strong interaction between concentration and temperature for xanthan gum.

The increase of apparent viscosity with increasing concentration was reported for carrageenan, pectin, starch, xanthan gums [31], basil seed gum [41], and sage seed gum [42]. Then, food gums have high potential of being a good thickener or stabilizer in increasing the consistency of food systems as it has been reported that higher viscosity of food products implied a better mouth feel [43].

3.4 Temperature Dependency

Commonly, the consistency coefficient (k) of food gums decreased with increasing temperature like carrageenan, pectin, xanthan gums [31], basil seed gum [41], sage

Table 3 Concentration dependence determined by Eqs. 9, 10, and 11 for apparent viscosity of selected hydrocolloids at different temperatures [34]

		Power			Exponential			Polynomial	
Gum	a	b	R^2	a	b	R^2	a	b	R^2
Carrageenan									
20 °C	0.668	4.4045	0.89	0.0947	2.292	0.78	6.8	3.09	0.8
40 °C	0.066	3.251	0.89	0.0108	1.877	0.97	−2.262	1.037	0.97
60 °C	0.0397	1.88825	0.93	0.0139	1.076	0.98	−0.039	0.054	0.97
80 °C	0.0229	1.7866	0.99	0.0091	0.995	0.99	−0.0006	0.019	0.99
Pectin									
20 °C	0.0476	2.733	0.99	0.019	1.156	0.98	−1.38	0.4421	1
40 °C	0.0247	2.652	0.99	0.0106	1.081	0.98	−0.953	0.231	0.95
60 °C	0.0148	2.519	0.98	0.0063	1.047	0.99	−0.895	0.185	0.84
Starch									
20 °C	0.00075	5.546	0.99	0.01855	1.126	0.99	−4.824	1.192	0.97
40 °C	0.0004	5.683	0.99	0.00985	1.155	0.99	−3.171	0.754	0.97
60 °C	0.0002	5.718	0.99	0.00545	1.161	0.99	−2.023	0.459	0.98
80 °C	0.0002	5.351	1	0.00495	1.085	0.99	−1.273	0.271	0.98
Xanthan									
20 °C	5.612	1.332	0.92	2.695	0.765	0.98	0.196	2.908	0.97
40 °C	4.826	1.352	0.97	2.348	0.765	0.99	0.889	2.179	0.99
60 °C	4.254	1.448	0.96	1.968	0.818	0.99	0.031	2.435	0.99
80 °C	3.582	1.695	1	1.541	0.929	0.97	0.749	2.219	1

seed gum [42], and canary seed starch [38]. However, the story for flow behavior index (n) is different. For example, n value increased with temperature increase for xanthan, carrageenan, pectin, and starch [31], but Hosseini-Paravar et al. [41], Razavi et al. [42], Irani et al. [38], Moreira et al. [44], and Albano et al. [45] reported unclear trend of it for basil seed gum, sage seed gum, canary seed starch, chestnut starch, and Peruvian carrot starch, respectively.

Table 4 shows the parameters of Arrhenius-type model (Eq. 12) determined for some selected food gums.

$$\eta = A \exp\left(E_a/RT_a\right) \tag{12}$$

where A is the proportionality constant (or apparent viscosity at infinite temperature, Pa.s), E_a is the activation energy (kJ/mol), R is the universal gas constant (kJ/mol K), and T_a is the absolute temperature (K).

As seen in Table 4, temperature dependency of all selected gums follows an Arrhenius-type model (acceptable R^2 values). The lower amount of activation energy (E_a) is related to the less temperature sensitivity of rheological properties of food gum [38].

Among selected food gums, xanthan gum illustrated the lower value of E_a at same concentration in comparison with other food gums like carrageenan, pectin, starch, sage seed gum, and basil seed gum (Table 4). Van Wazer et al. [46] informed that the more pseudoplastic behavior, the less temperature dependency. With a quick glance to the Table 2, it was noticed that xanthan and sage seed gums showed lower amount of n value and therefore they exhibited low values of E_a. Some literature presented the increase of E_a values with increasing concentration like carrageenan, xanthan, starch [31], basil seed gum [41], and sage seed gum [42]. However in some studies, the decrease of E_a values was observed by concentration for Salep [47], cress seed gum [20], and gelatin [31].

3.5 Time Dependency

Thixotropy is a key property of non-Newtonian fluid and has been described as the decrease in viscosity as a function of shearing time, which is going to be recovered when shearing is eliminated based on breaking down and later partial building up of some form of structure [48]. The time-dependent parameters of some selected food gums fitted with three famous models including second-order structural kinetic model (Eq. 13), first-order stress decay model, with a non-zero equilibrium stress value (Eq. 14), and Weltman model (Eq. 15) which are shown in Table 5.

$$\left[\frac{\boldsymbol{\eta} - \boldsymbol{\eta}_\infty}{\boldsymbol{\eta}_0 - \boldsymbol{\eta}_\infty}\right]^{(\mathbf{1}-\boldsymbol{n})} = (\boldsymbol{n} - \mathbf{1})\boldsymbol{kt} + 1 \tag{13}$$

where $\boldsymbol{\eta}_0$ and $\boldsymbol{\eta}_\infty$ (Pa s) are the initial (at t = 0) and equilibrium ($t \to \infty$) apparent viscosities, respectively, and k (1/s) is the rate constant of the thixotropic breakdown

Table 4 The activation energy determined for temperature dependence of apparent viscosity of some selected food gums

Gum	Concentration (%)	E_a (KJ mol^{-1})	R^2	References
Sage seed gum (at 50 s^{-1})	0.5 0.75 1.0 1.25 1.5 1.75 2.0	4.9 12.6 14.8 10.2 12.0 12.6 13.3	0.62 0.99 0.95 0.91 0.97 0.98 0.99	[42]
Carrageenan (at 50 s^{-1})	1 2 3	30.3 51.8 54.9	0.85 0.83 0.98	[31]
Xanthan (at 50 s^{-1})	1 2	5.74 0.36	0.88 0.20	[15]
Starch (at 50 s^{-1})	4 5 6	18.7 19.4 20.1	0.99 0.99 0.99	[15]
Pectin (at 50 s^{-1})	1 3 5	19.6 33.4 22.7	0.99 0.99 0.97	[15]
Gelatin (at 50 s^{-1})	2 3 4	19.6 16.8 15.8	0.93 0.88 0.85	[15]
Basil seed gum (at 100 s^{-1})	0.5 1 1.5 2	7.6 8.0 6.8 4.9	0.92 0.99 0.96 0.99	[41]

which is the function of shear rate, and eventually n is the breakdown order (generally $n = 2$).

$$\boldsymbol{\tau} - \boldsymbol{\tau}_{eq} = \left(\boldsymbol{\tau}_0 - \boldsymbol{\tau}_{eq}\right)\boldsymbol{e}^{-kt} \tag{14}$$

where $\boldsymbol{\tau}_0$ and $\boldsymbol{\tau}_{eq}$ (Pa) are the initial and equilibrium shear stresses, respectively; k (1/s) is the breakdown rate constant.

$$\boldsymbol{\tau} = \boldsymbol{A} + \boldsymbol{Blnt} \tag{15}$$

where, A (Pa) is the initial shear stress parameter and B (Pa) is the time coefficient of thixotropic breakdown.

Koocheki and Razavi [49] investigated the effect of shear rate, concentration, and temperature on thixotropic properties of *Alyssum homolocarpum* seed gum (AHSG). As seen in Table 5, the rate constant and extent of thixotropy were affected by concentration and temperature. The first order model was chosen to describe thixotropy behavior of AHSG and according to the results shown in Table 5, the structural breakdown value did not show any clear trend with increasing

Table 5 The time-dependent rheological parameters of some selected food gums fitted with three famous models including, second-order structural kinetic model (Eq. 13), first-order stress decay model, with a non-zero equilibrium stress value (Eq. 14), and Weltman model (Eq. 15)

Gum	Shear rate (s^{-1})	First-order stress decay model		Second-order structure kinetic model			Weltman model		References
		k (s^{-1})	τ_0 (Pa)	τ_{eq} (Pa)	k (s^{-1})	η_0/η_∞	A(Pa)	-B(Pa)	
Alyssum homolocarpum seed gum									[49]
3.0%, 25 °C	50	0.016	56.13	44.82	0.05	1.57	59.99	2.67	
3.5%, 25 °C		0.003	90.54	68.78	0.08	1.44	86.27	3.65	
4.0%, 25 °C		0.0099	128	115	0.09	1.25	133.32	2.97	
3.0%, 45 °C		0.029	39.55	30.84	0.07	1.49	39.30	1.59	
3.0%, 65 °C		0.020	30.52	24.85	0.07	1.41	35.62	1.42	
Salep gum	200	0.0054	408.49	69.39	0.0211	6.77	427.61	118.32	[50]
6.0%, 25 °C	300	0.028	855.92	144.0	0.0564	6.928	570.25	172.33	
	400	0.044	769.29	99.38	0.139	7.988	435.34	177.36	
Balangue seed gum	50	0.0374	194.96	92.4	0.0643	2.44	179.18	24.45	[50]
4.0%, 25 °C	100	0.0211	195.45	128.6130.3	0.0730	1.814	196.23	18.01	
	150	0.0208	157.99		0.0307	1.228	158.32	7.44	
Cress seed gum									[36]
6.0%, 25 °C	50	0.007	92.09	49.72	–	–	–	–	
6.0%, 60 °C–30 min		0.006	99.36	50.47	–	–	–	–	
6.0%, 80 °C–23 min		0.006	101.47	55.98	–	–	–	–	
6.0%, 100 °C–18 min		0.005	105.34	56.40	–	–	–	–	
6.0%, 121 °C–15 min		0.006	121.02	64.72	–	–	–	–	
Xanthan gum									[36]
6.0%, 25 °C	50	0.004	311.28	277.77	–	–	–	–	
6.0%, 60 °C–30 min		0.003	193.61	169.36	–	–	–	–	
6.0%, 80 °C–23 min		0.005	185.40	169.11	–	–	–	–	
6.0%, 100 °C–18 min		0.005	194.28	167.96	–	–	–	–	
6.0%, 121 °C–15 min		0.002	194.38	118.18	–	–	–	–	
Wheat starch									[51]
8.0%, 25 °C	50	0.027	38.50	28.07	0.135	1.92	39.63	1.51	
12.0%, 25 °C		0.010	75.40	33.36	0.013	3.48	106.50	8.75	

k, the breakdown rate constant; τ_0, initial shear stress; τ_{eq}, equilibrium shear stress; η_0, initial apparent viscosity; η_∞, equilibrium apparent viscosities; A, initial shear stress; B, coefficient of thixotropic breakdown

concentration, but it decreased with increasing temperature. However, the extent of thixotropy raised with gum concentration increase and decrease with increasing temperature. In another study, the time dependency of Salep and Balangu seed gums were affected by different shear rates [50] (Table 5). They introduced the first-order stress decay model with non-zero equilibrium as adequate model to describe thixotropy behavior of the selected gums. The rate of thixotropic breakdown of Salep increased with increasing shear rates; however, the extent of thixotropy increased and decreased for Salep and Balangu seed gum, respectively. Naji et al. [36] did research on time dependency of cress seed gum (CSG) and xanthan gum (XG) as a function of different heat treatments (Table 5). CSG exhibited higher rate of breakdown and lower values of initial stress (τ_0) and equilibrium stress (τ_{eq}) against XG. Opposite to XG, the heat treatment increased initial and equilibrium shear stresses of CSG, and it was concluded that at higher temperatures, links between polymeric chains of CSG become stronger. As can be seen in Table 5, the effect of concentration on thixotropic behavior of wheat starch gel was studied by Yousefi and Razavi [51]. The first-order stress decay model with a non-zero equilibrium stress value and the second-order structure kinetic models predicted time dependency well. It was reported that the both initial and equilibrium stress values increased by increasing concentration. On the other hand, decay rate constant (k), which determines how fast the gel gets in the equilibrium stress value, decreased with increasing concentration.

4 Dynamic Rheological Properties

4.1 Introduction

In many literatures, it was announced that dynamic rheological properties of food gums can provide practical information of their structure [52]. Actually, investigation of the viscoelastic proprieties of food gum dispersions is vital for food producer to adjust and recognize the processing parameters, consistency, stability, and textural features of formulated products. This is why dynamic rheology is one of the crucial methods which is used to evaluate the viscoelastic characteristic of polysaccharide dispersions or gels. In this section, oscillatory testing operating modes including amplitude sweep, frequency sweep, and time-temperature sweep of some food gums would be considered.

4.2 Amplitude Dependency

Table 6 shows some stress/strain ramp test parameters of some food gums including limiting values of strain (γ_c), loss tangent (tan δ_{LVE}), yield stress or stress at LVE range (τ_y), storage modulus (G'_{LVE}) and loss modulus (G''_{LVE}) along with flow-point stress (τ_f), and modulus at crossover point (G_f, $G' = G''$). It is obvious that different

conditions like changing temperature, concentration, and the source of the gums would change amplitude-dependent parameters.

In the linear viscoelastic region (LVE), storage modulus (G') and loss modulus (G'') are constant. However, in nonlinear viscoelastic region, both moduli (G' and G'') begin to decrease. The stress/strain point that moduli start to decrease is called critical point (critical stress (τ_y) or strain (γ_c)), which demonstrates the structural strength/deformability of the food gums.

As seen in Table 6, increasing gum concentration resulted in increase of the strength and rigidity of the *Lepidium perfoliatum* seed gum and wheat starch gel. Also, the magnitudes of G'_{LVE} and G''_{LVE} increased with increasing gum concentration. The ratio of G''_{LVE} to G''_{LVE} ($\tan \delta_{LVE}$) is a rheological parameter that shows the physical behavior of a gum system. The selected gum showed decreasing or unchanged $\tan \delta_{LVE}$ values by increasing gum concentration for wheat starch gel and *Lepidium perfoliatum* seed gum, respectively. Another important criterion which could be determined through stress sweep is yield stress (τ_y).

Yield stress of food gums is a criterion of the capacity or the ability of them to stabilize food systems [53]. By increasing gum concentration, the yield stress usually increases which means the stronger gel network formed. For example, the τ_y of wheat starch gel declined from 12 to 83 Pa as the concentration increased from 8% to 12% (Table 6). When the storage modulus is equal to loss modulus, called yield stress at flow point (τ_f), the structure rupture happened and with following the escalating of stress, the loss modulus is going to be more than storage modulus and then flow is occurred. The selected food gums (wheat starch and *Lepidium perfoliatum* seed gum) showed that the value of τ_f increased with increasing gum concentration. Finally, the stress at the crossover of the moduli (G_f, $G' = G''$) indicates the gel strength at the start of flow point which is also raised by increasing selected gums concentration.

The effect of temperature on stress sweep properties is different in various food gums. As seen in Table 6, Behrouzian et al. [33] reported completely different behavior of sage seed gum (SSG) and xanthan gum (XG) with increasing temperature. For example, G'_{LVE}, G''_{LVE}, γ_c, τ_y, and τ_f of SSG and XG increased and decreased, respectively, as temperature raised from 10 °C to 90 °C. In contrast, the values of $\tan \delta_{LVE}$ for SSG and XG decreased and increased, respectively. The effect of raising temperature for *Lepidium perfoliatum* seed gum was similar to SSG (Table 6), which demonstrated that increasing temperature strengthened the structure of SSG and *Lepidium perfoliatum* seed gum, while it weakened the XG structure. The different behavior of food gums with increasing temperature may be related to their molecular conformation changes. With a glance to the Sect. 2.2 (Table 1), it is obvious that the intrinsic viscosity of XG increased with increasing temperature, but SSG showed reversed trend. It was reported that increasing temperature transformed XG conformation from rigid rod to random coil, and in contrast it increased the rigidity of SSG [55].

As demonstrated in Table 6, SSG, BSG, and guar gum at the same experimental condition (1%, 20 °C, 1 Hz) showed different amplitude dependency behaviors. Obviously, these differences are related to different nature of them like molecular weight, molecular conformation, and so on.

Table 6 Dynamic moduli in the linear viscoelastic range (G'_{LVE} and G''_{LVE}), limiting strain (γ_c), and loss-tangent in the LVE range (tan δ_{LVE}), yield stress in the LVE range (τ_y), and flow-point stress (τ_f), with corresponding moduli (G_f: $G' = G''$) for some food gums

Gum	G'_{LVE} (Pa)	G''_{LVE} (Pa)	γ_c (%)	Tanδ_{LVE}	τ_y (Pa)	τ_f (Pa)	G_f (Pa)	References
Lepidium perfoliatum seed gum								
(1.5%, 5 °C, 1 Hz)	70.4	19.1	1.00	0.27	0.73	11.7	15.8	[54]
(3%, 5 °C, 1 Hz)	172	43.2	1.47	0.25	2.61	45.0	30.1	
(1.5%, 85 °C, 1 Hz)	72.7	13.3	1.45	0.18	1.49	10.7	16.7	
(3%, 85 °C, 1 Hz)	232	41.8	4.65	0.18	14.80	38.0	51.7	
Sage seed gum								
(1%, 10 °C, 0.16 Hz)	24.89	6.34	0.02	0.28	0.56	14.35	–	[55]
(1%, 30 °C, 0.16 Hz)	34.64	5.82	0.04	0.17	1.38	8.18	–	
(1%, 50 °C, 0.16 Hz)	43.65	5.39	0.03	0.12	1.47	5.64	–	
(1%, 70 °C, 0.16 Hz)	50.28	5.53	0.03	0.11	1.56	4.91	–	
(1%, 90 °C, 0.16 Hz)	68.08	12.51	0.04	0.10	2.30	16.93	–	
Xanthan gum							–	
(1%, 10 °C, 0.16 Hz)	66.16	15.54	0.17	0.24	9.26	21.96	–	
(1%, 30 °C, 0.16 Hz)	14.98	5.40	0.30	0.32	4.58	10.23	–	
(1%, 50 °C, 0.16 Hz)	7.33	3.34	0.40	0.46	3.06	7.52	–	
(1%, 70 °C, 0.16 Hz)	4.57	2.68	0.19	0.53	0.93	5.24	–	
(1%, 90 °C, 0.16 Hz)	3.01	1.61	0.10	0.54	0.66	4.12	–	
Sage seed gum								
(1%, 20 °C, 1 Hz)	13.30	5.78	8.15	0.44	–	1023.20	4.58	[34]
Guar gum								
(1%, 20 °C, 1 Hz)	8.05	6.55	35.82	0.81	–	1509.83	5.99	[34]
Basil seed gum								
(1%, 20 °C, 1 Hz)	227.00	55.20	–	0.24	3.59	13.90	54.49	[56]
Wheat starch gel (8%, 25 °C, 1 Hz)	344.1	58.5	1.5	0.17	11.9	27.7	88.5	[51]
Wheat starch gel (12%, 25 °C, 1 Hz)	4470.3	513.6	2.2	0.11	82.9	162.2	1450.2	

4.3 Frequency Dependency

Frequency sweep data can help to classify dispersions including dilute solution, entanglement network systems (concentrated solution), weak gel, and finally strong gel [48]. Strong gels of food gums behave like viscoelastic solids at small frequencies while they start to rupture above a critical frequency or deformation value. However, their weak gels behave like strong gels at small frequencies, but with increasing frequencies or deformation values, three-dimensional networks start to progressively breakdown into smaller clusters [51]. In weak gels, storage (G′) and loss moduli (G″) exhibit slight frequency dependency and G′ exceeding G″ at all frequencies [57]. Weak gel behavior was reported for *Lepidium perfoliatum* seed gum (1.5–3% concentration) [54], wheat starch gel (8–12% concentration) [51], basil seed gum (1 and 3% concentration) [56, 58, 59], sage seed gum, and xanthan gum (1% concentration) [19, 60], as the magnitudes of G′ and G″ slightly increase with frequency increasing, and G′ is always higher than G″ within frequency range and no crossover point occurred (Fig. 2). However, guar gum (1% concentration) [34], mucilage gum of *Opuntia ficus indica* (3% concentration) [61], and xanthan gum at 90 °C (1% concentration) [33] behave like entangled polymer solutions as a crossover point occurred at low frequency and G′ was higher than G″ at high frequency range (Fig. 3).

Commonly, when concentration of food gums increases in a solution, the magnitudes of both storage and loss moduli increase. At high concentrations, the formation of complex structure grows in the network system. The parallel increase of dynamic moduli (G′ and G″) is linked to the network defects [62]. In other words, in low concentration, there are lots of intermolecular zones, which could not take a part in

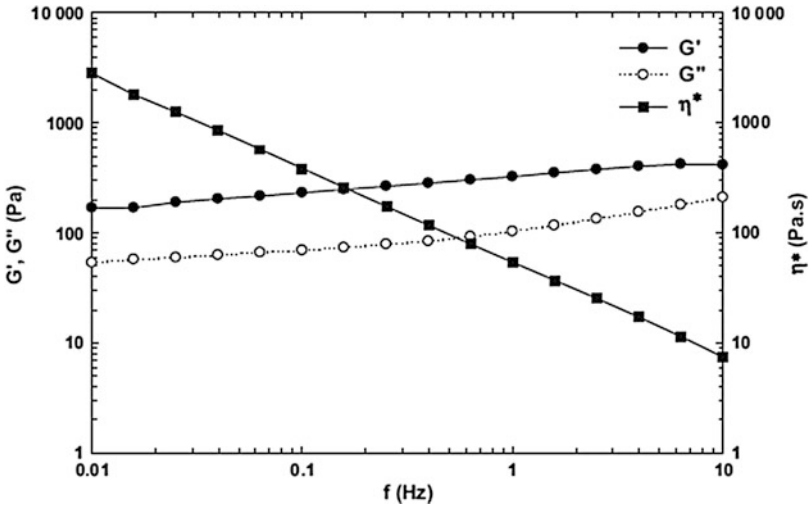


Fig. 2 Frequency sweep of 3% basil seed gum (20 °C; 0.5% strain), showing the variation of storage modulus (G′), loss modulus (G″) and complex viscosity (η*) with frequency (f, Hz) [63]

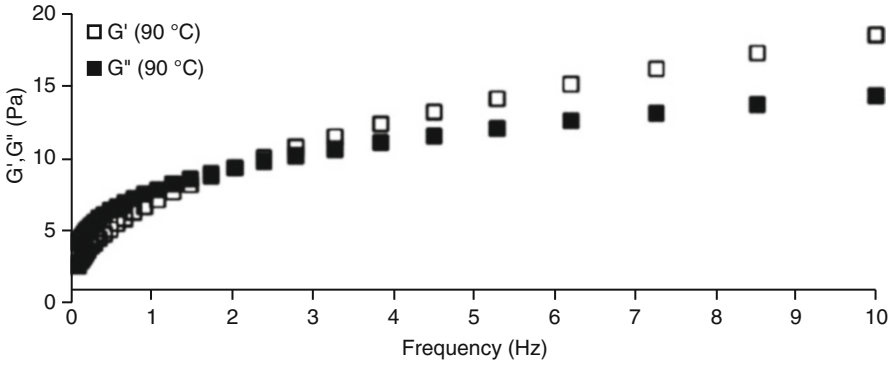


Fig. 3 The mechanical spectra for 1–3 sage seed gum-xanthan gum (1%w/w) at 90 °C [60]

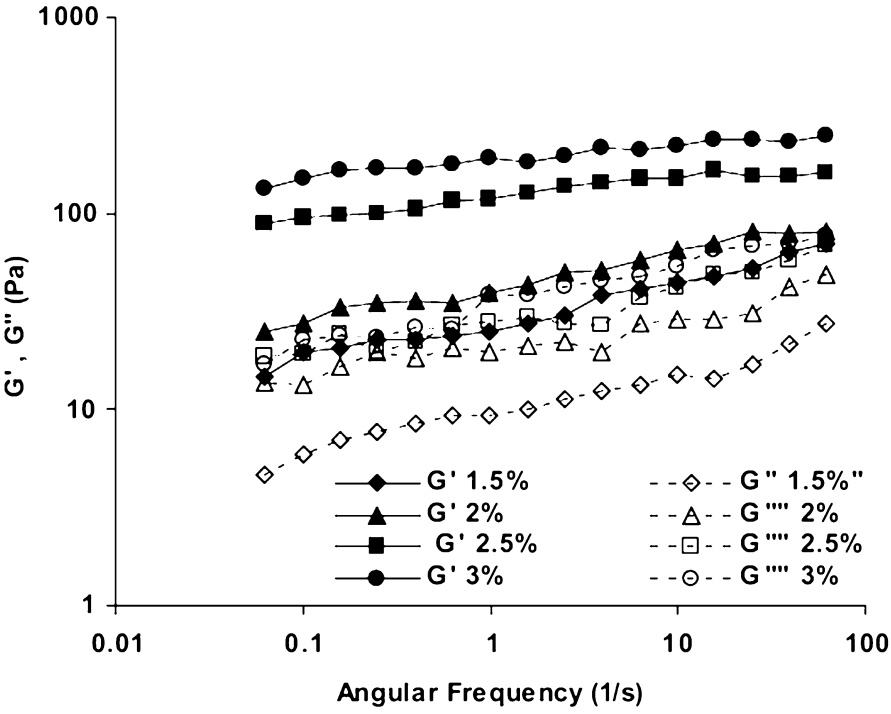


Fig. 4 The typical effect of concentration of *lipidium perfoliatum* seed gum on storage (G′) and loss (G″) moduli as functions of frequency at 5 °C [59]

noncovalence cross-junction [62–64]. However, at higher concentrations, the formation of junction zones increase. With increasing concentration, the values of G′ become higher than G″ (Fig. 4) showing a tendency to form macromolecular networks. This trend was reported for sage seed gum [60], wheat starch gel [51], basil seed gum [58],

Lepidium perfoliatum seed gum [54], gellan gum gels [65], psyllium gels [66], and xanthan gum [61]. It was observed that random coil polysaccharides have no dependency on frequency regardless of temperature [67] like *Lepidium perfoliatum* seed gum [54], mucilage gum of *opuntia ficus indica* [61], and sage seed gum [55].

According to polymer dynamics theory, there is a frequency dependency of G′ values based on power-law relation for a physical gel [68]. Thus, the power-law parameters can be used to describe the frequency dependency of G′ values of food gums:

$$G' = K' (\omega)^{n'} \tag{16}$$

where, ω (rad/s) is the oscillation frequency, K' is a constant, and the exponent n′ is the slope of log-log plot of G' versus ω. From structural perspective, $n' = 0$ is a covalent gel or a true gel, meanwhile for a physical gel, n' is larger than 0. Actually, the n′ value can be used as a criterion to compare a physical gel to a true gel. Lower n' values near to 0 are characteristic of an elastic gel, while n′ values near to 1 are considered as a viscous gel [51]. It was informed that the n' value is connected to the strength and nature of the food gum gel [69]. Some food gums showed decreasing n' with increasing concentration like wheat starch gel [51], *Lepidium perfoliatum* seed gum [54], and basil seed gum [58]; however, sage seed gum [60] showed no effect of concentration on n'. Also, different outcomes were reported for the effect of increasing temperature on n' values. A decrease in n' values was observed in *Lepidium perfoliatum* seed gum [54] and sage seed gum [55], when temperature increases from 5 °C to 85 °C and 10 °C to 50 °C, respectively, while Behrozian et al. [55] reported an increase in n' value for xanthan gum with escalating temperature from 10 °C to 90 °C.

The other dynamic rheological property is loss tangent (tan δ), the ratio of G″/G′, which describes the viscoelastic behavior. Tan δ is directly related to the energy lost per cycle divided by the energy stored per cycle. The values of tan δ <1 and tan δ >1 indicate predominantly elastic and viscous behaviors, respectively. Observations of polymer systems give the following numerical ranges for tan δ: very high for dilute solutions, 0.2–0.3 for amorphous polymers, low (near 0.01) for glassy crystalline polymers and true gels [48]. The *literatures demonstrated decreasing of tan δ with increasing concentration, like Lepidium perfoliatum seed gum* [54]*, psyllium gels* [66]*, and sage seed gum* [60]. With increasing temperature, tan δ changes similar to the n' of food gums mentioned in previous paragraph.

Since food gums are utilized as thickener or stabilizer in food products, the complex viscosity (η*) in frequency dependency becomes prominent. A linear decrease in η* was observed with increase in frequency for some food gums like wheat starch [51], corn starch [70], acorn starch [71], Peruvian carrot starch [45], sweet potato starch [72], sage seed gum, xanthan gum [55], and basil seed gum [56, 58], indicating non-Newtonian shear-thinning flow behavior of them. With increasing temperature, the η* of some gums like sage seed gum [55] and *Lepidium perfoliatum* seed gum [54] increased; however, xanthan gum [55] shows decreasing of η* with rising temperature. Morris [63] nominated that η* with slope near to

−0.76 describes "weak gel" characteristics of a polysaccharide gel formed by overlapping and entangled flexible random coil chains. Basil seed gum and xanthan gum, which generate "weak-gel" networks by tenuous association of rigid and ordered molecular structures in solution, show higher slope of η* than −0.76.

4.4 Time/Temperature Dependency

The melting temperature of a biopolymer could be determined as the temperature when G′ equals G″ (gel-sol transition). This feature easily can be measured by using a controlled stress rheometer in oscillatory mode. Distinguishing the temperature/time dependency of viscoelastic moduli is vital, and this dependency should be recognized when measuring the melting or gelling points [73].

Hesarinejad et al. [54] studied the effect of temperature ramp (5–85 °C) on *Lepidium perfoliatum* seed gum (LPSG) at different concentrations. The LPSG solution showed elastic-like behavior and stayed in the solid-like state in the whole temperature range. An increase in G′ was observed with increasing concentration, which connected to the creation of three-dimensional network structure and also conversion of the sol fraction into gel. Moreover, the thickening effect of gums can restrict the mobility of fluids. It may be clarified by the increase of G′ during heating/cooling, which related to get stronger of hydrophobic interaction with temperature. Also, a hysteresis was observed between heating and cooling curves. Because of the different energy requirements for association and disassociation of junction zones, the thermal hysteresis between gelation and melting occurs [74]. Rafe and Razavi [58] reported an increase in G′ value during heating/cooling (20–90 °C) for basil seed gum (BSG) (Fig. 5). G′ values during cooling were greater than heating; therefore, thermal hysteresis indicates the gel formation of BSG during cooling period [58]. Similar trends were reported in formation and melting of k-carrageenan [75–77] and xanthan-LBG mixture [78]. A decrease of dynamic moduli (G′ and G″) for xanthan gum (1% XG + Nacl (0.08 and 0.008 M)) and gellan gum (0.5%) was observed during heating from 25 °C to 80 °C and 5 °C to 85 °C, respectively. This trend was against the results reported for BSG and LPSG. As mentioned in Sect. 4.2, it may be related to the changing of XG conformation from rigid rod to random coil with increasing temperature.

4.5 Cox-Merz Rule

There is an empirical relationship between the apparent viscosity (η_a) of polymers as a function of shear rate ($\dot{\gamma}$) and the complex viscosity (η*) as a function of frequency (ω) developed by Cox and Merz (1958) as follows:

$$|\eta^*|\ (\omega) = \eta(\dot{\gamma})|_{\omega} = \dot{\gamma} \tag{17}$$

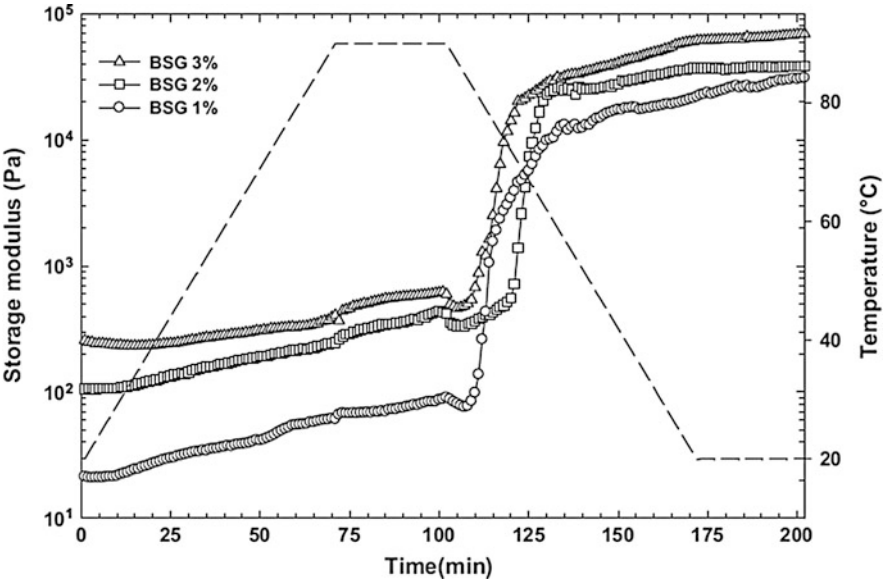


Fig. 5 Variation of storage modulus at different concentrations of basil seed gum as a result of heating (from 20 °C to 90 °C) at rate of 1 °C min^{-1} and holding for 30 min; then cooling to 20 °C with the same rate and keeping at 20 °C for 30 min ($f = 1$ Hz and 0.5% strain) [63]

The *Cox-Merz* rule equates η_a measured in shear flow to η^* measured with oscillatory rheometry, where ω is taken as $\dot{\gamma}$ in the frequency sweep test. It has been proved that the *Cox-Merz* rule can be used to forecast the steady shear properties of a material from the dynamic rheological properties [79]. Dynamic and steady shear flow curves should be the same when polymer solutions are devoid of strong interaction. Opposite to irregular biopolymers, polysaccharide solution with regular chains and rigid conformation that show gel-like behavior do not obey this law like gellan and xanthan gum [80–82]. A typical obey and disobey *Cox-Merz* rule is shown in Fig. 6. LBG and guar gum obey the *Cox-Merz* rule by using shift factor ($\eta^*(\alpha\omega) = \eta(\dot{\gamma})$) [83]. The deviations from *Cox-Merz* rule for galactomannans were reported at low shear rate frequencies [30]. It was related to the creation of high density of entanglements resulted from specific polymer/polymer interaction [84]. However, basil seed gum [56], Salep [83], and sage seed gum [34] disobey the *Cox-Merz* rule, suggesting a self-structured and ordered conformation for these gums.

5 Conclusion

Dilute regime, steady shear, and dynamic rheological properties of some different food gums in different situations were studied. Dilute solution properties of food gums provide deep understanding of molecular properties of their macromolecules in dilute regime and consequently the role of them in final food products as real

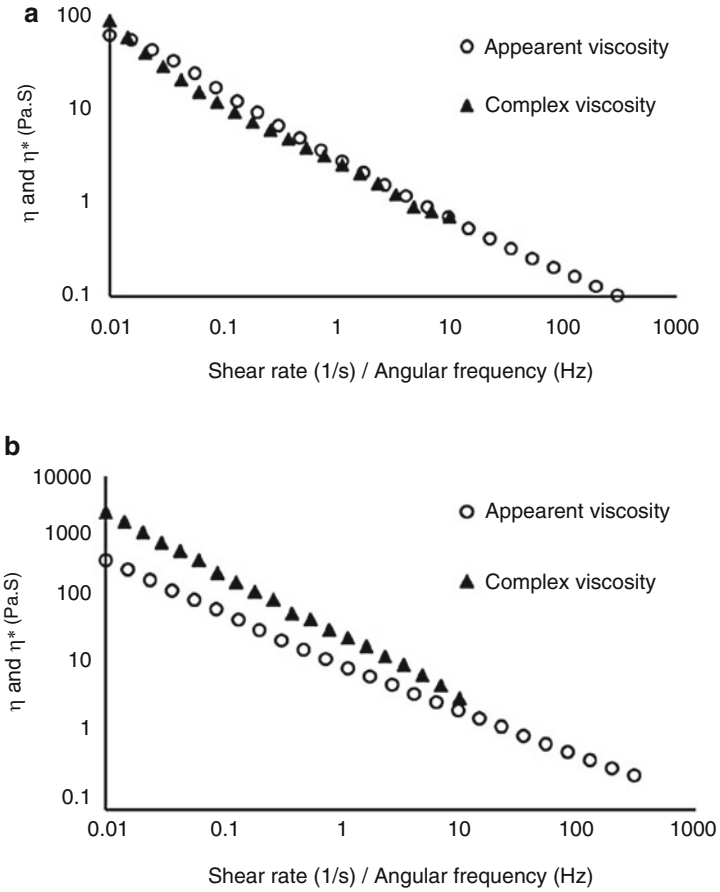


Fig. 6 Cox-Merz plot of complex viscosity (η*) against apparent viscosity (η_a) for SUPER-BSG (**a**) and PF-BSG (**b**). (SUPER-BSG, low molecular weight fraction of basil seed gum; PF-BSG, protein- free basil seed gum) [61]

systems. Also, steady shear and dynamic rheological features of food gums illustrate the high potential of them as thickener, stabilizer, and fat-replacer in food systems. So, knowing rheological features of food gums at different circumstances can help food companies to save time, choose appropriate gum for their food formulations, and produce new products with better mouth feeling.

References

1. Rao MA (2006) Influence of food microstructure on food rheology. In: Understanding and controlling the microstructure of complex foods. Woodhead Publishing, Cambridge, UK
2. Park S, Chung MG, Yoo B (2004) Rheological properties of octenylsuccinated corn starch pastes. Starch/Stärke 56:431–438

3. Mohammad Amini A, Razavi SMA (2012) Dilute solution properties of Balangu (*Lallemantia royleana*) seed gum: effect of temperature, salt, and sugar. Int J Biol Macromol 51:235–243. https://doi.org/10.1016/j.ijbiomac.2012.05.018
4. Pamies R, Cifre JGH, Martínez M d CL, de la Torre JG (2008) Determination of intrinsic viscosities of macromolecules and nanoparticles. Comparison of single-point and dilution procedures. Colloid Polym Sci 286:1223–1231
5. Huggins ML (1942) The viscosity of dilute solutions of long-chain molecules. IV. Dependence on concentration. J Am Chem Soc 64:2716–2718
6. Kraemer EO (1938) Molecular weights of celluloses and cellulose derivates. Ind Eng Chem 30:1200–1203
7. Tanglertpaibul T, Rao MA (1987) Intrinsic viscosity of tomato serum as affected by methods of determination and methods of processing concentrates. J Food Sci 52:1642–1645
8. Higiro J, Herald TJ, Alavi S (2006) Rheological study of xanthan and locust bean gum interaction in dilute solution. Food Res Int 39:165–175. https://doi.org/10.1016/j.foodres.2005.07.011
9. Rao MA (2007) Rheological Behavior of Processed Fluid and Semisolid Foods. In: Rheology of Fluid and Semisolid Foods. Food Engineering Series. Springer, Boston, MA
10. Khouryieh HA, Herald TJ, Aramouni F, Alavi S (2006) Influence of mixing temperature on xanthan conformation and interaction of xanthan–guar gum in dilute aqueous solutions. Food Res Int 39:964–973. https://doi.org/10.1016/j.foodres.2006.06.001
11. Wang F, Sun Z, Wang YJ (2001) Study of xanthan gum/waxy corn starch interaction in solution by viscometry. Food Hydrocoll 15:575–581. https://doi.org/10.1016/S0268-005X(01)00065-0
12. Richardson PH, Willmer J, Foster TJ (1998) Dilute solution properties of guar and locust bean gum in sucrose solutions. Food Hydrocoll 12:339–348. https://doi.org/10.1016/S0268-005X(98)00025-3
13. Wang S, He L, Guo J, Zhao J, Tang H (2015) Intrinsic viscosity and rheological properties of natural and substituted guar gums in seawater. Int J Biol Macromol 76:262–268. https://doi.org/10.1016/j.ijbiomac.2015.03.002
14. Khouryieh HA, Herald TJ, Aramouni F, Bean S, Alavi S (2007) Influence of deacetylation on the rheological properties of xanthan–guar interactions in dilute aqueous solutions. J Food Sci 72:C173
15. Higiro J, Herald TJ, Alavi S, Bean S (2007) Rheological study of xanthan and locust bean gum interaction in dilute solution: effect of salt. Food Res Int 40:435–447. https://doi.org/10.1016/j.foodres.2006.02.002
16. Viturawong Y, Achayuthakan P, Suphantharika M (2008) Gelatinization and rheological properties of rice starch/xanthan mixtures: effects of molecular weight of xanthan and different salts. Food Chem 111:106–114
17. Amini AM, Razavi SMA (2012) Dilute solution properties of Balangu (*Lallemantia royleana*) seed gum: effect of temperature, salt, and sugar. Int J Biol Macromol 51:235–243
18. Yousefi AR, Razavi SMA, Khodabakhsh Aghdam SH (2014) Influence of temperature, mono- and divalent cations on dilute solution properties of sage seed gum. Int J Biol Macromol 67:246–253. https://doi.org/10.1016/j.ijbiomac.2014.03.026
19. Behrouzian F, Razavi SMA, Karazhiyan H (2014) Intrinsic viscosity of cress (*Lepidium sativum*) seed gum: effect of salts and sugars. Food Hydrocoll 35:100–105. https://doi.org/10.1016/j.foodhyd.2013.04.019
20. Karazhiyan H, Razavi SMA, Phillips GO, Fang Y, Al-Assaf S, Nishinari K, Farhoosh R (2009) Rheological properties of *Lepidium sativum* seed extract as a function of concentration, temperature and time. Food Hydrocoll 23:2062–2068
21. Mirabolhassani SE, Rafe A, Razavi SMA (2016) The influence of temperature, sucrose and lactose on dilute solution properties of basil (*Ocimum basilicum*) seed gum. Int J Biol Macromol 93:623–629. https://doi.org/10.1016/j.ijbiomac.2016.09.021
22. Hanselmann R, Ehrat M, Widmer HM (1995) Molar masses and sizes of starches by high-performance size-exclusion chromatography with on-line multi-angle laser light scattering detection. J Agric Food Chem 44:3182–3188

23. Shon KJ, Lim ST, Yoo B (2005) Rheological properties of rice starch dispersions in dimethyl sulfoxide. Starch-Starke 57:363–369. https://doi.org/10.1002/star.20400353
24. Kowittaya C, Lumdubwong N (2014) Molecular weight, chain profile of rice amylopectin and starch pasting properties. Carbohydr Polym 108:216–223
25. Irani M, Razavi SMA, Abdel-Aal ESM, Hucl P, Patterson CA (2016) Dilute solution properties of canary seed (*Phalaris canariensis*) starch in comparison to wheat starch. Int J Biol Macromol 87:123–129. https://doi.org/10.1016/j.ijbiomac.2016.02.050
26. Draget KI, Moe ST, Skjak-Bræk G, Smidsrød O (2006) Alginates. In: Food Polysaccharides and Their Applications. Boca Raton: CRC Press.
27. Mohammadifar MA, Musavi SM, Kiumarsi A, Williams PA (2006) Solution properties of targacanthin (water-soluble part of gum tragacanth exudate from *Astragalus gossypinus*). Int J Biol Macromol 38:31–39
28. Harding SE (1997) The intrinsic viscosity of biological macromolecules. Progress in measurement, interpretation and application to structure in dilute solution. Prog Biophys Mol Biol 68:207–262
29. Millard MM, Dintzis FR, Willett JL, Klavons JA (1997) Light-scattering molecular weights and intrinsic viscosities of processed waxy maize starches in 90% dimethyl sulfoxide and H_2O. Cereal Chem 74:687–691
30. Morris ER, Cutler AN, Ross-Murphy SB, Rees DA, Price J (1981) Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. Carbohydr Polym 1:5–21
31. Marcotte M, Hoshahili ART, Ramaswamy HS (2001) Rheological properties of selected hydrocolloids as a function of concentration and temperature. Food Res Int 34:695–703. https://doi.org/10.1016/S0963-9969(01)00091-6
32. Razavi SMA, Naji-Tabasi S (2017) Chapter 16 - Rheology and Texture of Basil Seed Gum: A New Hydrocolloid Source A2 - Ahmed, J. In: Ptaszek P, Basu SBT-A in FR and IA (eds) Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, pp 405–435
33. Alghooneh A, Razavi SMA, Behrouzian F (2017) Rheological characterization of hydrocolloids interaction: a case study on sage seed gum-xanthan blends. Food Hydrocoll 66:206–215. https://doi.org/10.1016/j.foodhyd.2016.11.022
34. Razavi SMA, Alghooneh A, Behrouzian F, Cui SW (2016) Investigation of the interaction between sage seed gum and guar gum: steady and dynamic shear rheology. Food Hydrocoll 60:67–76
35. Salehi F, Kashaninejad M, Behshad V (2014) Effect of sugars and salts on rheological properties of Balangu seed (*Lallemantia royleana*) gum. Int J Biol Macromol 67:16–21. https://doi.org/10.1016/j.ijbiomac.2014.03.001
36. Naji S, Razavi SMA, Karazhiyan H (2012) Effect of thermal treatments on functional properties of cress seed (*Lepidium sativum*) and xanthan gums: a comparative study. Food Hydrocoll 28:75–81. https://doi.org/10.1016/j.foodhyd.2011.11.012
37. Lee HL, Yoo B (2011) Effect of hydroxypropylation on physical and rheological properties of sweet potato starch. LWT – Food Sci Technol 44:765–770. https://doi.org/10.1016/j.lwt.2010.09.012
38. Irani M, Razavi SMA, Abdel-Aal ESM, Taghizadeh M (2016) Influence of variety, concentration, and temperature on the steady shear flow behavior and thixotropy of canary seed (*Phalaris canariensis*) starch gels. Starch/Staerke 68:1203–1214. https://doi.org/10.1002/star.201500348
39. Hamza-Chaffai A (1990) Effect of manufacturing conditions on rheology of banana gelified milk: optimization of the technology. J Food Sci 55:1630–1633
40. Rao MA, Kenny JF (1975) Flow properties of selected food gums. Can Inst Food Sci Technol J 8:142–148
41. Hosseini-Parvar SH, Matia-Merino L, Goh KKT, Razavi SMA, Mortazavi SA (2010) Steady shear flow behavior of gum extracted from *Ocimum basilicum* L. seed: effect of concentration and temperature. J Food Eng 101:236–243. https://doi.org/10.1016/j.jfoodeng.2010.06.025
42. Razavi SMA, Taheri H, Quinchia LA (2011) Steady shear flow properties of wild sage (*Salvia macrosiphon*) seed gum as a function of concentration and temperature. Food Hydrocoll 25:451–458. https://doi.org/10.1016/j.foodhyd.2010.07.017

43. Yousefi AR, Eivazlou R, Razavi SMA (2016) Steady shear flow behavior of sage seed gum affected by various salts and sugars: time-independent properties. Int J Biol Macromol 91:1018–1024. https://doi.org/10.1016/j.ijbiomac.2016.06.046
44. Moreira R, Chenlo F, Torres MD, Glazer J (2012) Rheological properties of gelatinized chestnut starch dispersions: effect of concentration and temperature. J Food Eng 112:94–99
45. Albano KM, Franco CML, Telis VRN (2014) Rheological behavior of Peruvian carrot starch gels as affected by temperature and concentration. Food Hydrocoll 40:30–43
46. Van Wazer JR, Lyons JW, Kim KY, Viscosity REC (1963) Flow measurement: a laboratory handbook of rheology. Interscience, New York
47. Farhoosh R, Riazi A (2007) A compositional study on two current types of salep in Iran and their rheological properties as a function of concentration and temperature. Food Hydrocoll 21:660–666
48. Steffe JF (1996) Rheological methods in food process engineering. Freeman press, East Lansing
49. Koocheki A, Razavi SMA (2009) Effect of concentration and temperature on flow properties of Alyssum homolocarpum seed gum solutions: assessment of time dependency and thixotropy. Food Biophys 4:353–364. https://doi.org/10.1007/s11483-009-9134-7
50. Razavi SMA, Karazhiyan H (2009) Flow properties and thixotropy of selected hydrocolloids: experimental and modeling studies. Food Hydrocoll 23:908–912. https://doi.org/10.1016/j.foodhyd.2008.05.010
51. Yousefi AR, Razavi SMA (2015) Dynamic rheological properties of wheat starch gels as affected by chemical modification and concentration. Starch/Staerke 67:567–576. https://doi.org/10.1002/star.201500005
52. Clark AH, Ross-Murphy SB (1987) Structural and mechanical properties of biopolymer gels. In: Biopolymers. Advances in Polymer Science, vol 83. Springer, Berlin, Heidelberg
53. Guo Q, Cui SW, Wang Q, Goff HD, Smith A (2009) Microstructure and rheological properties of psyllium polysaccharide gel. Food Hydrocoll 23:1542–1547
54. Hesarinejad MA, Koocheki A, Razavi SMA (2014) Dynamic rheological properties of Lepidium perfoliatum seed gum: effect of concentration, temperature and heating/cooling rate. Food Hydrocoll 35:583–589. https://doi.org/10.1016/j.foodhyd.2013.07.017
55. Behrouzian F, Razavi SMA, Alghooneh A (2017) Evaluation of interactions of biopolymers using dynamic rheological measurements: effect of temperature and blend ratios. J Appl Polym Sci. https://doi.org/10.1002/app.44414
56. Naji-Tabasi S, Razavi SMA (2017) New studies on basil (*Ocimum bacilicum* L.) seed gum: part III – steady and dynamic shear rheology. Food Hydrocoll 67:243–250. https://doi.org/10.1016/j.foodhyd.2015.12.020
57. Martínez-Ruvalcaba A, Chornet E, Rodrigue D (2007) Viscoelastic properties of dispersed chitosan/xanthan hydrogels. Carbohydr Polym 67:586–595
58. Rafe A, Razavi SMA (2013) Dynamic viscoelastic study on the gelation of basil seed gum. Int J Food Sci Technol 48:556–563. https://doi.org/10.1111/j.1365-2621.2012.03221.x
59. Hosseini-Parvar SH (2009) Basil seed gum (BSG): Physico-chemical, rheological and emulsifying characterization and its synergistic interactions in combination with locust bean gum and guar gum. Department of Food Science and Technology. Ferdowsi University, Mashhad
60. Razavi SMA, Taheri H, Sanchez R (2013) Viscoelastic characterization of sage seed gum. Int J Food Prop 16:1604–1619. https://doi.org/10.1080/10942912.2011.604888
61. Medina-Torres L, Brito-De La Fuente E, Torrestiana-Sanchez B, Katthain R (2000) Rheological properties of the mucilage gum (*Opuntia ficus indica*). Food Hydrocoll 14:417–424. https://doi.org/10.1016/S0268-005X(00)00015-1
62. Ross-Murphy SB, Harding SE (1994) Physical techniques for the study of food biopolymers. Trends Food Sci Technol 5:126
63. Morris ER (1990) Shear-thinning of "random coil" polysaccharides: characterisation by two parameters from a simple linear plot. Carbohydr Polym 13:85–96
64. Rincón F, Muñoz J, De Pinto GL, Alfaro MC, Calero N (2009) Rheological properties of Cedrela odorata gum exudate aqueous dispersions. Food Hydrocoll 23:1031–1037

65. Rodrıguez-Hernandez AI, Durand S, Garnier C, Tecante A, Doublier JL (2003) Rheology-structure properties of gellan systems: evidence of network formation at low gellan concentrations. Food Hydrocoll 17:621–628
66. Farahnaky A, Askari H, Majzoobi M, Mesbahi G (2010) The impact of concentration, temperature and pH on dynamic rheology of psyllium gels. J Food Eng 100:294–301
67. Robinson G, Ross-Murphy SB, Morris ER (1982) Viscosity-molecular weight relationships, intrinsic chain flexibility, and dynamic solution properties of guar galactomannan. Carbohydr Res 107:17–32
68. Ferry JD (1980) Viscoelastic properties of polymers. Wiley, New York
69. Khondkar D, Tester RF, Hudson N, Karkalas J, Morrow J (2007) Rheological behaviour of uncross-linked and cross-linked gelatinised waxy maize starch with pectin gels. Food Hydrocoll 21:1296–1301
70. Rosalina I, Bhattacharya M (2002) Dynamic rheological measurements and analysis of starch gels. Carbohydr Polym 48:191–202
71. Kim W, Yoo B (2009) Rheological behaviour of acorn starch dispersions: effects of concentration and temperature. Int J Food Sci Technol 44:503–509
72. Kim D, Yoo B (2010) Rheological behaviors of hydroxypropylated sweet potato starches influenced by guar, locust bean, and xanthan gums. Starch-Stärke 62:584–591
73. Djabourov M, Leblond J, Papon P (1988) Gelation of aqueous gelatin solutions. I. Structural investigation. J Phys 49:319–332
74. Nishinari K, Takaya T, Kohyama K, Watase M (1994) Effects of Sugars on the Gel-Sol Transition of Agarose and k -Carrageenan. In: Yano T, Matsuno R, Nakamura K (eds) Developments in Food Engineering. Springer, Boston, MA
75. Rochas C, Rinaudo M (1980) Activity coefficients of counterions and conformation in kappa-carrageenan systems. Biopolymers 19:1675–1687
76. Hermansson A-M, Eriksson E, Jordansson E (1991) Effects of potassium, sodium and calcium on the microstructure and rheological behaviour of kappa-carrageenan gels. Carbohydr Polym 16:297–320
77. Kohyama K, Sano Y, Nishinari K (1996) A mixed system composed of different molecular weights konjac glucomannan and κ-carrageenan. II. Molecular weight dependence of viscoelasticity and thermal properties. Food Hydrocoll 10:229–238
78. Williams PA, Phillips GO (2007) Gums and stabilisers for the food industry 11. Royal Society of Chemistry, Cambridge
79. Da Silva PMS, Oliveira JC, M A R (1998) Rheological properties of heated cross-linked waxy maize starch dispersions. Int J Food Prop 1:23–34. https://doi.org/10.1080/10942919809524562
80. Chronakis IS, Kasapis S (1995) Food applications of biopolymer – theory and practice. Dev Food Sci 37:75–109
81. Miyoshi E, Nishinari K (1999) Non-Newtonian flow behaviour of gellan gum aqueous solutions. Colloid Polym Sci 277:727–734
82. Fang Y, Takemasa M, Katsuta K, Nishinari K (2004) Rheology of schizophyllan solutions in isotropic and anisotropic phase regions. J Rheol 48:1147–1166
83. Yaşar K, Kahyaoglu T, Şahan N (2009) Dynamic rheological characterization of Salep glucomannan/galactomannan-based milk beverages. Food Hydrocoll 23:1305–1311. https://doi.org/10.1016/j.foodhyd.2008.11.005
84. Rao MA (1999) Rheological of fluids and semisolids. Principal and applications. An Gaitherburg, Maryland, USA An Aspen Publ Inc

GLC/HPLC Methods for Saffron (*Crocus sativus* L.)

68

Armin Amanpour, Hasim Kelebek, and Serkan Selli

Contents

Abstract

Saffron (*Crocus sativus* L.) is provided from the dried and dark-red stigmas of flowers belonging to the family of Iridaceae. Concerning the total content of saffron production, the biggest producer territory in the world is Iran, followed by Spain, India, Italy, Greece, and Morocco. Crocetin, crocin, picrocrocin, and safranal are the four main bioactive compounds in saffron which contribute both organoleptic profile of saffron (pigment, pigment, taste, and odor, respectively) and the health-progressing features. Isolation, identification, and quantification of bioactive compounds from complex and natural matrix of food stuffs are a main and common trouble of initial interest in food quality measurement and characterization. Chromatography is a set of constituents' separation techniques in a complex mixture. Recently, chromatographic methods were widely used for

A. Amanpour · S. Selli (✉)
Department of Food Engineering, Faculty of Agriculture, Cukurova University, Adana, Turkey
e-mail: dr.aamanpour@gmail.com; sselli@cu.edu.tr

H. Kelebek
Department of Food Engineering, Faculty of Engineering and Natural Sciences, Adana Science and Technology University, Adana, Turkey
e-mail: hkelebek@adanabtu.edu.tr

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_42

the isolation, identification, quantification, and analysis of saffron components. Although there are various kinds of chromatographic techniques, more recently a gas chromatography (GC) with a mass spectrometer (MS) detector for the volatile compounds and reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with a UV-Vis detector are the techniques of choice, permitting the isolation on an analytical value and the quantification and identification of the metabolites of interest in saffron. However, this chapter principally conducted the analysis of saffron compounds comprising the aroma and most aroma-active compounds using GC-MS and GC-MS-olfactometry setups and bioactive compounds such as carotenoids, flavonoids, and phenolic compounds using HPLC techniques.

Keywords

Saffron · *Crocus sativus* · Extraction techniques · Aroma and aroma-active · Bioactive compounds · GC-MS-olfactometry · HPLC-MS-DAD

Abbreviations

1-D	One-dimension
2-D	Two-dimension
3-D	Three-dimension
AEDA	Aroma extract dilution analysis
Charm	Combined hedonic aroma response measurement
DAD	Diode array detector
ESI	Electrospray ionization
FD factor	Flavor dilution factor
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
GC-MS-O	Gas chromatography-mass spectrometry-olfactometry
HD	Hydrodistillation
HPLC-DAD-MS	High-performance liquid chromatography with diode array detection and mass spectrometry
HS	Headspace
HTCC	4-Hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde
LLE	Liquid-liquid extraction
MSDE	Microsimultaneous hydrodistillation-extraction
MSDE	Micro-steam distillation extraction
PDA	Photodiode array
PTE	Purge and trap extraction
RP-HPLC	Reversed-phase high-performance liquid chromatography
SAFE	Solvent-assisted flavor evaporation
SBSE	Stir bar sorptive extraction
SD	Steam distillation
SDE	Simultaneous distillation/extraction

SFE	Supercritical fluid extraction
SPME	Solid-phase microextraction
TD	Thermal desorption
USAE/UV-Vis	Ultrasound-assisted extraction/ultraviolet−visible spectroscopy
USE	Ultrasonic solvent extraction
VHS	Vacuum headspace

1 Introduction

Saffron (*Crocus sativus* L.) is provided from the dried and dark-red stigmas of flowers belonging to the Iridaceae family. The taxonomic categorization of *C. sativus* is described as follows:

Kingdom: Plantae
Division: Magnoliophyta
Class: Liliopsida
Order: Asparagales
Family: Iridaceae
Genus: *Crocus*
Species: *sativus*

Among the 85 species of *Crocus* distributed around the world, *C. sativus* is the most expensive at nearly US$50–60 per gram with its characteristic color, aroma, and taste in addition to medicinal features which has received more cultivation interest in numerous countries [1–4]. To produce 1 pound of this valuable crop, nearly 75,000 blossoms of flowers or a number of 225,000 stigmas are required [2].

Regarding the total content of saffron production, the biggest producer in the world is Iran, which has cultivated and traded the spice for centuries around the world. After Iran, Spain, India, Italy, Greece, and Morocco are the main producers [5]. Furthermore, saffron has successfully been cultivated in France, Pakistan, Turkey, Azerbaijan, Egypt, the United Arab Emirates, Japan, and Switzerland, and lately this unique spice has also been introduced to nontraditional areas such as the USA, Chile, Argentina, New Zealand, and Australia [4]. Today, Iran produces approximately more than 90% of the total production of saffron in the world [5, 6].

Saffron (*Crocus sativus* L.) has been applied as a main dietary ingredient in diverse regions worldwide since ancient times; however, over the last decade, saffron consumption has experienced an extensive increase worldwide. The main saffron-importing countries include the USA, Germany, Italy, France, Switzerland, and the UK (International Trade Centre, 2006). Spain also imports large amounts of saffron, particularly from Iran, Morocco, and Greece, for its internal market consumptions and for re-export [7].

Saffron usage historically predates around 3,000 years, covering plenty of cultures, civilizations, and continents [8]. Today, the usage of saffron is well-known not only for coloring and flavoring food agents but also for the therapeutic interests and

even in the mediation of the different health disorders [2]. Moreover, saffron is less widely applied as an ink or dye, especially in the textile, perfume, and cosmetics industries [4, 9]. Saffron provides a luminous yellow-orange color to items soaked with it. The honey with hay-like, green/grassy, and metallic notes is depicted by cooking experts and saffronologists as the descriptors of this unique spice. The taste of saffron is similar that of hay and is bitter [1]. Based on these properties, saffron is extensively applied in the food industry such as baked crops, meat, fish, soups, bread, confectionary and pastry, dairy products, cheese, butter, ice cream, dressings, puddings, sausages, curries, liquors, and alcoholic and nonalcoholic beverages [1]. It is also applied as a good condiment for rice in Iran, India, Spain, and other territories. The abovementioned pleasant interests have been attributed to the diverse chemical compounds with various contents existing in the stigmas of the flower [2].

Overall, saffron can be divided from the point of view of the chemical composition into major and minor fractions. Indeed, along with the present primary metabolites (e.g., fats, carbohydrates, vitamins, and minerals) [10], the saffron plant includes a large number of compounds ascribing to various groups of secondary metabolites such as monoterpenoids, carotenoids, anthocyanins, and flavonoids [7]. Crocetin (a natural carotenoid dicarboxylic acid as a precursor of crocin), crocin (mono-glycosyl or di-glycosyl polyene esters), picrocrocin (monoterpene glycoside precursor of safranal and a product of zeaxanthin degradation), and safranal (Fig. 1) [7] are the four main bioactive compounds in saffron which contribute both organoleptic profile of saffron (pigment, pigment, taste, and odor, respectively) and health-progressing features [11]. There are several literature reviews on diverse aspects of

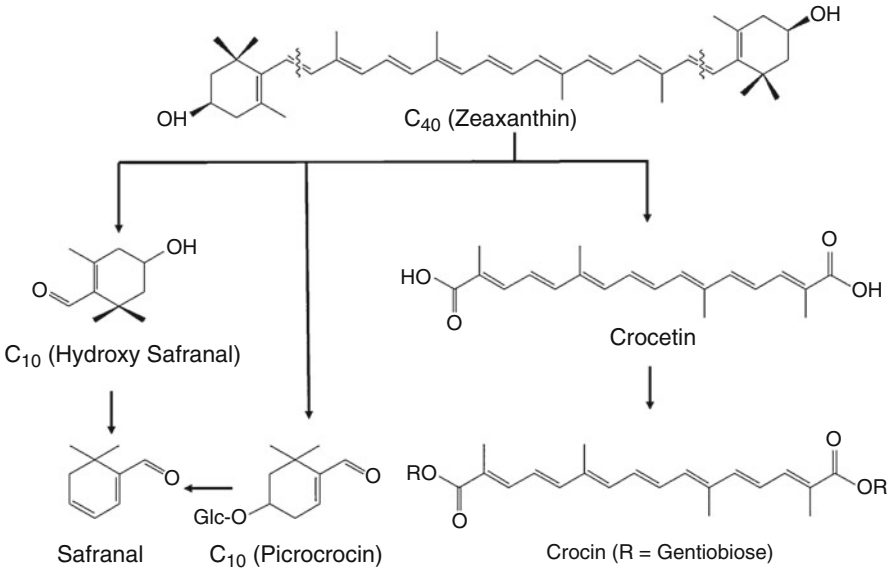


Fig. 1 Commonly admitted hypothesis for the formation of the secondary metabolites in saffron from the zeaxanthin precursor

C. sativus comprising its chemical composition [12, 13]; traditional and modern uses; pharmacology and clinical uses [14]; usefulness in medicine, cosmetics, and coloring industries [14]; reproductive biology [12]; production and distribution [15]; and cultivation, harvesting, processing, and yield [1].

Isolation, identification, and quantification of bioactive compounds from complex and natural matrix of foodstuffs are a primary challenge of initial interest in food quality measurement and characterization. The perception of the composition in saffron matrix has received more attention in last decades. Therefore, due to the complicated structure of saffron matrix, there are challenges for the extraction, isolation, identification, and quantification of its chemical profile. Chromatography is a set of constituents' separation techniques in a complex mixture. Recently, chromatographic methods were widely used for the isolation, identification, quantification, and analysis of saffron components. However, this chapter principally focuses on the gas chromatography (GC) and high-performance liquid chromatography (HPLC) techniques conducted to analysis of saffron compounds.

2 Saffron Chemistry

The proximate analyses of the chemical composition in saffron regarding dried and red stigmas of the flower have been disclosed by weight (% w/w), including 10% moisture, 12% protein (N*6.25), 6% inorganic matter (ash), 6% nonvolatile oils, 1% volatile oils, 5% crude fiber, and 53% water-soluble matters comprising 14% sugars, 10% gums, 8% pentosans, 6% pectin, 6% starch, 2% α-crocin, and 1% other carotenoids [10, 16, 17]. Additionally, trace elements of thiamine and riboflavin vitamins have been found in saffron [11]. The amounts of all chemical compounds in saffron may alter considerably because of growing situations and geographical origin [9].

The quality of saffron is determined by its common combination of intense red color, aromatic smell, and bitter taste [14]. The literature data shows the red stigmas of the saffron flower involve three major metabolites: (1) crocins being saffron-colored compounds (uncommon water-soluble carotenoids because of their elevated glycosyl extents), (2) picrocrocins being the principle constituents responsible for bitter taste of saffron, and (3) safranal being a volatile compound responsible for the unique aroma of saffron [4, 11, 13]. High-quality saffron is composed of approximately 30% of crocins, from 5% to 15% of picrocrocin, and generally up to 2.5% of volatile compounds involving safranal [14].

The word saffron probably originates from the Persian word sahafaran derived from asfar which means yellow or white to redden [7]. This name mentioning the elevated concentration of carotenoid pigments existing in the stigmas of the flowers contributes mostly to the profile of color in this unique spice [2]. Both lipophilic and hydrophilic carotenoids were reported in saffron [18]. A number of lipophilic carotenoids have been reported comprising trace extents of lycopene, α- and β-carotene, zeaxanthin, phytoene, and phytofluene, being the oil-soluble color pigments in saffron [10, 19]. From the hydrophilic carotenoids, crocins are either

mono- or di-lycosyl polyene esters of crocetin in which D-glucose and/or D-gentiobiose happen as carbohydrate residues [20, 21].

α-Crocin (crocin 1), a digentiobioside, is the most predominant crocin with an elevated solubility which is ascribed to these sugar moieties. Crocin with deep red pigment promptly dissolves in water to generate a solution with an orange color, thereby introducing crocin extensively applied as a natural food colorant. Apart from this feature, crocin also has an antioxidant properties such as free radical quenching and cells and tissues protecting against oxidation [22–24].

The bitter taste of saffron comes from picrocrocin, the β-D-glucoside of hydroxysafranal (4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde), being the second most predominant compound (by weight), altering between 1% and 13% in dry matter of saffron [13, 18]. Its structure was first reported by Kuhn and Winterstein [25]. The stereochemistry at the chiral center of the aglycone moiety was identified to be (4*R*) [26] being in relationship with the chirality of its assumed precursor, the carotenoid zeaxanthin. The hypothesized bio-oxidative cleavage of zeaxanthin gave rise to picrocrocin and crocin, the intensively water-soluble pigment of saffron [19]. During the drying process, high temperature and/or the action of glycosidases willingly changes picrocrocin into the aroma-active safranal [19].

Saffron's aroma profile is relatively complicated, originating principally from safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) [27]. The stigmas of saffron freshly picked are practically odorless, containing the ordinary saffron flavor being promoted during the drying process. Safranal is particularly generated throughout the bitter glycoside picrocrocin hydrolysis by the enzymatic activity, the reaction producing safranal, and D-glucose. Along with picrocrocin, there are several other glycosides that might undergo the dehydration to produce a mixture set of compounds, which includes the volatile profile of saffron. Safranal and these further dehydration products might undergo other degradation to produce other volatile compounds [28].

Apart from safranal, 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (an isomer of safranal), 3,5,5-trimethyl-2-cyclohexen-1-one (isophorone), 3,5,5-trimethyl-3-cyclohexen-1-one (an isomer of isophorone), 2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-ketoisophorone), 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one, 4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde (HTCC), and 2,2,6-trimethyl-1,4-cyclohexanedione are also significant aroma compounds in saffron [29]. From these compounds, safranal contributes about 30–70% of volatile oil and 0.001 to 0.006% of dry matter [5, 30]. In addition to its common spicy aromatic features, safranal has also demonstrated an elevated antioxidant capacity [22, 31] as well as cytotoxicity toward certain cancer cells in vitro [32].

3 Chromatographic Methods

Chromatography is described as an array of techniques applying for the isolation of analytes in a structure. There are two phases in this technique, namely, the stationary and mobile phases. The isolation of analytes is due to the diversity between partition coefficients of the two phases. Chromatography is the most well-known and

extensively applied analytical technique. Nevertheless, the possibility of the isolation in every compound in a complicated instance when applying a one-dimensional (1-D) isolation procedure has been appraised as varying between 19% and 37%, in a random mix, belonging to the complication and compound purity demanded [33].

Though one dimension of isolation represents adequate resolution for a large number of instances and analytical issues, a considerable number of applications still necessitate that the promoted resolution potency be utilized more than that supplied via a single dimension. In these situations, the isolation potency could be increased by applying more than one mechanism or isolation technique. Multidimensional chromatography is an isolation technology that applies two orthogonal isolation mechanisms to enhance the resolution potency and peak capacity of an experiment by raising the experiment selectivity.

Multidimensional chromatography itself could be divided into two groups: (1) only certain parts from the first dimension are introduced into a second dimension for other isolation, named multidimensional chromatography, 2-D, [34], and (2) every part eluting from the first dimension is introduced into the second dimension for further isolation named comprehensive multidimensional chromatography [35]. The technology could be led not only in the gas phase, 2-D comprehensive GC (GC × GC) but also in the liquid phase, LC × LC. A third dimension of information could be coupled by applying the information-rich detection setups, including a mass spectrometer [36–38].

Application of these and other techniques is almost accompanied by an enhancing number of biological instances needing prompt quantitative analysis, along with a decrement in the eligible quantitation amounts, as the bioavailability of plenty of analysis is at a low level and thus objective concentrations are very low. Therefore, suitably designed, quick, useful, and sensitive bioanalytical techniques are required. Usually a reliable bioanalytical technique, which is convenient for an intended target, should accomplish the requirements of validity guidelines such as precision, accuracy, sensitivity, selectivity, stability, and reproducibility [39]. There are various kinds of chromatographic techniques, comprising paper chromatography, gas chromatography, thin-layer chromatography, ion-exchange chromatography, liquid chromatography, and high-performance liquid chromatography. More recently, reversed-phase high-performance liquid chromatography (RP-HPLC), equipped with a UV-Vis detector or, more often, a gas chromatography (GC), with a mass spectrometer (MS) detector for the volatile compounds [29, 40, 41] and UV-Vis-DAD for nonvolatile compounds [42], is the method of choice, allowing the separation on an analytical level and the identification and quantification of the metabolites of interest [7]. Therefore, this work principally focuses on the application of the GC and HPLC in saffron spice as a food ingredient.

3.1 Gas Chromatography (GC)

The types of analysis that could be performed by GC are extensive. GC has been utilized for the determination of cholesterol and other sterols, triglycerides, fatty acids, gases, alcohols, solvent analysis, water and simple sugars, as well as vitamins,

amino acids and peptides, oligosaccharides, herbicides, pesticides, food additives, nitrosamines, antioxidants, drugs, polychlorinated biphenyls (PCBs), volatile compounds, and so forth. The fact that GC has been employed for these diverse applications does not necessarily mean it is the most suitable technique as better selections are often present.

GC is commonly suitable for the analysis of thermally stable volatile compounds. Compounds that do not meet these requirements such as vitamins, peptides, amino acids, oligosaccharides, and sugars are more suitable for the analysis by a technique comprising supercritical fluid chromatography (SFC) or high-performance liquid chromatography (HPLC).

Saffron volatile compounds can be categorized into two groups. The first group includes compounds which have structures that bear an explicit similarity to that of safranal and are disclosed also as isophorone-related compounds (C9 and C10 group of compounds) [40, 43]. These compounds are 4-ketoisophorone, isophorone, 2,2,6-trimethyl-1,4-cyclohexanedione, 4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one, 2-hydroxy-3,5,5-trimethylcyclohex-2-en-1,4-dione, 4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde, and 4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-diene-1-carboxaldehyde. The second group includes C_{13}-norisoprenoids. These compounds are formed from lipophilic carotenoids [40]. These compounds are 3-[(*E*)-but-1-enyl]-2,4,4-trimethylcyclohex-2-enol, *E*-4-(2,6,6-trimethylcyclohexyl)-but-3-en-2-one, (*E*)-4-(2,6,6-trimethyl-7-oxa-bicyclo-[4.1.0]-heptan-1-yl)-but-3-en-2-one, and 3-(but-1-enyl)-2,4,4-trimethylcyclohexan-1-ol.

Moreover, the profile of the volatile compounds that are acquired is highly related to the extraction techniques that are utilized for the separation [29, 30, 44]. The number of volatile compounds has been detected in saffron spice achieved over 160 [5, 30]. Nevertheless, it is known that only a trace part of the numerous volatile compounds occurring in food indeed contributes to the overall aroma. Hence, a significant function in flavor investigations is to isolate the powerfully aroma-active compounds from the less odorous or odorless compounds existing in food [45].

Separation of the volatile compounds in saffron could be obtained by numerous extraction methods prior to the gas chromatography analyses [46]. In order to specify which compounds contribute significantly to the aroma of a crop or which compounds are responsible for the variations between the aromas of two crops, it is essential to ensure that the extraction technique supplies an extract with an aroma that is representative of the main crop. The GC system could be equipped with three different detectors such as (i) a mass selective detector (MSD), (ii) flame ionization detector (FID), and (iii) sniffing port (olfactometric detection). This system can provide an opportunity to simultaneously achieve an MS signal for the identification, an FID signal for the quantification, and the odor characteristics of each compound discovered via the sniffing port [46].

3.1.1 Sample Preparation Techniques Prior to GC Analyses

Preparation of sample affects almost all the later trial stages and is therefore critical for unerring qualification, confirmation, and quantification of compounds. It consists

of both the separation and pre-concentration of compounds of interest from different matrices as well as making the compounds more appropriate for isolation and identification. As analytical chemistry flourishes, gentle preparation of the sample becomes a main section of analysis, generally consuming up to 80% of the total analytical analysis time, including five stages, which are sampling, preparation of sample, isolation, identification, and data analysis [47].

While chromatographic techniques are contributed in the organic molecules analysis, preparation of the sample in bioanalytical techniques usually utilizes solid-phase and liquid-liquid extractions. Despite ultra-prompt chromatographic analyses, prevalent sample preparation methods are still more laborious and time-consuming due to an abundant number of stages.

Subsequently, sample preparation has increasingly progressed over the last few years [39, 47]. Development in analysis methods has led to numerous volatile compounds detected in foods [48]. Over the last few decades, serious investigations have been performed based on the organoleptic activity of the individual odor compounds of foods and beverages and the affiliation between the odor and the composition of the volatile section of these products. Most of the achievements within this field could be ascribed to the coupling of GC with olfactometric detection [49].

Determination of aroma compounds applying instrumental techniques includes two steps. The first and most important step of the analysis is isolation of the constituents from the food matrices. The genesis of the olfactograms extensively links to the separation process, as several comparative investigations disclosed that the utilization of various sample preparation methods (even employing diverse solvents in the case of liquid-liquid isolation) may impact the composition and amounts of the separated compounds [50–52]. The extract separated should be representative; therefore, the selection of a suitable sample preparation process is very important [53, 54].

Separates acquired utilizing perfect extraction techniques, comprised of distillation and solvent extraction, do not always reverberate the composition of the aroma reaching the aroma and taste receptors through drinking and eating. It ought to be emphasized that only some of the volatiles measure up to the characteristic aroma of food and beverages. The volatile fraction composition of the food products could vary belonging to the solubility of the compounds and the features of the matrices such as the amount of sugar.

Subsequently, it is more advantageous to employ separation techniques which reverberate the volatile compounds' liberation from the matrix rather than ascertaining the overall amount of such compounds, as this facilitates the correlation with organoleptic analysis outcomes. Both dynamic and static headspace methods could be utilized for this target; nevertheless, due to the feasibility of compound enrichment, dynamic techniques are applied widely [55].

The prevalent condition and last developments in sample preparation techniques were completely reviewed by [47]. The current review discussed some of the aroma extraction techniques used in saffron sample studies up to now, simultaneous distillation/extraction (SDE) and solvent-assisted flavor evaporation (SAFE) [46],

steam distillation (SD) [29], stir bar sorptive extraction (SBSE) [56], hydrodistillation (HD), microsimultaneous hydrodistillation-extraction (MSDE) [40, 56], vacuum headspace (VHS) [29], liquid-liquid extraction (LLE) [58], supercritical fluid extraction (SFE) [57], ultrasonic solvent extraction (USE) [60], and solid-phase microextraction (SPME) [59], described below in details.

Steam Distillation (SD)

Steam distillation is a remarkable technique which allows for the separation of volatile compounds from plants comprised of some amines, organic acids, and volatile oils, as well as relatively volatiles, insoluble in water (Fig. 2). Furthermore, steam distillation has been utilized to separate the volatile oil fraction from both plant material and a previously provided extract in a solvent with low boiling points such as diethyl ether or petroleum ether [61]. This method contains shortcomings, as it requires considerable energy consumption. A high temperature (ca. 100 °C) might result in the thermal decomposition of compounds. This could also impact the volatile oil compounds, thus causing flavor alterations [62].

Tarantilis et al. [29] investigated the volatiles of the dried and dark-red stigmas of saffron. For the separation of the aroma, they applied three distillation techniques such as micro-steam distillation extraction (MSDE), steam distillation (SD), and vacuum head space method (VHS) for the output to the extraction of representative volatile composition of saffron, and their results presented some qualitative variations among the three extraction techniques which revealed 16, 13, and 8 compounds, respectively [29].

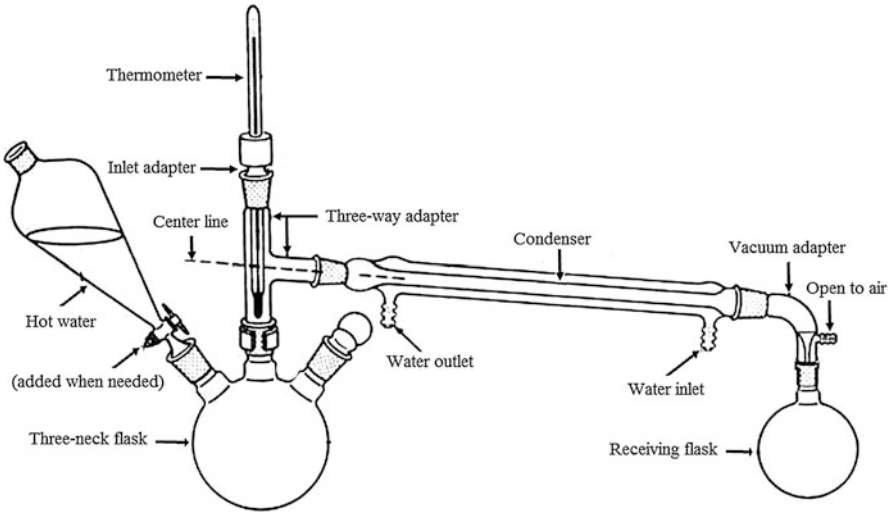


Fig. 2 A schematic exhibition of a steam distillation extractor

The abovementioned techniques disclosed the major characteristic compounds including 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde, namely, safranal (ca. 70% of the material in the extract); 3,5,5-trimethyl-2-cyclohexen-1-one, namely, isophorone (ca. 14%); 3,5,5-trimethyl-3-cyclohexen-1-one, isomer of isophorone (ca. 5%); 2,6,6-trimethyl-2-cyclohexene-1,4-dione (ca. 4%); and 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde, isomer of safranal (ca. 3%). Plenty of volatile compounds (ca. 1–0%) were also available.

The intensive statuses of the SD technique formed substantially more elevated boiling point compounds than in the saffron main aroma. Numerous such compounds are presumably artifacts, including 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxylic acid. This compound may have resulted from oxidation of safranal during the separation. Furthermore, this means that safranal as are all aldehydes is simply oxidized, especially when subjected to the elevated temperatures confronted through SD.

It is important to note that the steam distillation technique is sorely inappropriate for the determination of the volatile compounds in saffron due to the existence of carotenoids. Numerous volatiles in the concentrate were likely the degradation compounds of saffron carotenoids forming from the action of heat and oxygen on these ingredients [29, 63].

The MSDE technique formed both low and high boiling point compounds. This technique was less intensive than that of the SD technique. Therefore, odorants comprising 3,7-dimethyl-1,6-octadiene generated through the thermal degradation of carotenoids existed [29]. The eight compounds determined through the VHS technique were also discovered in the volatile oil formed by the two other techniques. Many of these compounds were the safranal and isophorone derivatives. Additionally, only the isomer of isophorone (3,5,5-trimethyl-3-cyclohexen-1-one) was not detected. It is hypothesized that the VHS extraction more resembled MSDE rather than SD [29].

Zareena et al. [64] investigated the effect of γ-irradiation at doses of 2.5 and 5 kGy, obligatory for the decontamination of microbes, in aroma features of saffron using the steam distillation extraction techniques. According to their outcomes, no significant qualitative alterations were detected in the aroma compounds upon the irradiation, though a trained panel in the sensory analysis can find slight quality deterioration at a dose of 5 kGy. They observed that the control samples, safranal, composed of the main compound detected, accounting for 30% of the oil, being far lower than the reported data values (60–70%). Ketoisophorone (3%), α-isophorone (5%), 2,4,4-trimethyl-3-carboxaldehyde-5-hydroxy-1-cyclohexanone-2,5-diene (3.4%), and dihydro-beta-ionone (3.71%) were the further main compounds determined in oil [64].

According to earlier reports [29], intensive situations are utilized in the SD and MSDE techniques terminated in the volatile oil over-enriched with elevated boiling point compounds due to the degradation of low boiling-sensitive compounds. Zareena et al. [64] reported that these kinds of impacts, besides the source or origin of instance, can account for the low amount of safranal. They reported that no

detectable qualitative variations in the volatile compounds can be detected between the irradiated and control samples.

Liquid-Liquid Extraction (LLE)

Solvent extraction technique utilizes nonpolar solvents being solvable with water to isolate the target compound from water by applying more solubility of the target compound in the solvent than water. Preferably, one selectively extracts the purpose compound by applying a solvent whose polarity is close to that of the purpose compound. Volatile solvents including benzene, hexane, ether, dichloromethane, and ethyl acetate are commonly employed for the separation of semi-volatile compounds from water. Hexane is appropriate for extraction of nonpolar compounds including aliphatic hydrocarbons; benzene is appropriate for aromatic compounds; and ether and ethyl acetate are appropriate for relatively polar compounds consisting of oxygen. Dichloromethane has high efficiency extracted for an extant range of nonpolar to polar compounds. It is appropriate for simultaneous analysis due to the following advantages: its boiling point is low and simple to reconcentrate after extraction, it is simple to isolate from water owing to its more elevated specific gravity, and it is nonflammable.

However, dichloromethane, like benzene, is carcinogenic, and the current consensus is to avoid utilizing these solvents in liquid-liquid extractions. Indeed, despite its simplicity, the modern trend is to replace LLE by other techniques because high-purity solvents are demanded for small analysis and due to the need to diminish the environmental and health risks associated with their manipulation. Furthermore, in the case of biological aroma, LLE cannot be applied to live samples [65]. Cadwallader et al. [66] used the LLE method for extracting aroma compounds of the Spanish "Mancha Superior" and compared it with the SDE method. The explanation of the comparison of these two methods was described in detail in Sect. 3.1.1.3. Additionally, Amanpour et al. [46] utilized the LLE method for the isolation of the aroma compounds in the Iranian saffron in a representative study.

Simultaneous Distillation-Extraction (SDE)

Simultaneous distillation-extraction (SDE) is a prevalent distillation-based sample preparation technique for aroma, which is well-known as the Likens-Nickerson technique (Fig. 3). An extent of the aroma-forming sample (e.g., sliced plant tissue, beverage, food), together with distilled water for dry samples, is subjected in flask II, and flask III attains an appropriate bulk of an extracting solvent more condensed than water (e.g., chloroform, dichloromethane). Flasks II and III are heated; then the water and solvent vapors are led to the extractor body, where they compress through the surface of cold tube IV [67]. In the procedure of this technique, aroma compounds are separated from the food matrices via the water vapor and transferred to the organic phase when the liquids condense together on the cold tube. Solvent and water are gathered together in the extractor body after their condensation and return to the corresponding functions, premising consecutive reflux. A modified apparatus provided an opportunity to use solvents lighter than water comprised of pentane or ethyl acetate as extractors [67].

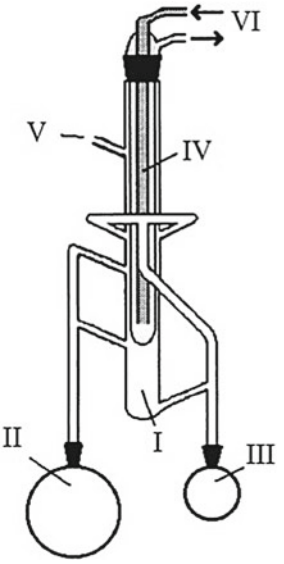


Fig. 3 A schematic shape of Likens-Nickerson apparatus/ simultaneous distillation-extraction extractor

I) Body, II) Sample flask, III) Extracting solvent flask, IV) Cold tube, V) Inlet for purge gas, VI) Cold water inlet and outlet

This technique has been employed for separating the volatiles in saffron [40, 68]. Cadwallader et al. [66] separated volatile compounds from Spanish "Mancha Superior" saffron by two different methods such as SDE and LLE extraction. A total of 46 volatile compounds were determined. Among the detected volatiles, a total of 30 volatile compounds were common to both extracts, the LLE and SD. 2,6,6-Trimethyl-l,3-cyclohexadiene-l-carboxaldehyde (safranal) was the most prevailing volatile compound, followed by 3,5,5-trimethyl-2-cyclohexen-l-one (isophorone) and 2,6,6-trimethyl-2-cyclohexene-l,4-dione [68]. The concentrations of safranal were analogous to reported studies [69]. Moreover, the relative abundances (based on the safranal) for dominant volatile compounds were in proper agreement with former investigations [40, 43, 68]. Despite the agreement with reported data, volatile compounds of extracts using the SDE and DE techniques are altered from each other with regard to amounts of main volatile compounds as well as in the kinds of volatile compounds determined. LLE was released for the separation of acidic compounds (acetic acid, 2-methylpropanoic acid, and hexanoic acid), while no acids were found in the SDE extracts. Additionally, SDE extracts included higher amounts of main saffron volatile compounds such as 3,5,5-trimethyl-2-cyclohexen-1-one (isophorone), 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (safranal) and 2,6,6-trimethyl-2-cyclohexene-1,4-dione. Elevated amounts of these compounds might have been an outcome of thermally induced hydrolysis of their glucoside precursors over SDE [43].

Additionally, it is probable that further thermally formed compounds were generated over SDE. For instance, the sugar breakdown products such as

5-methyl-2-furancarboxaldehyde and 2-furancarboxaldehyde were only found in the extracts using SDE technique. SDE extracts also included four megastigmatriene isomers (megastigma-7,9,13-triene, megastigma-4,6,8-triene isomer, megastigma-4,6,8-triene isomer, and megastigma-4,6,8-triene isomer), which have been formerly disclosed as volatile compounds of star fruit [68]. In addition to this research, Amanpour et al. [46] studied the Iranian saffron volatile compounds, and they used four different extraction methods in a representative study which included SDE and LLE methods described in detail in Sect. 3.1.1.10.

Ultrasonic Solvent Extraction (USE)

Ultrasonic solvent extraction includes the application of elevated intensity, elevated frequency sound waves, varying between 16 kHz and 1 GHz, and their interaction with materials [9]. Indeed, ultrasonic vibrations are the energy facilitating source to liberate some compounds from the sample matrix [70]. USE is a common technology as it does not demand complicated instruments and is normally inexpensive (Fig. 4) [71]. It can be applied both in large and small scale [72]. USE comprises ultrasonic impacts of acoustic cavitation. As for the ultrasonic action, liquid and solid particles are vibrated and accelerated, and owing to that solute swiftly diffuses out from solid phase to solvent [68]. Numerous feasible mechanisms for ultrasonic increment of extraction, comprising cell disruption, developed penetration and increased swelling and capillary impact, and hydration procedure have been proposed [73]. If the severity of ultrasound is enhanced in a liquid, then it attains at a point at which the intramolecular powers are not able to hold the molecular structure intact, so it breaks down and bubbles are generated; this procedure is named

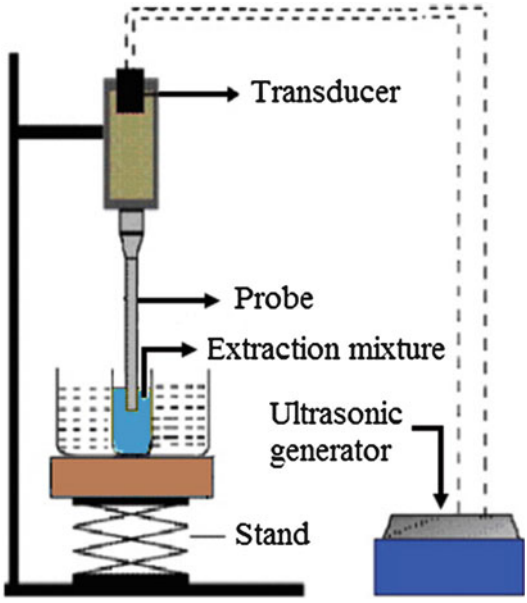


Fig. 4 A schematic exhibition of UAE setup

cavitation. Collapse of bubbles can generate physical, chemical, and mechanical impacts which terminate in the disruption of biological membranes to facilitate the liberation of extractable compounds and increase penetration of solvent into cellular materials and progress mass transfer [68, 74].

The useful impacts of sound waves on extraction are ascribed to the generation and asymmetrical collapse of micro-cavities near the cell walls leading to the formation of micro-jets rupturing the cells. The bubbles' pulsation is believed to result in acoustic streaming which develops a mass transfer rate by inhibiting the solvent layer surrounding the plant texture from attaining saturated and therefore development of convection [75]. The skin of external glands of the plant cell wall is very thin and could be readily ruined by sonication which liberates the volatile oil amounts into the extraction solvent, hence causing decreased extraction time and enhanced extraction output [76].

Jalali-Heravi et al. [60] studied the volatile compounds of Iranian saffron via the USE technique applying GC-MS. Variables impacting the extraction process were screened by applying a 25–1 fractional factorial scheme and between them; sample content, volume of solvent, solvent ratio (in binary mixture of solvents), and time of extraction were optimized by using a rotatable central composite scheme. The best levels of factors were 2.38 g instance, 29.04-mL solvent, 69.23% MeOH solvent ratio, and 71.8 min for the time of extraction. A total of 40 compounds were determined for Iranian saffron using GC-MS, accounting approximately 90% of the total peak area. The main compounds were safranal (26.29%); bicyclo[3,2,0] hept-2-ene-,4-ethoxy-,endo (5.69%); linoleic acid (4.77%); 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, namely, HTCC (4.44%); and non-adecanol (3.32%) [60].

Maggi et al. [5] designed a comparative study to analyze the 418 commercial saffron instances depending on diverse ISO classes. UAE with an organic solvent and dynamic headspace desorption followed by gas chromatography/mass spectrometry were employed to characterize for the volatile profile of saffron. For both techniques, the saffron aromatic composition was specified by spicy aromatic features because of safranal, the most predominant volatile compound, by a flowery contribution imputable to 2,2,6-trimethyl-1,4-cyclohexanedione and isophorone, together with spicy and citrus aromatic features from 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one and 4-ketoisophorone, respectively. UAE permitted the discovery of a more number of volatiles, whereas the dynamic headspace technique was faster and a fewer amount of saffron was demanded. Compared with the ultrasound-assisted extraction technique, the dynamic headspace technique specified the instances as possessing a spicier and more floral aromatic contribution, hence corroborating that the isolation technique remarkably alters the aromatic fingerprint of saffron instances.

Maggi et al. [77] assayed the saffron quality control by using UAE of safranal optimizing on the basis of the solvent, diethyl ether, hexane, and chloroform, the extraction time, and the saffron concentration in each organic solvent. The most suitable extraction conditions were acquired when 20 g/L of saffron was extracted with chloroform for 15 min. The comparison of safranal content acquired from 40

saffron instances by applying the UV-Vis is based on the ISO 3632 (2003) and previously reported technique (Maggi et al., 2009). There was no correlation between the safranal content acquired by ISO 3632 (2003) and validated GC technique, because there are further compounds existing in saffron absorbing at 330 nm, comprising the isomers of *cis*-crocetin esters [77].

Supercritical Fluid Extraction (SFE)

The SFE technique penetrates plant materials approximately as well as gases, and this is caused from their low viscosity and elevated diffusion coefficients. SFE is employed at temperature close to ambient, hence inhibiting the material from causing in thermal denaturation [78]. SFE is a well-known method of solvent extraction; however, its commercial application occurred gently because of the sophisticated and expensive high pressure equipment and technology demanded [78]. Simultaneously, their dissolving potency is similar to that of liquids. However, the desired transport features of fluids near their critical points permit deeper infiltration into solid plant matrix and more efficient and faster extraction than with conventional organic solvents [9]. The most widely utilized isolating agent is carbon dioxide (CO_2), due to its low price, low toxicity, and desired critical parameters ($Tc = 31.1$ °C, $Pc = 74.8$ atm). CO_2 as a nonpolar material is able of dissolving nonpolar or mildly polar compounds. An admixture of CO_2 with modifiers (polar organic solvents) is applied for extracting the polar materials. The modifiers enhance the solubility of compounds, preventing them from adsorption on the active sites of sample matrix. The most substantial advantages of SFE involve the following: remarkable decrement in the bulk of solvent utilized, decreased the time of extraction, automation facility, the trace amount of instance demanded, feasibility of online coupling with the isolation and identification methods (SFE/HPLC, SFE/GC), elevated purity and trace bulk of the extract, and elevated selectivity [70]. SFE is moderately efficient even for compounds with compact and extremely available mixture. It is particularly well adjusted for the separation of materials of low and medium polarity and elevated volatility. As a rule, carbon dioxide or carbon dioxide with a volatile polar modifier, comprising methyl diethyl ether, acetate, methanol, ammonia, or formic acid, is applied as supercritical fluids [70]. A supercritical fluid extraction system is demonstrated in Fig. 5 [79].

Lozano et al. [80] have developed a supercritical carbon dioxide extraction technique to acquire selectively volatiles of saffron without sample destruction. The impact of both temperature and pressure was investigated, 100 °C and 20 MPa being the most appropriate situations to isolate the total safranal amount. A reduction in supercritical fluid density was demonstrated to be a crucial factor for increasing the extraction potency of carbon dioxide. For all the experiment circumstances, the extracts principally concluded HTCC and safranal, as indicated by GC and HPLC techniques. Both chromatographic techniques were appropriate for safranal quantification and exhibited perfect compromise.

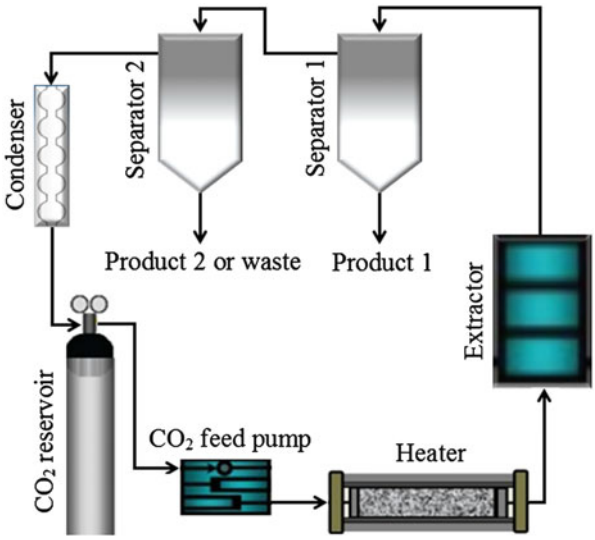


Fig. 5 Supercritical fluid extraction setup

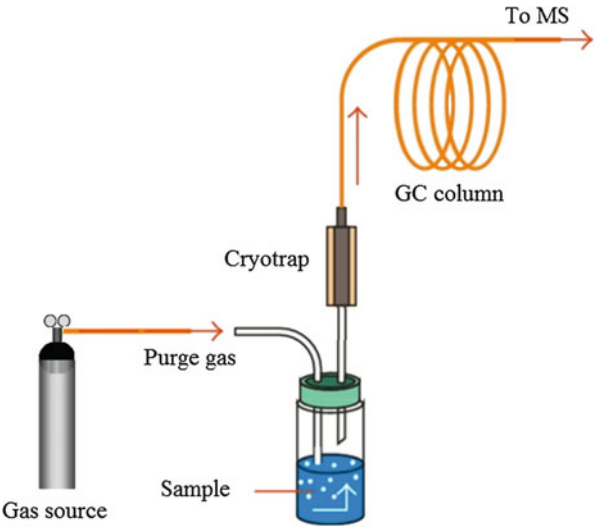


Fig. 6 Purge and trap extraction system

Purge and Trap Extraction (PTE) System

The purge and trap system is also referred to as the dynamic headspace method (Fig. 6) that diminishes matrix impacts and enhances sensibility, respective to static headspace methods. Instances including volatile compounds are subjected into a purge vessel, and a flow of inert gas such as nitrogen or helium is passed over the instance to remove the volatiles from the instance matrix at a steady flow rate for a determined time. Volatiles are desorbed from the instance into the headspace

(purged) above the instance and are then isolated from the stream of gas (trapped) by adsorbent filters.

After the process of purging is complete, the trap is quickly heated and back-flushed with carrier gas such as pure helium to desorb and transfer the compounds to the column of GC. Typical trapping (adsorbent) materials are activated charcoal, porous polymer beads, silica gel, further GC column packing materials, or combinations of such materials [65].

Cullere et al. [81] evaluated the aroma profile of a saffron instance from Valle de Jiloca (Teruel, Spain) by applying a purge and trap technique prior to GC-O. The LiChrolut EN cartridge (400 mg) was subjected on the top of a bubbler flask including 10 g of saffron. This instance was purged by a stream of nitrogen at ambient temperature for 4 h. Volatile compounds of saffron transferred in the headspace were trapped in the cartridge including the sorbent, and others were eluted with 3.2 mL of dichloromethane including 5% methanol.

Solid-Phase Microextraction (SPME)

Pawliszyn and Arthur [82] improved the SPME technique as a solvent-free technique concerning the sorption (adsorption-absorption) generality. Though it is a balance (non-comprehensive) technique, SPME was swiftly admitted as an easy, green, and miniaturized technique, which merges sampling, extraction, concentration, cleanup, and instance presentation in a solitary stage. Regarding its efficiencies, SPME rapidly became one of the 204 most extensively employed methods in different fields of analytical chemistry, involving environmental and biological applications [82]. Analyte enrichment by SPME has two stages. In the first stage, a fiber, coated with an adsorbent or stationary liquid, is exposed to a liquid sample, or the headspace above a sample and the analytes are sorbed on the fiber. In the second stage, the fiber is presented into the injection chamber of a gas chromatograph, where it is introduced to a high temperature or it is subjected into the injector of a liquid chromatograph. The liberated analytes are swept into the chromatographic column.

According to the position of the fiber coating in the extraction setup, the extraction techniques are categorized commonly into two modes: direct immersion (DI, the coating is completely immersed in the sample matrix) and headspace (HS, the coating is exposed to the gas phase above the sample matrix) modes. Solid-phase microextraction device is shown in Fig. 7. SPME is a sample preparation technique most suited for gas chromatography. Though SPME has been successfully mixed with HPLC, this needs moderately complex processes and supplementary devices. Hence, it could be anticipated that the method will be applied frequently with GC [9].

Auria et al. [59] analyzed the three saffron samples from various regions of Italy (Salerno, Sardinia, and Abruzzo) and one sample from Iran using SPME-GC/MS. They reported that the major compounds were those detected in ethereal extract of saffron in a study disclosed in 1971. Numerous compounds detected by applying further extraction-distillation methods were not detected. They detected 18 volatiles never found in saffron. In all the saffron samples, 3,5,5-trimethyl-2-cyclohexen-1,4-dione, 3,5,5-trimethyl-2-cyclohexen-1-one, 2,4,4-trimethyl-6-

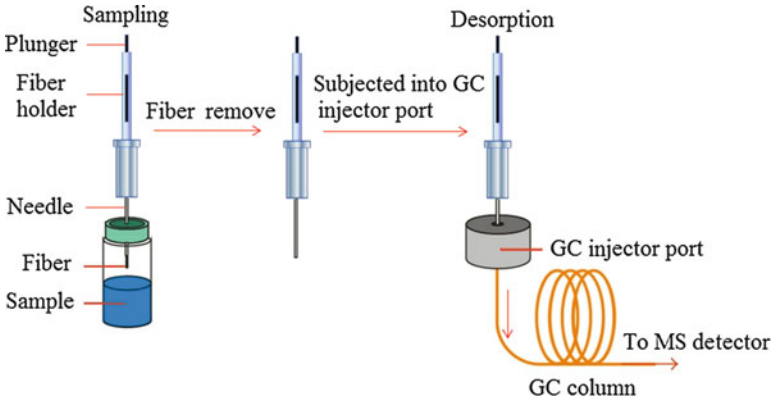


Fig. 7 Solid-phase microextraction setup

hydroxy-3-carboxaldehyde-2,5-cyclohexadien-1-one, and safranal were detected. 3,5,5-Trimethyl-1,4-cyclohexandione was detected in three samples. *β*-Ionone and 5,5-dimethyl-2-methylene-1-carboxaldehyde-3-cyclohexene were detected in six samples, and 2,6-di-t-butylphenol and nonanal were detected in two samples.

These volatiles demonstrate the compounds detected with the highest frequency. Concerning the relevant content of the compounds, safranal is the major compound in all the samples. The analyses indicate that saffron from various cultivation sites have some peculiarities because of the attendance of some uncommon compounds. The Iranian saffron sample indicated the attendance of 2,7,7-trimethyl-2,4-cycloheptadien-1-one, 3,5,5-trimethyl-2-hydroxy-2-cyclohexen-1-one, 1,3,3-trimethyl-2-(E-2-butenylidene)-3-cyclohexene, 1,1,3-trimethyl-2-(Z-2-butenylidene)-3-cyclohexene, 5,5-dimethyl-1,3-cyclohexadien-1-carbaldehyde, *β*-ionol, and 1-acetyl-5,5-dimethyl-bycyclo[3.1.0]hexane [59]. In another study, the influence of storage factor on the aroma profile of saffron was investigated [83].

Six samples of saffron from various regions of Italy are evaluated by SPME-GC/MS. Samples 1, 2, and 3 originated from cultivations of *C. sativus* in the zone of Salerno (Southern Italy) from 2000 to 2002. Samples 4, 5, and 6 are originated from cultivations in Sardinia, Italy (from 1998, 2000, and 2001, respectively). In all samples, 3,5,5-trimethyl-2-cyclohexen-1,4-dione, 3,5,5-trimethyl-2-cyclohexen-1-one, 2,4,4-trimethyl-6-hydroxy-3-carboxaldehyde-2,5-cyclohexadien-1-one, and safranal are detected. 3,5,5-Trimethyl-1,4-cyclohexanedione, 5,5-dimethyl-2-methylene-1-carboxaldehyde-3-cyclohexene, and β-ionone are discovered with 2,6-di-t-butylphenol, nonanal, and dihydro-β-ionone. Safranal is the principle compound in the all samples. The most significant variations are in the attendance of aldehydes and alcohols and oxidation products of the main terpenoid compounds. Moreover, the attendance of safranal, the most significant compound of the flavor, alters through the time, enhancing over 3 years and then declining after 5 years.

Du et al. [84] studied the quantitative structure-retention relationship (QSRR) investigations for the prediction of the retention times in 43 saffron aroma compounds being performed by using SPME-GC. The chemical descriptors were calculated from the molecular structures of the compounds of saffron aroma alone, and the linear and nonlinear QSRR models were constructed using the best multi-linear regression (BMLR) and projection pursuit regression (PPR) techniques. The foretell outcomes of the two approaches were in line with the data of experiment. The proposed models can also determine and supply some insights into structural properties that can play a role on the retention behaviors of the compounds of saffron aroma in the SPME-GC. The current investigation provides an easy but efficient approach for the investigation of the retention behaviors of further analogous herbs and plants [84].

To develop the stability of fibers, Sarafraz-Yazdi et al. [85] synthesized PEG/CNTs fiber, PDMS fiber, and PEG fiber by a sol-gel approach, for determining some polycyclic aromatic hydrocarbons (PAHs) such as fluorene, naphthalene, phenanthrene, and anthracene in aqueous saffron instance by direct immersion SPME and GC. A sol-gel method is employed for preparing the SPME fibers. Three types of sol-gel coatings on the fibers were examined and compared. They are consisted of poly(dimethyl siloxane) (PDMS), poly(ethylene glycol) (PEG), and a poly(ethylene glycol) modified with multiwalled carbon nanotubes (PEG/CNTs). The impacts of fiber coating, time of desorption, temperature of deorption, extraction time, rapid of stirring, and salting impact were optimized. Among the three kinds of the fibers, the sol-gel-derived PEG/CNT fiber has the best affinity for PAHs due to the particular features of multiwalled carbon nanotubes (MWCNTs).

Stir Bar Sorptive Extraction (SBSE)

SBSE was improved by Baltussen et al. [86] on the basis of the sorptive extraction. It is similar to SPME technique which prepared solventless sample. SBSE has the higher surface area and content of coating than the SPME fiber. Hence, SBSE in comparison with SPME was developed as extraction efficiency, and this could account for its major advantage [87]. Nevertheless, SBSE has typically longer extraction and desorption time (over 60 min) than the SPME because of the distribution of the volatiles in the large volume of coating. At the end of the extraction process, after rinsing with distilled water and drying the stir bar, sample can be straightly subjected to the thermal desorption (TD) setup in GC to analyze. From 70% to 130% is the recovery ranges of the compounds [9]. SBSE and TD coupled with GC-ion trap tandem mass spectrometry were first developed simultaneously as a technique for determining the 46 semi-volatile organic contaminants and pollutants of saffron sample. The designed analytical technique not only was easy, sensitive, and quick but also showed excellent repeatability, linearity, reproducibility, and accuracy through the limit of concentration examined. Additionally, target compounds exhibited greater correlation coefficients (>0.98), and detection ranges exhibited lower than 1 μg/kg except for simazine [27].

Maggi et al. [5] first applied saffron as an environmental biomarker to the characterization of the attendance of a complicated polycyclic aromatic

hydrocarbons (PAHs) admixture. To confirm the feasibility to utilize the saffron as an environmental bioindicator and to specify the bioaccumulation of compounds, current study was arranged to identify the quantitative information respecting to PAHs contamination in saffron. In this study, a total of 27 saffron samples obtained from various territories were applied for analysis using SBSE coupled with GC-ion trap tandem mass spectrometry. Consequently, the samples contained diverse values of contaminants, but some of them have greater concentrations than the maximum residue ranges of pollutants confirmed by the European Commission for spices.

Thermal Desorption (TD)

After adsorption, thermal desorption could introduce the whole trapped purpose compounds into GC-MS (Fig. 8). This technique is suited for volatiles' analyses and needed small amounts of sample which included low concentrations of purpose compounds. Heating the adsorbent materials desorbs the adsorbed compounds thermally. By altering the direction of GC carrier gas flow by applying a three-way valve, these compounds are introduced into the column of GC. All these procedures are automated and this equipment is available on the market. In this setup, cold trap is also equipped in order to trap the desorbed samples in a concentration tube cooled via liquid nitrogen and then subjected into the GC column through the heating. This kind of cold trapping system provides the faster sample preparation and makes sharper peaks. In fact, the advantages of TD are included in economy, swift, sensitivity, and facility of both liquid and solid sample analyses. Therefore, such

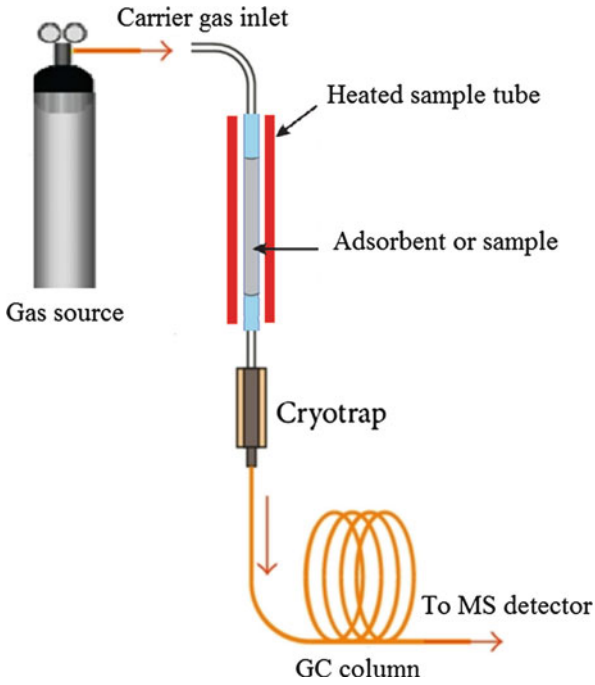


Fig. 8 Thermal desorption setup

system was improved to prepare solventless and easy sample to subject the volatile and semi-volatile compounds of foods into the GC or GC-MS [88].

Alonso et al. [27] utilized the TD-GC in saffron samples for the quantification of the safranal content. Seventy-two percent of the total volatile fraction of saffron is composed of safranal. β-cyclocitral was used as an internal standard, and samples were submitted to 70 °C for 8 min. Therefore, the determination percentages of the total volatiles for the first, first-second, and third desorption were 83, 95, and 100%, respectively.

Recently, Amini et al. [89] studied the impact of cold plasma on crocin esters and volatile oils of saffron using thermal desorption system. After the treatments (Ar, Ar/5% O_2 and Ar/10% O_2 at 8 and 12 kV of voltage), a decrement in crocin esters and saffranal and an enhancement in isophorone and 4-ketoisophorone were confirmed. After 4 min, the saffron samples treated with Ar/20% O_2 had blackened and the treatment was discontinued. The outcomes exhibit that raising the input voltage and enhancing the extent of added oxygen to argon gas promoted the variations in the safranal and crocin esters. There was no *trans*-2G, *cis*-4GG or *cis*-3Gg compounds observed after the Ar/10% O_2 cold plasma treatment at 12 kV.

Solvent-Assisted Flavor Evaporation (SAFE)

The SAFE technique was progressed as a well-set and versatile distillation setup for the swift and precise separation of volatile compounds from complicated food matrix by Engel et al. [90] (Fig. 9). The SAFE unit contained a high vacuum pump (5×10^{-3} Pa) and permits to separate the volatile fractions of either solvent extracts; aqueous materials comprising fruit pulps, beer, and milk; or even matrix containing an elevated oil amount. In comparison to formerly employed methods for either solvent extracts or fatty matrices (50% fat) like elevated vacuum transfer, the SAFE application exhibited greater outputs in model solutions of chosen aroma compounds.

Furthermore, distilling the aqueous foods directly including orange juice or beer resulted in flavorful aqueous distillates free from the matrix of nonvolatile compounds. The following problems of the equipment operated the scholars to develop this method better: (i) aroma compounds with higher boiling points may partially condense inside the tubings before reaching the traps; (ii) only dichloromethane and diethyl ether extracts could be utilized; otherwise, frozen solvent might block the tubes and traps; (iii) extracts including elevated concentrations of saturated fat such as butter might plug up the stopcock of the dropping funnel; and (iv) the SAFE unit takes a lot of bench space and at least 1 h is demanded to get the distillation begun. Additionally, this unit is brittle [90]. This method, due to low pressure used, extracts volatile compounds at low temperatures (40 °C), preventing the artifacts' formation, and has already shown its reliability for the volatile compounds' extraction in saffron [46], dill, savory [91], and golpar [92] spices from Iran, orange juice [93], and coffee [94].

Amanpour et al. [46] evaluated the aroma profile and aroma-active compounds of saffron obtained from Iran using GC-MS-olfactometry. For selecting a suitable and

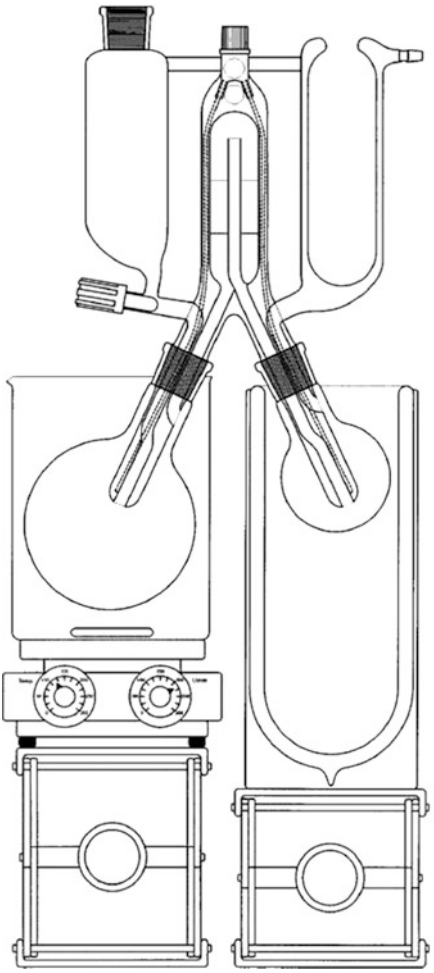


Fig. 9 Solvent-assisted flavor evaporation (SAFE) apparatus

representative aromatic extract from saffron, they used four diverse extraction methods such as the liquid-liquid extraction (LLE), simultaneous distillation-extraction (SDE), solid-phase extraction (SPE), and solvent-assisted flavor evaporation (SAFE). Based on the organoleptic evaluation results, SAFE aromatic extract gave the most representative aroma of saffron. A total of 28 compounds were detected in the saffron sample. The ketone group contained the most prevailing volatile compounds in the sample, followed by aldehydes and acids. Aroma extract dilution analysis (AEDA) was applied for determining the odor-active compounds in studied saffron. Nine odor-active compounds, in total, were discovered in the extract. According to the flavor dilution (FD) factor, the most potent odor-active compounds were safranal with 512 FD, 4-ketoisophorone with 256 FD, and dihydrooxophorone 128 FD values [46].

3.1.2 Selecting the Suitable Extraction Technique/Representativeness Test of the Aromatic Extract

There are different extraction techniques used in saffron aroma characterization as explained some of them above. Remarkably, the extraction technique employed ought to supply an extract containing the organoleptic properties as close as feasible to the source product. The efficiency of a selected extraction method and the representativeness of the sample are often evaluated via primary conventional organoleptic analysis and a comparison of the aroma features of the samples and their corresponding extracts [95, 96]. When one considers that the number of aroma-active compounds discovered by GC-O belongs to the extraction technique, involving variables are often arbitrarily chosen, comprising content of sample, factor of concentration, and volume of the injected sample. Therefore, it is clear that the technique for extraction of volatiles as well as all the variables must be carefully selected [48]. Various techniques could be applied to assess the representativeness of the aroma of aromatic extracts belonging to the kind of the study such as duo-trio, triangular, notation, similarity, and intensity tests. Recently, Amanpour et al. [46] used the similarity and intensity tests to choose the representativeness extraction technique for the GC-MS-olfactometric characterization of the Iranian saffron. The procedure of this suitable sensory analysis which they used in their investigation is explained in detail below:

Panel. The panel consisted of seven assessors (five males and two females from 27 to 48 years old) from the Food Biotechnology Laboratory, Food Engineering Department, Cukurova University. The assessors had good experiences in GC-MS-O analyses, and formerly trained in aroma diagnosis and organoleptic assessment methods, and become familiar with the aroma of saffron.

Sample preparation and presentation. In the investigation, they applied a cardboard smelling strip (reference 7140 BPSI, Granger-Veyron, Lyas, France) to evaluate the representativeness of the aromatic extracts acquired by four various separation methods. Smelling strips have already given excellent outcomes for the representativeness of aroma extract in cherry tomato extracts [97] and orange juice [93]. First, a 10-mL bulk of aqueous solution of saffron was subjected in a 25-mL brown-coded flask as an original sample (reference) for the tests of representativeness. Aromatic extracts of saffron acquired by four various solation methods were adsorbed onto a cardboard smelling strip. After 1 min (the required time for the evaporation of solvent), the strips' extremities were cut off, and then they were subjected in four various dark-coded flasks (25 mL) and introduced to the panel after 15 min. As dichloromethane is a very volatile solvent, it evaporates very quickly; therefore no panelists discovered the aroma of the solvent. Evaluation of samples was carried out at temperature of 20 °C (close to room temperature).

Similarity test. A similarity test was performed to evaluate the closeness between the odor of extracts and the saffron (reference sample). The panelists were instructed to sniff and memorize the aroma of the reference sample and, for the extracts, to sniff the smelling strip and determine the similarity of their odors. A 100-mm unstructured scale was used, anchored with "far from the reference" on the left and "near to

Fig. 10 A 100-mm unstructured scale for evaluating the similarity test

Fig. 11 A 100-mm unstructured scale for evaluating the intensity test

the reference" on the right (Fig. 10). The position of the sample on the unstructured scale was read as the distance in millimeters from the left anchor.

Intensity test. The evaluation of the odor intensity of the extract was requested from panelists. A 100-mm unstructured scale was utilized, anchored with "no odor" on the left and "very strong odor" on the right (Fig. 11). The sample position on the unstructured scale was read as the distance in millimeters from the left anchor. Outcomes were assessment by analysis of variance using Statgraphics Plus program (Manugistic, Inc., Rockville, MD).

The median intensities and similarities of aroma in Iranian saffron observed by the panelists for the aromatic extracts acquired by four different extraction methods are indicated in Fig. 12. The goal of the intensity and similarity assessment tests was to the comparison of the representativeness of the aroma of the extract with that of original sample (reference). Between these methods, the SAFE technique was first used on saffron, and perfect outcomes were attained. The aromatic extracts of SAFE, SPE, LLE, and SDE techniques exhibited 71.8, 61.1, 55.4, and 37.8 mm on a 100-mm unstructured scale, respectively, for the similarity scores. The median similarity scores acquired from four various extractions were detected to be statistically diverse from each other ($p < 0.05$).

The SAFE technique demonstrated an acceptable value for the similarity score. In comparison with further investigations, 70.4 mm in a cherry tomato extract by Selli et al. [97], 64.2 and 63.5 mm in extracts of oils obtained from Turkish olives using pentane/dichloromethane and dichloromethane solvents by Kesen et al. [98], 66.7 mm in banana by Selli et al. [99], and 60.6 mm in blood orange juice extract by Selli and Kelebek [93] were detected for similarity scores. The aromatic extracts of SAFE, SPE, LLE, and SDE techniques indicated 63.9, 69.1, 53.5, and 58.7 mm on a 100-mm unstructured scale, respectively, for the intensity scores.

3.1.3 GC-Olfactometric Characterization of Saffron Key Odorants

GC coupled with MS and FID has been applied for identification and quantification of volatiles. Nevertheless, GC-MS-O or GC-O techniques are a potent approach to specify powerful odor-active compounds of food aroma. The identified volatiles in saffron have been reached over 160 compounds [5, 100]. Nevertheless, it is well-

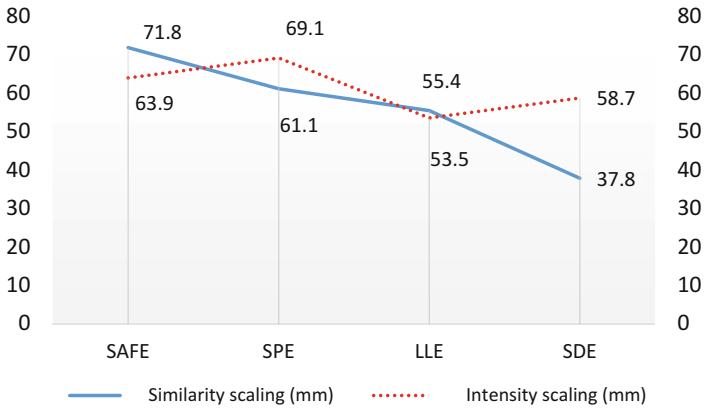


Fig. 12 Similarity and intensity scores of the different extraction techniques [46]

accepted that only a small fraction of the large number of volatile compounds occurring in food actually contributes to the overall aroma. Therefore, a major task in flavor studies is to separate the strongly odor-active compounds from the less odorous or odorless compounds present in food [45]. An interesting way is sniffing the gas chromatographic effluent of a representative separate of volatiles of a food in order to associate odor activity with the eluting compounds. Plenty of the "chemical" detectors are not as sensitive as the human nose for many aroma-active compounds. Experience indicates that plenty aroma-active compounds happen at very low concentrations; their organoleptic relevance is because of low odor thresholds.

Hence, the profile of peak acquired by any "chemical" detector does not presently reflect the profile of aroma in a food. GC-O was designed by Fuller et al. as early as 1964 and has demonstrated to be a valuable technique for the chosen of aroma-active compounds from a complicated structure [45]. Olfactory detection, a variant of organoleptic evaluation, places the nose of a trained expert at the outlet of a column in GC. The expert prompts a slide rheostat with settings altering from no odor to moderate odor to extreme odor, and the attached time versus intensity recorder forms a trace looking like a chromatogram [101].

Numerous ways have been extended to gather and provide GC-O data and to evaluate the organoleptic contribution of single potent aroma-active compounds, which could be categorized in the following four classes [48]:

1. **Dilution analysis techniques** for generating vigor amounts according to stepwise dilution to the threshold, e.g., mixed aroma extraction dilution analysis (AEDA) and hedonic response measurement (Charm analysis).
2. **Detection frequency techniques** for registering discovered aromas by a class of assessors. The number of panelists discovering an aroma (detection frequency) is applied as an assessment of intensity of aroma.
3. **Posterior intensity techniques** for generating assessments of comprehended intensity, being registered after a peak has eluted.

4. **Time-intensity techniques** for generating assessments of comprehended intensity registered at the same time with the elution of the chromatographic peak, e.g., [48].

Among these methods, AEDA technique, which is one of the detection concentration methods, is the most frequently used method in olfactometric analysis. The greatest advantage of this method, which is simple to fulfil, is that the number of panelists participating in the olfactometric analysis is low compared to other methods [102].

Although GC-MS-olfactometry, a very sensitive instrumental device, is used to identify aroma-active compounds, from this point of view, there are limited studies. Indeed, four papers have undertaken olfactometric investigations of saffron samples so far and their olfactometric results are summarized in Table 1 [40, 46, 68, 81]. Rodel and Petrzika [40] were the first to employ GC-O to characterize saffron aroma obtained from Absheron Peninsula, Baku, Azerbaijan. They observed the highly intense and characteristic odor related with the peak produced by safranal, considered to be the main volatile compound in saffron, the key aroma compounds. Although as is typical of such characteristic impact compounds, safranal provides the main impact of the flavor of saffron; however, they also detected several unidentified aroma-active components. In fact, it is apparent that many other compounds contribute to the complete flavor of the material [40].

Cadwallader et al. [66] determined the odor-active compounds in Spanish "Mancha Superior" saffron via direct solvent extraction (DE) and simultaneous steam distillation solvent extraction (SDE) applying AEDA. They confirmed that 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one (saffron, dried hay-like) has much more significant contribution to the aroma of the Spanish saffron than safranal. Furthermore, other several volatiles also contribute to the aroma of saffron based on the GC-O and AEDA results. Although extracts of both DE and SDE techniques had characteristic saffron-like aroma, they showed gentle differences between each other. Floral, spicy, and sweet aroma notes were found in the extracts of DE, and these features were similar to the dried saffron; whereas nutty, cooked, and hay- and rice-like aroma notes were found in the extracts of SDE but sustained certain saffron-like attributes. A mixed total of 25 odor-active compounds were discovered in the extracts of DE and SDE by using GC-O and AEDA techniques. Eighteen of them existed in both of the extracts in common. The majority of the 25 odor-active compounds could be nearly classified applying the organoleptic characteristic terms such as floral, sweet, spicy, herbal, barky, fatty, and harsh/acrid [41]. It is clear that plenty of compounds can be classified in more than one term, while some of them cannot fall under any of these ones. The highest FD factor was found in 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-l-one, followed by safranal. However, previous studies showed reverse results, and safranal was regarded as the most potent aroma-active compound in saffron. This may be depicted by the fact that the amount of 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-l-one has been commonly detected in a far lower values (nearly 10–20-fold less) than safranal [68].

Table 1 Identified aroma-active compounds in saffron by different authors

No.	Aroma-active compounds	Aroma descriptions		
		Cadwallader et al. [68]	Cullere et al. [81]	Amanpour et al. [46]
1	2,3-Butanedione	Buttery, cream cheese	Butter, cream	
2	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Cotton candy, strawberries		
3	3,5,5-Trimethyl-3-cyclohexen-1-one	Saffron, floral, hay		
4	Linalool	Floral, honeysuckle		Floral
5	2-Phenylethanol	Floral, rose		
6	2,6,6-Trimethyl-1,3-cyclohexadien-1-carboxaldehyde (safranal)	Saffron, tea	Saffron	Saffron
7	2-Hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one	Saffron, stale, dried hay		
8	(E,Z)-2,6-Nonadienal	Sweet, cucumber		
9	(E,E)-2,4-Decadienal	Fatty, fried fat		
10	l-Octen-3-one	Mushroom earthy	Mushroom	
11	3-Methylbutanoic acid	Rotten, sour, dried fruit		
12	Acetic acid	Vinegar, acidic	Vinegar	
13	2-Acetyl-1-pyrroline	Nutty, popcorn		
14	3-(Methylthio)propanal	Baked potato		
15	Hexanal		Grass	
16	3-Hexen-2-one		Grass, geranium	
17	Octanal		Lemon	
18	6-Methyl-5-hepten-2-one		Clove, spicy	
19	Isophorone		Saffron	Saffron, herbal
20	(Z)-2-Nonenal		Green, metallic	
21	(E)-2-Nonenal		Melon, aldehydic	
22	(E,Z)-2,6-Nonadienal		Cucumber	
23	Butyric acid		Cheese	
24	Isovalerianic acid		Cheese	
25	(E,E)-2,4-Nonadienal		Rancid oil	
26	(E,E)-2,4-Decadienal		Fatty, deep-fried	
27	b-Phenylethanol		Roses	
28	Furaneol		Cotton candy	
29	Homofuraneol		Cotton candy	
30	4-Ketoisophorone			Saffron

(*continued*)

Table 1 (continued)

No.	Aroma-active compounds	Aroma descriptions		
		Cadwallader et al. [68]	Cullere et al. [81]	Amanpour et al. [46]
31	Dihydrooxophorone			Saffron
32	Phenylethyl alcohol			Floral, rose
33	4-Hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde			Green

Along with these compounds, 3,5,5-trimethyl-3-cyclohexen-l-one and numerous unknown compounds derived from carotenoid compounds had also slightly elevated FD factors and the contribution in the saffron-like aroma properties. Plenty of other volatile compounds were formed through (i) lipid oxidation such as (*E,Z*)-2,6-nonadienal, l-octen-3-one, and (*E,E*)-2,4-decadienal; (ii) Strecker degradation/Maillard reaction such as 2-acetyl-l-pyrroline, 2,3-butanedione, 3-methylbutanoic acid, and 3-(methylthio)propanal; and (iii) hydrolysis of non-carotenoid glycoside precursors such as 2-phenylethanol, benzenemethanol, and linalool, which contributes to the overall saffron aroma. The attendance of 4-hydroxy-2,5-dimethyl-3(2H)-furanone in saffron can be described by either Maillard reaction or possibly by its liberate from a glycoside precursor. Concerning the above results, it could be summarized that together with the carotenoid-derived compounds, plenty further non-carotenoid compounds influence the saffron aroma [28].

Cullere et al. [81] investigated the extract of aroma in a Spanish "Teruel" saffron sample applying a purge and trap setup to isolate volatiles of saffron prior to GC-O. Via an olfactometric method employing the mixed evaluations of intensity and frequency of detection, they demonstrated 20 different aroma-active compounds in the GC-O analysis. The specificity of all the chemicals responsible for these compounds could be established with various classes of certainty. Only two of them were unknown. The elevated modified frequency level of safranal (93%) was considerable. (*E*)-2-Nonenal, hexanal, 2,3-butanedione, and a fourth unidentified compound with a distinctive aroma note of burnt curry also released elevated modified frequencies. Majority of the sustaining aromas had modified frequency levels that were quite elevated (50–70%). Additionally, carbonyl compounds had an important role in the aroma of saffron. Only 6 of the 20 compounds were not included in carbonyl compounds. The odor-active compounds involved an alcohol (β-phenylethanol), two furans (furaneol and homofuraneol), and three acids (acetic, butyric, and isovaleric). The carbonyl compounds composed of three five alkenals, ketones, two aliphatic aldehydes (octanal and hexanal), and two carbonyls with a cyclohexane base (isophorone and safranal). Thirteen of the compounds were formerly considered related [68]. Three of these odor-active compounds were unknown then but were detected to be (*Z*)-2-nonenal, (*E*)-2-nonenal, and hexanal in the current examine. Seven of these compounds such as butyric acid, octanal, (*E,E*)-2,4-nonadienal, 6-methyl-5-hepten-2-one, homofuraneol, and two unidentified

compounds were first determined as related to odor-active compounds in a GC-O analysis in the aroma of saffron. 6-Methyl-5-hepten-2-one and octanal have been discovered formerly by GC-MS experiment in saffron [83, 84]. The majority of these compounds (except homofuraneol and butyric acid) had modified frequency levels greater than 55%. This might demonstrate that some of these newly determined compounds of saffron may be substantial volatiles in its aroma. Additionally, some odor-active compounds found in a former GC-O analysis [68], including 2-acetylpyrroline and three unidentified volatiles with RI (DB-Wax) 1682, 1734, and 1980, respectively, were unknown in the present assay in the profile of aroma. The odor-active compound with RI (DB-Wax) 1734 is specified as 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one [68]. This compound in the La Mancha (Spain) saffron was reported as the most odor-active compound, even greater than safranal.

Nevertheless, 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one was found in the current investigation containing a MF of less than 30% (exactly 19%) in saffron and is hence not included as an odor-active compound. Thus, it can be concluded that in spite of the attendance of this odor-active compound in both types of saffron, its concentration was only elevated enough to be related in one of the saffron varieties [81].

Recently, Amanpour et al. [46] characterized the odor-active compounds in Iranian saffron by GC-MS-O. They used the SAFE technique for the isolation of aroma extracts, which showed a most representative extraction technique close to saffron aroma. The powerful aromas were specified employing AEDA for the evaluation of flavor dilution factors (FD factor). Therefore, the AEDA application on the SAFE extract of saffron liberated nine odor-active compounds such as two three ketones, alcohols, two aldehydes, and two unknown compounds discovered by GC-O but were unknown by GC-MS. The range of FD factors of odor-active compounds was altered between 16 and 512.

Two odor-active aldehyde compounds were found in the sample, namely, safranal and HTCC; safranal with 512 FD showed the most potent odor-active compound, supplying distinct saffron aroma note. HTCC was first discovered as an odor-active compound in the Iranian saffron. According to the aroma chemistry of saffron, safranal is an important volatile compound in the volatile oil and is generated from picrocrocin and HTCC over the drying process of fresh saffron [80, 103]. 4-Ketoisophorone with 256 FD, dihydrooxophorone 128 FD, and isophorone 32 FD factors were found as odor-active ketone compounds in the examined sample. Among these compounds, isophorone was detected in the most elevated concentration (845 μg/g) in Iranian saffron. This ketone is remarkable in saffron to the formation of the saffron aroma note [81].

Dihydrooxophorone and 4-ketoisophorone were first discovered as odor-active compounds generating saffron aroma note in the assayed sample. In addition, these compounds were determined in Spanish “Mancha Superior” [68] and in Iranian saffron [60]. Derivatives of isophorone are the major volatile compounds of the volatile oil and are responsible for the distinct aroma of saffron [103].

On the basis of the β-isophorone biosynthesis, it was disclosed that its generation happened via a mechanism including the zeaxanthin degradation, with

the concomitant generation of both (1R)-3,5,5-trimethyl-3-cyclohexen-1-ol *O*-*β*-D-glucopyranoside and (4R)- and (4S)-4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one *O*-*β*-D-glucopyranoside [13]. These two glycosides are feasible precursors of *β*-isophorone and *α*-isophorone. Additionally, alcohols are considerable as odor-active compounds in the saffron sample. Phenylethyl alcohol and linalool were found in Iranian saffron with the identical aroma note (floral) and flavor dilution factor (FD = 32). Linalool was found in Spanish "Mancha Superior" as an odor-active compound with honeysuckle and floral aroma attributes [68]. Nonetheless, phenylethyl alcohol was first found as an odor-active compound generating floral aroma note in this investigation. Moreover, two unidentified compounds might chip up to the overall aroma in saffron. Unidentified 1 (LRI = 1044) was found in saffron providing a buttery odor (FD = 64). Unidentified 2 (LRI = 1221) formed buttery and burnt odor features (FD = 16) [46].

3.2 High-Performance Liquid Chromatography (HPLC)

HPLC device is a widely used chromatographic method that could isolate compounds' structure and is applied in analytical chemistry for identifying, quantifying, and purifying the individual compounds of the structure [104]. This method is well-known between numerous analytical methods in order to fingerprint, control quality, and determine the saffron adulteration [105]. This method is preferably suitable for the swift processing of such multi-compound instances on both an analytical and preparative scale [106]. The majority of researchers pronounce the utilization of HPLC in order to characterize and quantify the secondary metabolites in the extracts of plant such as steroids, flavonoids, alkaloids, and phenolic compounds [107]. Reversed-phase chromatography is the most generally employed isolation method in HPLC because of its wide application limit. Consequently, its advantages comprise the versatility, simplicity, and scope of the reversed-phase technique as it is capable of handling compounds of a different polarity and molecular mass.

Chromatographic setup must be able to resolve from the peak of interest all other compounds that give a detector signal. The success of method validation depends mainly on the correct determination of the peak purity and selectivity of given HPLC system. It is clear that acceptable selectivity and specificity can result in pure peak. Therefore, the appropriate determination of the purity of a chromatographic peak is of primary interest. One of the most important steps in HPLC studies is the detector selection. After the detector is selected, a chromatographic separation assessed must be developed. The UV, VIS, and PDA (photodiode array) detectors are classified as absorbance detectors. They supply excellent sensitivity for light-absorbing compounds at small value. They are simple to prompt and supply excellent stability. UV detector is a very generally employed detector for HPLC analyses. A standard UV detector permits user to select wavelength from 195 to 370 nm. In comparison with a UV detector, a Vis detector applies longer wavelength between 400 and 700 nm. There are detectors that supply more extensive wavelength choice, which cover both UV and Vis ranges (between 195 and 700 nm) named UV/Vis detector. PDA detects

a complete spectrum simultaneously. UV and Vis detectors conceive the acquired outcome in two dimensions (light intensity and time), but PDA adds the third dimension (wavelength). This is proper to specify the most appropriate wavelength without repeating analyses. Phenolic compounds are often specified utilizing UV-Vis and photodiode array (PDA) detectors at wavelengths between 190 and 380 nm [108].

Further detection techniques are also being used to find phytochemicals among which is the diode array detector (DAD) coupled with mass spectrometer (MS) [106]. Liquid chromatography coupled with mass spectrometry (LC-MS) is an intensive method for the analysis of complicated botanical extracts [109]. HPLC is impressive in isolating chemical compounds in an admixture, and MS supplies numerous knowledge for structural elucidation of the compounds when tandem mass spectrometry (MS) is employed. Therefore, the blend of HPLC and MS comforts swift and accurate specification of chemical compounds in medicinal herbs, particularly when a pure standard is not accessible. Newly, LC-MS has been vastly utilized for phenolic compounds' analysis [106]. Electrospray ionization (ESI) is a preferred source because of its elevated ionization efficiency for phenolic compounds.

The extraction of bioactive compounds from the saffron and their quantitative and qualitative analysis is important for exploration of new biomolecules for use in pharmaceutical and agrochemical industry. These biomolecules are used directly or can be used as a lead molecule to synthesize more potent molecules. Saffron includes a vast limit of constituents that could be applied to treat chronic as well as infectious illnesses [110]. In the literature, more than hundreds of phytochemicals in saffron were disclosed as a safe and widely impressive alternative spice containing less adverse impact. Plenty useful biological activities comprising anti-inflammatory, antioxidant, antimicrobial, anticancer, wound healing, and analgesic activity were disclosed.

However, clinical experiments are essential for the demonstration of the effectiveness of a bioactive compound to confirm this traditional claim. Owing to the fact that saffron extracts commonly happen as a blend of different kinds of bioactive compounds or phytochemicals with various polarities, their isolation still sustains as large challenge for the process in order to identify and characterize them [111].

More than 150 compounds were reported in saffron stigmas [14]. Numerous analytical investigations have been led to specify the big number of possible biologically active compounds detected in saffron. The compounds generating saffron features are crocins ($C_{44}H_{64}O_{24}$), picrocrocin ($C_{16}H_{26}O_7$), and safranal ($C_{10}H_{14}O$) [112].

The first reference to crocin [crocetin di(β-gentiobiosyl) ester] was disclosed in 1818 by Aschoff [113] who gave it its name. Pfander et al. [19] were the first to separate six glycosides of crocetin in saffron sample. In 1984, Speranza et al. [114] characterize their *cis* and *trans*-isomers via HPLC-UV-Vis. Tarantilis et al. [115] characterize a more number of crocetin esters and their *cis* and *trans* isomers using high-performance liquid chromatography with diode array detection and mass spectrometry (HPLC-DAD-MS), and Carmona et al. [116] identified four more

by HPLC-DAD-MS. Thus far, the quantification of crocetin esters by HPLC has been carried out without high precision [117–119], and the use of commercial standards in calibration curves gives low correlation coefficients, owing to the phenomenon of aggregation that some authors have demonstrated that happens in highly concentrated aqueous solutions of crocetin esters [120, 121]. The kinetics of individual crocetin ester degradation in aqueous extracts of saffron were studied to determine their stability [117]. Different methodologies and analytical devices have been employed to recover bioactive compounds of saffron preparative high-performance liquid chromatographic purification of saffron secondary metabolites [69, 103, 115, 122].

Analysis of saffron chemicals coming from different geographical zones demonstrates that the content of compounds belongs highly to processing, drying, extraction techniques and quantification. Saffron metabolites' extraction is optimized by testing different parameters such as the temperature, solvent, light, and stirring time utilized in the procedure. Diverse solvents such as diethyl ether, ethanol, and water are applied for crocin extraction [103, 123]. Last investigations show that a solution of methanol-water (50%, v/v) with magnetic stirring during 1 h in darkness at 25 C is optimal for acquiring all the saffron compounds, comprising crocin [58, 122, 124].

Picrocrocin [4-(*β*-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde] has been identified only in the genus *Crocus*, of which the only edible species is *Crocus sativus* L. Therefore, picrocrocin is a molecular marker of this spice that is able to give a unique taste that cannot be imitated from other spices or seasonings. This compound was isolated for the first time in the stigmas of *C. sativus* by Winterstein et al. [125]. Its molecular structure was defined in 1934 by Kuhn et al. [25]. Glycosidic compounds that are structurally related to picrocrocin have been identified. Tarantilis et al. [115] identified three glycosidic compounds in 1995; the Winterhalter research team [126, 127] recognized eight other glycosidic compounds between 1997 and 1998; and Carmona et al. [128] identified four more by HPLC-DAD-MS in 2006, which were found in considerably lower amounts than picrocrocin. The picrocrocin stability was determined by Sanchez et al. [129], and picrocrocin is more stable than the crocetin esters. The principled usage of the identifications recommended by the ISO 3632 has managed to categorize saffron in world trade by its coloring strength, supplied that the sustaining necessities are accomplished. Subsequently, this categorization has been conducted to the being of a spectrophotometer in approximately all saffron companies. The coloring strength is representative of the crocetin ester amount.

Nevertheless, the specification of picrocrocin over the parameter at 257 nm indicates a trouble of selectivity since further compounds of saffron extract, primarily crocetin esters, also have absorbance at this wavelength because of the glycoside bonds, which cause interferences in assessment [58, 116, 117, 130]. Until now, further methods comprising thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC) have been employed for the quantification of picrocrocin, with this last method remarked as being the most impressive [69, 115, 131].

Safranal is a nonpolar compound, but its solubility in water has not been studied. Different analytical techniques have been developed for its quantification, such as GC-O [40, 46, 68, 81], GC-MS [5], HPLC [58], or ultrasound-assisted extraction/ultraviolet-visible spectroscopy (USAE/UV-Vis) [77]. The results obtained are quite different between these various methods.

Numerous investigations previously disclosed have explained their biological activities: crocins, safranal, and picrocrocin possess a cytotoxic impact on human tumor cells [130]; crocins indicate antioxidant potency [132] and memory-promoting influence [133]; and safranal possesses a relaxant impact [134]. Crocins compose approximately 6–16% of saffron's total dry matter belonging to the growing circumstances, variety, and processing techniques [20]. Picrocrocin, which is the second most prevailing compound (by weight), accounts for nearly 1–13% of saffron's dry matter [18]. Another important chemical compound is safranal which contains nearly 30–70% of volatile oil and 0.001–0.006% of dry matter vastness [5]. Quality of saffron belongs to the amount of these three principle metabolites generating the incomparable color, taste, and aroma to the saffron stigmas [80].

Saffron stigmas are identified by the attendance of vitamins, fats, minerals, sugars, and secondary metabolites including carotenoids, anthocyanins, flavonoids, and terpenes. Among the secondary metabolites, carotenoids are the most substantial compounds since they characterize the spice color and taste [135]. Of these compounds, lycopene, α- and β-carotene, zeaxanthin, crocetin (liposoluble), and crocins (hydrosoluble) derived by crocetin esterification with sugars could be mentioned. Crocins are *trans*-crocetin di-(*β-D*-gentiobiosyl) ester (named *trans*-4-GG), *trans*-crocetin (*β-D*-glucosyl)-(β-D-gentiobiosyl) ester (named *trans*-3-Gg), *trans*-crocetin (β-D-gentiobiosyl) ester (named *trans*-2-G), *cis*-crocetin di-(β-D-gentiobiosyl) ester (named *cis*-4-GG), *trans*-crocetin di-(β-D-glucosyl) ester (named *trans*-2-gg), and *cis*-crocetin (*β-D*-glucosyl)-(β-D-gentiobiosyl) ester (named *cis*-3-Gg) [135]. Crocetin is classified as a natural carotenoid dicarboxylic acid that generates brick red crystals with a melting point of 285 °C. Its chemical structure is the central core of crocins [136].

The saffron-colored compounds are crocins, a family of uncommon water-soluble carotenoid mono- and di-glycosyl esters of a polyene dicarboxylic acid, called crocetin, where D-glucose and D-gentobiose happen as carbohydrate residues. The digentiobiosyl ester of crocetin, named α-crocin, is the main compound [19, 58]. Picrocrocin is the major compound responsible of the bitter taste in saffron, it is a colorless glycoside, the sugar moiety of which is D-glucose, and HTCC the aglycone. Safranal is the volatile oil responsible of the distinct saffron aroma note. This compound originated from HTCC and picrocrocin over the drying process of saffron [10, 43, 137]. Techniques used to detect and quantify saffron metabolites are not integrated. In the last few years, there has been increasing attention to guarantee and to defend the quality of saffron historically produced in specific regions. Among the methods used for saffron characterization, currently recommended by the International Standardisation Organisation (ISO/TS 3632, 2003), is UV-Vis spectrophotometry [138]. The absorbance measurements in saffron at 250 nm (λ_{max} of both HTCC and picrocrocin), 310 nm (λ_{max} of safranal), and 440 nm (λ_{max} of crocins) are

considered to be proper for the identification of the taste, aroma, and color, respectively [131]. Unfortunately, this technique is not specific and able to adequately distinguish between original saffron and adulterated one and hence incapable of providing a quality class on the international market [139].

For the quantitative analysis of picrocrocin and crocetin, a new high-performance thin-layer chromatographic method was developed [140]. A rapid and nondestructive technique to determine safranal and HTCC contents was performed using supercritical carbon dioxide extraction combined with high-resolution gas chromatography (HRGC) and reversed-phase high-performance liquid chromatography (RP-HPLC) [80]. For HPLC analysis, a DAD (diode array detector) was commonly used [112], sometimes in combination with electrospray ionization (ESI) mass spectrometry (MS) [141]. Sánchez et al. [61] developed a solid-phase extraction procedure coupled with RP-HPLC-DAD analysis.

According to the isolation and purification of bioactive compounds in saffron, an aqueous water, ethanol, or methanol is usually utilized for the extraction of numerous bioactive compounds [2]. An easy, susceptible, and peculiar HPLC-UV technique has been first improved for simultaneous quantification of the five main biologically active compounds in saffron such as crocin 1, crocin 2, crocin 3, crocin 4, and crocetin. Calibration curves were provided by spiking authentic compounds and internal standard, 13-*cis*-retinoic acid, into herbal samples prior to extraction. Extraction was led easily by stirring dried herb (20 mg) with 80% aqueous methanol (5 ml) at room temperature in the dark for 2 h. The HPLC technique was carried out on a reversed-phase C_{18} column with linear gradient elution employing methanol and 1% aqueous acetic acid. Calibrations were linear ($r^2 = 50.999$) for all five analytes, with global intra- and interday RSDs of less than 11%.

The technique was successfully used to determine the four crocins and crocetin in three saffron samples and two Zhizi, another crocin-containing herb. Findings show that the progressed HPLC technique could be readily employed as a quality control technique for crocin-containing medicinal herbs [124]. In addition, crocins' extraction and purification are well depicted in the published literatures [64, 142, 143]. After defatting with diethyl ether, stigmas are usually extracted 2 or 3 times utilizing 70–90% methanol or ethanol (~10-mL solvent/g saffron). Solvents involving extract are pooled, evaporated to dryness, and then purified applying silica gel column chromatography with ethanol-ethyl acetate-water (~6:3:1) as the mobile phase. Crocin and crocetin esters are eluted singly [143]. Picrocrocin could be effectively separated from saffron stigma by successive, exhaustive Soxhlet extraction applying light petroleum, methanol, and diethyl ether to acquire three fractions [58].

The diethyl ether phase involving picrocrocin and lipids is evaporated to dryness, defatted applying Soxhlet for picrocrocin purification, and then dissolved in methanol for filtration. The filtrate is then analyzed applying HPLC device. Tarantilis et al. [58] effectively analyzed picrocrocin applying an HPLC LiChroCART 125-4 Superspher 100 RP-18 column and 20–100% ACN in water linear gradient mobile phase. Several studies reported in the literature about the bioactive compounds in saffron using HPLC.

Eleven authenticated saffron samples obtained from Iran, Spain, Greece, India, Italy, Azerbaijan, Turkey, New Zealand, France, China, and the Sigma Chemical Company were used for analyzing by applying an HPLC photodiode array detection technique. Also, for determination of chemical composition of 11 saffron samples, the reversed-phase C18 HPLC was applied. Ten main compounds in each sample were identified, and a well-resolved baseline isolation was attained. Each compound was specified in comparison with its retention time as formerly explained in the published papers [115, 124, 131] as well as by LRFAB-MS analysis through the detection (m/z) of its corresponding pseudomolecular ion $[M + H]^+$ [115, 144]. The peak specification is as permits: picrocrocin, HTCC, and 3-gentiobiosyl-kaempferol were discovered at 250 nm, safranal at 310 nm, and *trans*-crocin 4, *trans*-crocin 3, *trans*-crocin 20, *cis*-crocin 4, *trans*-crocin 2, and *cis*-crocin 2 at 440 nm. With respect to this study, various saffron samples did not alter in their chemical composition but did alter in the amount of each compound. Findings demonstrated that the Indian, Greek, Spanish, and New Zealand saffron extracts had the highest amounts of water-soluble glycosidic carotenoids.

Cossignani et al. [138] evaluated the quality of saffron belongs to the amount of secondary metabolites such as crocins, picrocrocin, and safranal. The purpose of the current study was to assess the impact of drying circumstances on the secondary metabolite amounts of saffron obtained from Cascia region, in the center of Italy. Diverse aliquots of the identical saffron sample were submitted to different dehydration conditions and analyzed by UV-Vis spectrophotometry for the determination of crocins, picrocrocin, and safranal. Safranal was also analyzed by elevated resolution gas chromatography, while the crocins and picrocrocin were identified by HPLC-MS-DAD. The chromatographic analyses resulted in that the samples dried in the milder conditions possessed the lowest amounts of secondary metabolites. Furthermore, the sample dried at 60 C for 55 min had the highest amounts of *trans*-crocin-4 and picrocrocin, while safranal was the most represented in saffron dried at 55 C for 95 min [138].

A research was to perform saffron as a supportable replacement product with elevated added level in some Moroccan agricultural zones with low and erratic rainfalls, for their socioeconomical improvement. The saffron quality must be determined prior to recommendation for commercial production. For the sake of this target, saffron was first cultivated in experimental plots in 11 various experimental areas with a disparity of the different climates, soils, and altitudes. HPLC was utilized for the quantification of the most significant saffron compounds, namely, crocins, picrocrocin, and safranal. The related mean levels, in % dry matter, across all sites altogether are 29.01 ± 5.6, 14.04 ± 7.1, and 0.22 ± 0.11, respectively. The analysis statistically indicates that crocins are stable under each specific environment examined ($p > 5\%$) for 3 years of investigation. Simultaneously, there was a large alteration in the safranal amount for the identical stage ($p < 0.05$). Results showed that the post-harvest processing of saffron produced under various environment conditions might need to be developed. Analysis of environmental influence on the quality of saffron indicated that just the altitude impacts crocins ($R2 = 0.84$, $p < 0.05$) [112].

García-Rodríguez et al. [145] carried out a comparative assessment of the ISO 3632 (2011) technique and an HPLC-DAD technique for determining the safranal amount in saffron. Analyses of samples from various areas were performed by UV-Vis based on the ISO 3632 (2011) and by HPLC-DAD technique. Both techniques were compared with each other, and there was no correlation between the safranal amount acquired by UV-Vis and HPLC-DAD. An overestimation in the UV-Vis test was confirmed, being linked to the *cis*-crocetin esters amount, as well as further compounds. The findings illustrated there was no relationship between ISO quality classified and safranal amount applying HPLC-DAD device. Hence, HPLC-DAD may be preferred to UV-Vis to the determination of the safranal amount and the categorization of saffron for commercial targets. Additionally, HPLC-DAD was enough to the determination of the three foremost factors that describe the saffron quality such as crocetin esters, picrocrocin, and safranal; thus, this assay can be contained in the ISO 3632 technique (2011) [145].

D'Archivo et al. [146] used HPLC-DAD for analyzing the 144 Italian saffron samples obtained from five distinct zones placed in four diverse districts comprising Sardinia, Umbria (Cascia and Città della Pieve), Tuscany (Florence), and Abruzzo (L'Aquila) cultivated in the years from 2009 to 2015. In order to attempt geographical discrimination of saffron samples, intensities of the chromatographic peaks ascribed to crocins, safranal, picrocrocin and its derivatives, and flavonoids were remarked as variations in linear discriminant analysis. The findings exhibited that samples produced at various regions of Italy could discriminate with excellent accuracy from each other. The discrimination of the saffron sample obtained from Sardinia from those obtained from Central Italy was principally ascribed to diverse amounts of the most dominant crocins. Also, excellent discrimination of samples obtained from close regions of Central Italy was confirmed, 88% of validation saffron samples being correctly categorized; some minor crocins are responsible for this differentiation [146].

The impact of various cooking times at 100 °C (in boiling water) in "La Mancha" saffron samples on the bioactive compounds such as safranal, picrocrocin, *trans*-crocin 3, *trans*-crocin 4, and *cis*-crocin 4 was studied. HPLC-photo diode array-mass spectrometry was applied as a confirmatory method in crocin specification. When the samples are introduced to various cooking times, they exhibited diverse behaviors, belonging to the bioactive compound. Moreover, no alterations were confirmed in the amount of picrocrocin, while heat culinary treatment adversely impacts the crocins and safranal amounts [147].

García-Rodríguez et al. [148] proposed an HPLC-DAD technique to determine the three principal compounds responsible for the determination of the saffron quality such as crocetin esters, picrocrocin, and safranal by providing an aqueous extract with regard to the ISO 3632 standard to solve the difficulty that this standard has for aroma and taste specification by ultraviolet−visible spectroscopy. According to this target, laboratory-isolated picrocrocin; a safranal standard with a purity of ≥88%; *trans*-crocetin di(β-D-gentiobiosyl) ester (*trans*-4-GG) and *trans*-crocetin (β-D-glucosyl)-(β-D-gentiobiosyl) ester (*trans*-3-Gg) standards, both with a purity

of ≥99%; and 50 different saffron spice samples from Italy, Iran, Greece, and Spain were applied in the intralaboratory validation of the HPLC technique.

The analytical technique proposed was enough in terms of selectivity, linearity, accuracy, and sensitivity for the determination of the three foremost factors that describe the saffron quality employing only a saffron solution provided based on the ISO 3632 standard.

Sujata et al. [69] have applied gas chromatography (GC), high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC) techniques to determine the saffron quality. HPLC and TLC liberated comparable outcomes for crocin and crocetins (color principles), picrocrocin (bitter substance), and safranal (aroma). In a similar manner, the specification of safranal by GC was in line with analysis by HPLC and TLC. Isolation of the compounds was performed by silica gel G TLC applying an n-butanol-acetic acid-water (4:l:l) system. The resolution of crocin, crocetins, and picrocrocin by HPLC was acquired applying a Shimadzu 15-cm CLC-ODS column with 20–80% acetonitrile in water as the eluent; for safranal an isocratic run with 76% acetonitrile in water was proper. GC was adopted only for the determination of safranal applying a Shimadzu 5% SE-30 column. HPLC was most appropriate for the adulterants' detection and was easier and more effective for saffron quality analysis. The TLC technique was time-consuming and also released an overestimation of the color sources.

Numerous authors have indicated that spices involving flavonoid and phenolic compounds showed antioxidant potencies, so they are often utilized as antioxidant food supplements [149, 150]. The antioxidant potency of the saffron stigma can be due to its phenolic amount as well as to its active compounds comprising safranal, crocin, crocetin, and carotene, all of which have been disclosed to possess antioxidant potencies [149]. Picrocrocin could be impressively separated from stigma of saffron by successive, exhaustive Soxhlet extraction applying light methanol, diethyl ether, and petroleum to attain three fractions [58]. The diethyl ether phase involving picrocrocin and lipids is evaporated to dryness, defatted applying Soxhlet for picrocrocin purification, and then dissolved in methanol for filtration. The filtrate is then analyzed applying HPLC devise. Tarantilis et al. [58] effectively analyzed picrocrocin applying an HPLC LiChroCART 125-4 Superspher 100 RP-18 column and 20–100% ACN in water linear gradient mobile phase [58].

The antioxidant potencies of spices are principally ascribed to their flavonoid and phenolic compounds. Flavonoid in saffron was first identified in saffron using mass spectrometry by Tarantilis et al. [115], proposing a kaempferol structure with a disaccharide moiety. Straubinger et al. [126] specified kaempferol 7-O-glucopyranosyl-3-O-sophoroside and kaempferol 7-O-sophoroside by NMR and MS after countercurrent preparative chromatography. According to this determination, the authors similarly remarked that the specification of a new flavonoid called kaempferol 3-O-gentiobioside fulfilled by Lozano et al. [131] was not correct [13]. Additionally, further flavonoids might be detected in saffron, as they have already been explained in further *Crocus* species [151]. Saffron is one of the spices believed to have antioxidant potencies, but knowledge on its antioxidant potency, flavonoids, and phenolic compound are rather limited; hence a study was accomplished to assess

the antioxidant potency of saffron stigmas isolated with various solvents. The flavonoid and phenolic compounds in saffron were also tested applying reversed-phase (RP)-HPLC.

Outcomes illustrated that stigma of saffron has antioxidant potency. The free radical-scavenging and ferric diminishing power activities were greater for the methanolic extract of saffron stigma at a concentration of 300 μg/mL, with levels of 68.2% and 78.9%, respectively, as compared to the corresponding boiling water and ethanolic extracts, but the activities were lower than those of antioxidant standards such as BHT and α-tocopherol. The acquired total phenolic compounds' level for methanolic saffron extract was 6.54-mg gallic acid equivalent (GAE)/g dry weight (DW) and for total flavonoids, 5.88-mg rutin equivalent/g DW, which were also greater than levels acquired from the ethanolic and boiling water extracts. Furthermore, the RP-HPLC analyses showed the presence of gallic acid and pyrogallol as two bioactive compounds. Briefly, stigmas of saffron demonstrated antioxidant potency and methanol appeared to be the best solvent to extract the active compounds, among which the presence of gallic acid and pyrogallol might contribute toward the antioxidant activities of stigma. Therefore, stigma of saffron can be utilized as a source of natural antioxidant for industrial aims [100].

Makhlouf et al. [152] reported that the total amount of polyphenols in saffron (16-mg gallic acid/l) showed greater than that in white grape juice (6.285-mg gallic acid/l). Their experiment indicated that saffron extract attained from the saffron flower possessed elevated contents of polyphenol compounds, which could impressively decline activity of free radicals and could supply an intensive protection for the various organs such as the heart, lung, kidneys, and liver against some oxidative damages under a dose-dependent behavior. Therefore, saffron exhibited a good and natural antioxidant in comparison with the antioxidant capacity of white grape source.

The elevated content of saffron was detected to be more in counteracting the manifestation of hyperlipidemia than elevated content of crocin. These propose that together with crocin of saffron, there are further compounds responsible for synergistic antihyperlipidemic and antioxidant potential of saffron. The potent hyperlipidemic activities could be straightly connected to the presence of flavonoids in saffron as it is known that flavonoids have potent hypolipidemic features [153].

The compounds considered to be pharmacologically active in saffron are the bitter principles and the pigment derivatives from the carotenoid crocetin [11]. In addition to picrocrocin, that is to say 4-(β-D-glucopyranosyl)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, the main compound responsible for saffron bitterness, further compounds with this sensory feature have been identified in saffron. These are linked to picrocrocin and flavonoids [127, 154]. Flavonoids have several functions in the ecology, physiology, and biochemistry of plants, and they are significant in the nutrition of both animal and human [155]. The antioxidant potency of flavonoids toward free radicals and reactive oxygen species, plus their potential estrogenic and anticancer activity, draws attention to their health-protecting role in human and animal foods [156]. Some of these health features might be because of

the flavonoid amount, as has been disclosed for anti-inflammatory and anti-conceptive influences [157].

There is also an important interest in accurate techniques for the characterization of saffron that can be employed to prevent adulteration and to categorize saffron from various geographical areas or territories [124, 131]. The differentiation and discrimination of saffron from diverse territories could be achieved by screening of color compounds present in the saffron matrix [18]. Since this time, various analytical methods such as ultraviolet-visible (UV-Vis) spectrophotometry [130, 158, 159], spectrofluorometry [160, 161], TLC [103, 159], GC [5, 69], LC [124, 140, 162, 163], HPLC [64, 80, 115, 131, 140, 164], capillary electrophoresis (CE), infrared spectroscopy (IR) [119, 144, 165], Fourier transform near-infrared (FT-NIR) spectroscopy [165], LC-MS [115], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [166], liquid chromatography-diode array detection-tandem mass spectrometry (LC-DAD-MS/MS) [100], hydrogen-1 nuclear magnetic resonance (1H-NMR) [166], Raman spectroscopy [167], and chemometrics [139, 168] are widely used.

Among these methods, HPLC with DAD has been indicated to be the most efficient method for analyzing the sensitive compounds in complicated extracts of natural products [112, 115]. However, HPLC and most of the other methods can hardly be used for routine analysis since they require equipment that are hardly ever detected in small- or medium-sized companies that process and package saffron spice [58].

References

1. Kumar R, Singh V, Devi K et al (2008) State of art of saffron (*Crocus sativus* L.) agronomy: a comprehensive review. Food Rev Int 25:44–85. https://doi.org/10.1080/87559120802458503
2. Melnyk JP, Wang S, Marcone MF (2010) Chemical and biological properties of the world's most expensive spice: saffron. Food Res Int 43:1981–1989. https://doi.org/10.1016/j.foodres.2010.07.033
3. Baghalian K, Sheshtamand MS, Jamshidi AH (2010) Genetic variation and heritability of agro-morphological and phytochemical traits in Iranian saffron (*Crocus sativus* L.) populations. Ind Crop Prod 31:401–406. https://doi.org/10.1016/j.indcrop.2009.12.010
4. Fernández J-A (2004) Biology, biotechnology and biomedicine of saffron. Recent Res Dev Plant Sci 2:127–159. ISBN 81-7736-239-9
5. Maggi L, Carmona M, del Campo CP et al (2009) Worldwide market screening of saffron volatile composition. J Sci Food Agric 89:1950–1954. https://doi.org/10.1002/jsfa.3679
6. Ghorbani M (2008) The efficiency of saffron's marketing channel in Iran. World Appl Sci J 4:523–527. ISSN 1818-4952
7. Gresta F, Lombardo GM, Siracusa L, Ruberto G (2008) Saffron, an alternative crop for sustainable agricultural systems. A review. Agron Sustain Dev 28:95–112. https://doi.org/10.1051/agro:2007030
8. Deo B (2003) Growing saffron – the World's most expensive spice. Crop Food Res (New Zealand Institute for Crop & Food Research) 20:1–4
9. Sereshti H, Heidari R, Samadi S (2014) Determination of volatile components of saffron by optimised ultrasound-assisted extraction in tandem with dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry. Food Chem 143:499–505. https://doi.org/10.1016/j.foodchem.2013.08.024

10. Sampathu SR, Shivashankar S, Lewis YS, Wood AB (1984) Saffron (*Crocus Sativus* Linn.) – cultivation, processing, chemistry and standardization. CRC Crit Rev Food Sci Nutr 20:123–157. https://doi.org/10.1080/10408398409527386
11. Ríos JL, Recio MC, Giner RM, Máñez S (1996) An update review of saffron and its active constituents. Phyther Res 10:189–193. https://doi.org/10.1002/(SICI)1099-1573(199605)10:3<189::AID-PTR754>3.0.CO;2-C
12. Grilli Caiola M (2004) Saffron Reproductive Biology. Acta Hortic 650:25–37. https://doi.org/10.17660/ActaHortic.2004.650.1
13. Winterhalter P, Straubinger M (2000) Saffron – Renewed Interest In An Ancient Spice. Food Rev Int 16:39–59. https://doi.org/10.1081/FRI-100100281
14. Schmidt M, Betti G, Hensel A (2007) Saffron in phytotherapy: pharmacology and clinical uses. WMW Wien Med Wochenschr 157:315–319. https://doi.org/10.1007/s10354-007-0428-4
15. Dhar AK, Mir GM (1997) Saffron in Kashmir-VI: a review of distribution and production. J Herbs Spices Med Plants 4:83–90. https://doi.org/10.1300/J044v04n04_09
16. Basker D, Negbi M (1985) Crocetin equivalent of saffron extracts-comparison of 3 extraction methods. J Assoc Publ Analysts 23:65–69. ISSN: 0004-5780
17. Skrubis B (1989) The cultivation in Greece of *Crocus sativus* L. In Proceedings of the International Conference on Saffron (*Crocus Sativus* L.), L'Àquilla, Italy, 1990:171–182
18. Alonso GL, Salinas MR, Garijo J, Sanchez-fernandez MA (2001) Composition of crocins and picrocrocin from spanish saffron (*Crocus Sativus* L.). J Food Qual 24:219–233. https://doi.org/10.1111/j.1745-4557.2001.tb00604.x
19. Pfander H, Schurtenberger H (1982) Biosynthesis of C20-carotenoids in *Crocus sativus*. Phytochemistry 21:1039–1042. https://doi.org/10.1016/S0031-9422(00)82412-7
20. Gregory MJ, Menary RC, Davies NW (2005) Effect of drying temperature and air flow on the production and retention of secondary metabolites in saffron. J Agric Food Chem 53:5969–5975. https://doi.org/10.1021/jf047989j
21. Alavizadeh SH, Hosseinzadeh H (2014) Bioactivity assessment and toxicity of crocin: a comprehensive review. Food Chem Toxicol 64:65–80. https://doi.org/10.1016/j.fct.2013.11.016
22. Assimopoulou AN, Sinakos Z, Papageorgiou VP (2005) Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. Phyther Res 19:997–1000. https://doi.org/10.1002/ptr.1749
23. Soeda S, Ochiai T, Shimeno H et al (2007) Pharmacological activities of crocin in saffron. J Nat Med 61:102–111. https://doi.org/10.1007/s11418-006-0120-9
24. Papandreou MA, Kanakis CD, Polissiou MG et al (2006) Inhibitory activity on amyloid-β aggregation and antioxidant properties of *Crocus sativus* stigmas extract and its Crocin constituents. J Agric Food Chem 54:8762–8768. https://doi.org/10.1021/jf061932a
25. Kuhn R, Winterstein A (1934) Über die Konstitution des Pikro-crocins und seine Beziehung zu den Carotin-Farbstoffen des Safrans. Ber Dtsch Chem Ges 67:344–357. https://doi.org/10.1002/cber.19340670239
26. Buchecker R, Eugster CH (1973) Absolute konfiguration von picrocrocin. Helv Chim Acta 56:1121–1124. https://doi.org/10.1002/hlca.1973056033
27. Alonso GL, Salinas MR, Esteban-Infantes FJ, Sánchez-Fernández MA (1996) Determination of Safranal from saffron (*Crocus sativus* L.) by thermal desorption−gas chromatography. J Agric Food Chem 44:185–188. https://doi.org/10.1021/jf940665i
28. Cadwallader KR (2002) Flavor chemistry of saffron. ISSN: 0065-7727
29. Tarantilis PA, Polissiou MG (1997) Isolation and identification of the aroma components from saffron (*Crocus sativus*). J Agric Food Chem 45:459–462. https://doi.org/10.1021/jf960105e
30. Carmona M, Zalacain A, Salinas MR, Alonso GL (2007) A new approach to saffron aroma. Crit Rev Food Sci Nutr 47:145–159. https://doi.org/10.1080/10408390600626511
31. Kanakis CD, Tarantilis PA, Tajmir-Riahi HA, Polissiou MG (2007) Crocetin, Dimethylcrocetin, and Safranal bind human serum albumin: stability and Antioxidative properties. J Agric Food Chem 55:970–977. https://doi.org/10.1021/jf062638l

32. Escribano J, Alonso G-L, Coca-Prados M, Fernández J-A (1996) Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. Cancer Lett 100:23–30. https://doi.org/10.1016/0304-3835(95)04067-6
33. Davis JM, Giddings JC (1983) Statistical theory of component overlap in multicomponent chromatograms. Anal Chem 55:418–424. https://doi.org/10.1021/ac00254a003
34. Cortes HJ (1992) Developments in multidimensional separation systems. J Chromatogr A 626:3–23. https://doi.org/10.1016/0021-9673(92)85324-M
35. Liu Z, Phillips JB (1991) Comprehensive two-dimensional gas chromatography using an on-column thermal modulator interface. J Chromatogr Sci 29:227–231. https://doi.org/10.1093/chromsci/29.6.227
36. Kajdan T, Cortes H, Kuppannan K, Young SA (2008) Development of a comprehensive multidimensional liquid chromatography system with tandem mass spectrometry detection for detailed characterization of recombinant proteins. J Chromatogr A 1189:183–195. https://doi.org/10.1016/j.chroma.2007.11.031
37. Mondello L, Tranchida PQ, Dugo P, Dugo G (2008) Comprehensive two-dimensional gas chromatography-mass spectrometry: a review. Mass Spectrom Rev 27:101–124. https://doi.org/10.1002/mas.20158
38. Cortes HJ, Winniford B, Luong J, Pursch M (2009) Comprehensive two dimensional gas chromatography review. J Sep Sci 32:883–904. https://doi.org/10.1002/jssc.200800654
39. Nováková L, Vlčková H (2009) A review of current trends and advances in modern bio-analytical methods: chromatography and sample preparation. Anal Chim Acta 656:8–35. https://doi.org/10.1016/j.aca.2009.10.004
40. Rödel W, Petrzika M (1991) Analysis of the volatile components of saffron. J High Resolut Chromatogr 14:771–774. https://doi.org/10.1002/jhrc.1240141118
41. Narasimhan S, Chand N, Rarjalakshmi D (1992) Saffron: quality evaluation by sensory profile and gas chromatography. J Food Qual 15:303–314. https://doi.org/10.1111/j.1745-4557.1992.tb00994.x
42. Caballero-Ortega H, Pereda-Miranda R, Abdullaev FI (2007) HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. Food Chem 100:1126–1131. https://doi.org/10.1016/j.foodchen.2005.11.020
43. Zarghami NS, Heinz DE (1971) Monoterpene aldehydes and isophorone-related compounds of saffron. Phytochemistry 10:2755–2761. https://doi.org/10.1016/S0031-9422(00)97275-3
44. Kanakis CD, Daferera DJ, Tarantilis PA, Polissiou MG (2004) Qualitative determination of volatile compounds and quantitative evaluation of Safranal and 4-Hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC) in Greek saffron. J Agric Food Chem 52:4515–4521. https://doi.org/10.1021/jf049808j
45. Grosch W (1993) Detection of potent odorants in foods by aroma extract dilution analysis. Trends Food Sci Technol 4:68–73. https://doi.org/10.1016/0924-2244(93)90187-F
46. Amanpour A, Sonmezdag AS, Kelebek H, Selli S (2015) GC-MS-olfactometric characterization of the most aroma-active components in a representative aromatic extract from Iranian saffron (*Crocus sativus* L.). Food Chem 182:251–256. https://doi.org/10.1016/j.foodchem.2015.03.005
47. Chen Y, Guo Z, Wang X, Qiu C (2008) Sample preparation. J Chromatogr A 1184:191–219. https://doi.org/10.1016/j.chroma.2007.10.026
48. van Ruth SM (2001) Methods for gas chromatography-olfactometry: a review. Biomol Eng 17:121–128. https://doi.org/10.1016/S1389-0344(01)00070-3
49. Plutowska B, Wardencki W (2008) Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – a review. Food Chem 107:449–463. https://doi.org/10.1016/j.foodchem.2007.08.058
50. Bonino M, Schellino R, Rizzi C et al (2003) Aroma compounds of an Italian wine (Ruché) by HS–SPME analysis coupled with GC–ITMS. Food Chem 80:125–133. https://doi.org/10.1016/S0308-8146(02)00340-0

51. López EF, Gómez EF (2000) Comparison of solvents for determination of monoterpenes in wine using liquid-liquid extraction. Chromatographia 52:798–802. https://doi.org/10.1007/BF02491007
52. Nonato EA, Carazza F, Silva FC et al (2001) A headspace solid-phase microextraction method for the determination of some secondary compounds of Brazilian sugar cane spirits by gas chromatography. J Agric Food Chem 49:3533–3539. https://doi.org/10.1021/jf000896r
53. Plutowska B, Wardencki W (2007) Aromagrams–aromatic profiles in the appreciation of food quality. Food Chem 101:845–872
54. Sides A (2000) Developments in extraction techniques and their application to analysis of volatiles in foods. TrAC Trends Anal Chem 19:322–329. https://doi.org/10.1016/S0165-9936(99)00225-3
55. Pollien P, Ott A, Montigon F et al (1997) Hyphenated headspace-gas chromatography-sniffing technique: screening of impact odorants and quantitative Aromagram comparisons. J Agric Food Chem 45:2630–2637. https://doi.org/10.1021/jf960885r
56. Maggi L, Carmona M, del Campo CP et al (2008) Multi-residue contaminants and pollutants analysis in saffron spice by stir bar sorptive extraction and gas chromatography–ion trap tandem mass spectrometry. J Chromatogr A 1209:55–60. https://doi.org/10.1016/j.chroma.2008.09.026
57. Zougagh M, Ríos A, Valcárcel M (2006) Determination of total safranal by in situ acid hydrolysis in supercritical fluid media: application to the quality control of commercial saffron. Anal Chim Acta 578:117–121. https://doi.org/10.1016/j.aca.2006.06.064
58. Tarantilis PA, Polissiou M, Manfait M (1994) Separation of picrocrocin, cis-trans-crocins and safranal of saffron using high-performance liquid chromatography with photodiode-array detection. J Chromatogr A 664:55–61. https://doi.org/10.1016/0021-9673(94)80628-4
59. D'Auria M, Mauriello G, Rana GL (2004) Volatile organic compounds from saffron. Flavour Fragr J 19:17–23. https://doi.org/10.1002/ffj.1266
60. Jalali-Heravi M, Parastar H, Ebrahimi-Najafabadi H (2009) Characterization of volatile components of Iranian saffron using factorial-based response surface modeling of ultrasonic extraction combined with gas chromatography–mass spectrometry analysis. J Chromatogr A 1216:6088–6097. https://doi.org/10.1016/j.chroma.2009.06.067
61. Sánchez AM, Carmona M, del Campo CP, Alonso GL (2009) Solid-phase extraction for picrocrocin determination in the quality control of saffron spice (*Crocus sativus* L.). Food Chem 116:792–798. https://doi.org/10.1016/j.foodchem.2009.03.039
62. Ammann A, Hinz DC, Addleman RS et al (1999) Superheated water extraction, steam distillation and SFE of peppermint oil. Fresenius J Anal Chem 364:650–653. https://doi.org/10.1007/s002160051406
63. Kanasawud P, Crouzet JC (1990) Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium. 1. .beta.-Carotene degradation. J Agric Food Chem 38:237–243. https://doi.org/10.1021/jf00091a052
64. Zareena AV, Variyar PS, Gholap AS, Bongirwar DR (2001) Chemical investigation of gamma-irradiated saffron (*Crocus sativus* L.). J Agric Food Chem 49:687–691. https://doi.org/10.1021/jf0009221
65. Golumbic C (1951) Liquid-liquid extraction analysis. Anal Chem 23:1210–1217. https://doi.org/10.1021/ac60057a004
66. Cadwallader KR, Baek HH, Cai M (1997) Characterization of saffron flavor by aroma extract dilution analysis. ACS Symp Ser 660:66–79
67. Augusto F, Leite e Lopes A, Zini CA (2003) Sampling and sample preparation for analysis of aromas and fragrances. TrAC Trends Anal Chem 22:160–169. https://doi.org/10.1016/S0165-9936(03)00304-2
68. Cares MG, Vargas Y, Gaete L et al (2010) Ultrasonically assisted extraction of bioactive principles from *Quillaja Saponaria* Molina. Phys Procedia 3:169–178. https://doi.org/10.1016/j.phpro.2010.01.024
69. Sujata V, Ravishankar GA, Venkataraman LV (1992) Methods for the analysis of the saffron metabolites crocin, crocetins, picrocrocin and safranal for the determination of the quality

of the spice using thin-layer chromatography, high-performance liquid chromatography and gas chromatography. J Chromatogr A 624:497–502. https://doi.org/10.1016/0021-9673(92)85699-T
70. Romanik G, Gilgenast E, Przyjazny A, Kamiński M (2007) Techniques of preparing plant material for chromatographic separation and analysis. J Biochem Biophys Methods 70:253–261. https://doi.org/10.1016/j.jbbm.2006.09.012
71. Jadhav D, Rekha BN, Gogate PR, Rathod VK (2009) Extraction of vanillin from vanilla pods: a comparison study of conventional soxhlet and ultrasound assisted extraction. J Food Eng 93:421–426. https://doi.org/10.1016/j.jfoodeng.2009.02.007
72. Dai J, Mumper RJ (2010) Plant Phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15:7313–7352. https://doi.org/10.3390/molecules15107313
73. Xu H, Zhang Y, He C (2007) Ultrasonically assisted extraction of Isoflavones from stem of *Pueraria lobata* (Willd.) Ohwi and its mathematical model. Chin J Chem Eng 15:861–867. https://doi.org/10.1016/S1004-9541(08)60015-4
74. Metherel AH, Taha AY, Izadi H, Stark KD (2009) The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed). Prostaglandins Leukot Essent Fat Acids 81:417–423. https://doi.org/10.1016/j.plefa.2009.07.003
75. Kadkhodaee R, Hemmati-Kakhki A (2006) Ultrasonic extraction of active compounds from saffron. II Int Symp Saffron Biol Technol 739:417–425
76. Huie CW (2002) A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. Anal Bioanal Chem 373:23–30. https://doi.org/10.1007/s00216-002-1265-3
77. Maggi L, Sánchez AM, Carmona M et al (2011) Rapid determination of safranal in the quality control of saffron spice (*Crocus sativus* L.). Food Chem 127:369–373. https://doi.org/10.1016/j.foodchem.2011.01.028
78. Tonthubthimthong P, Chuaprasert S, Douglas P, Luewisutthichat W (2001) Supercritical CO2 extraction of nimbin from neem seeds – an experimental study. J Food Eng 47:289–293. https://doi.org/10.1016/S0260-8774(00)00131-X
79. Wang L, Weller CL (2006) Recent advances in extraction of nutraceuticals from plants. Trends Food Sci Technol 17:300–312. https://doi.org/10.1016/j.tifs.2005.12.004
80. Lozano P, Delgado D, Gómez D et al (2000) A non-destructive method to determine the safranal content of saffron (*Crocus sativus* L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography. J Biochem Biophys Methods 43:367–378. https://doi.org/10.1016/S0165-022X(00)00090-7
81. Culleré L, San-Juan F, Cacho J (2011) Characterisation of aroma active compounds of Spanish saffron by gas chromatography–olfactometry: quantitative evaluation of the most relevant aromatic compounds. Food Chem 127:1866–1871. https://doi.org/10.1016/j.foodchem.2011.02.015
82. Arthur CL, Pawliszyn J (1990) Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal Chem 62:2145–2148. https://doi.org/10.1021/ac00218a019
83. D'Auria M, Mauriello G, Racioppi R, Rana GL (2006) Use of SPME-GC-MS in the study of time evolution of the constituents of saffron aroma: modifications of the composition during storage. J Chromatogr Sci 44:18–21. https://doi.org/10.1093/chromsci/44.1.18
84. Du H, Wang J, Hu Z, Yao X (2008) Quantitative structure-retention relationship study of the constituents of saffron aroma in SPME-GC–MS based on the projection pursuit regression method. Talanta 77:360–365. https://doi.org/10.1016/j.talanta.2008.06.038
85. Sarafraz-Yazdi A, Piri moghadam H, Es'haghi Z, Sepehr S (2010) Comparative study of the three sol–gel based solid phase microextraction fibers in extraction of BTEX from water samples using gas chromatography-flame ionization detection. Anal Methods 2:746. https://doi.org/10.1039/c0ay00175a
86. Baltussen E, Sandra P, David F, Cramers C (1999) Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. J

Microcolumn Sep 11:737–747. https://doi.org/10.1002/(SICI)1520-667X(1999)11:10<737::AID-MCS7>3.0.CO;2-4
87. He M, Chen B, Hu B (2014) Recent developments in stir bar sorptive extraction. Anal Bioanal Chem 406:2001–2026. https://doi.org/10.1007/s00216-013-7395-y
88. Wilkes JG, Conte ED, Kim Y et al (2000) Sample preparation for the analysis of flavors and off-flavors in foods. J Chromatogr A 880:3–33. https://doi.org/10.1016/S0021-9673(00)00318-6
89. Amini M, Ghoranneviss M, Abdijadid S (2017) Effect of cold plasma on crocin esters and volatile compounds of saffron. Food Chem 235:290–293. https://doi.org/10.1016/j.foodchem.2017.05.067
90. Engel W, Bahr W, Schieberle P (1999) Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. Eur Food Res Technol 209:237–241. https://doi.org/10.1007/s002170050486
91. Amanpour A, Kelebek H, Selli S (2017) Aroma constituents of shade-dried aerial parts of Iranian dill (*Anethum graveolens* L.) and savory (Satureja sahendica Bornm.) by solvent-assisted flavor evaporation technique. J Food Meas Charact 11:1430–1439. https://doi.org/10.1007/s11694-017-9522-5
92. Amanpour A, Kelebek H, Selli S (2016) Aroma components of Iranian dried Heracleum persicum fruit (golpar) using solvent-assisted flavour evaporation technique. J Food Nutr Res 55:141–147. ISSN: 1336-8672
93. Selli S, Kelebek H (2011) Aromatic profile and odour-activity value of blood orange juices obtained from Moro and Sanguinello (*Citrus sinensis* L. Osbeck). Ind Crop Prod 33:727–733. https://doi.org/10.1016/j.indcrop.2011.01.016
94. Sanz C, Czerny M, Cid C, Schieberle P (2002) Comparison of potent odorants in a filtered coffee brew and in an instant coffee beverage by aroma extract dilution analysis (AEDA). Eur Food Res Technol 214:299–302. https://doi.org/10.1007/s00217-001-0459-9
95. Silva Ferreira AC, Hogg T, Guedes de Pinho P (2003) Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. J Agric Food Chem 51:1377–1381. https://doi.org/10.1021/jf025847o
96. Priser C, Etiévant PX, Nicklaus S, Brun O (1997) Representative champagne wine extracts for gas chromatography olfactometry analysis. J Agric Food Chem 45:3511–3514. https://doi.org/10.1021/jf970123b
97. Selli S, Kelebek H, Ayseli MT, Tokbas H (2014) Characterization of the most aroma-active compounds in cherry tomato by application of the aroma extract dilution analysis. Food Chem 165:540–546. https://doi.org/10.1016/j.foodchem.2014.05.147
98. Kesen S, Kelebek H, Selli S (2014) Characterization of the key aroma compounds in Turkish olive oils from different geographic origins by application of aroma extract dilution analysis (AEDA). J Agric Food Chem 62:391–401. https://doi.org/10.1021/jf4045167
99. Selli S, Gubbuk H, Kafkas E, Gunes E (2012) Comparison of aroma compounds in Dwarf Cavendish banana (Musa spp. AAA) grown from open-field and protected cultivation area. Sci Hortic (Amsterdam) 141:76–82. https://doi.org/10.1016/j.scienta.2012.04.008
100. Carmona M, Sánchez AM, Ferreres F et al (2007) Identification of the flavonoid fraction in saffron spice by LC/DAD/MS/MS: comparative study of samples from different geographical origins. Food Chem 100:445–450. https://doi.org/10.1016/j.foodchem.2005.09.065. Published: 2007
101. Pollien P, Fay LB, Baumgartner M, Chaintreau A (1999) First attempt of odorant quantitation using gas chromatography−olfactometry. Anal Chem 71:5391–5397. https://doi.org/10.1021/ac990367q
102. Ferreira V, Pet'ka J, Aznar M (2002) Aroma extract dilution analysis. Precision and optimal experimental design. J Agric Food Chem 50:1508–1514. https://doi.org/10.1021/jf010933u
103. Iborra JOL, Castellar MR, Canovas MA, Manjon AR (1992) TLC preparative purification of Picrocrocin, HTCC and Crocin from saffron. J Food Sci 57:714–716. https://doi.org/10.1111/j.1365-2621.1992.tb08079.x

104. Piana M, Zadra M, de Brum TF et al (2013) Analysis of Rutin in the extract and gel of *Viola tricolor*. J Chromatogr Sci 51:406–411. https://doi.org/10.1093/chromsci/bms155
105. Heidarbeigi K, Mohtasebi SS, Foroughirad A et al (2015) Detection of adulteration in saffron samples using electronic nose. Int J Food Prop 18:1391–1401. https://doi.org/10.1080/10942912.2014.915850
106. Sasidharan S, Chen Y, Saravanan D et al (2011) Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med 8:1. ISSN: 0189-6016
107. de la Torre-Carbot K, Jauregui O, Gimeno E et al (2005) Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS. J Agric Food Chem 53:4331–4340. https://doi.org/10.1021/jf0501948
108. Gazdag M (2000) 2.7 high performance liquid chromatography (HPLC) and related techniques. Prog Pharm Biomed Anal. 4:210–239. Elsevier
109. Peng J, Elias JE, Thoreen CC et al (2003) Evaluation of multidimensional chromatography coupled with tandem mass spectrometry (LC/LC−MS/MS) for large-scale protein analysis: the yeast proteome. J Proteome Res 2:43–50. https://doi.org/10.1021/pr025556v
110. Atanasov AG, Waltenberger B, Pferschy-Wenzig E-M et al (2015) Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 33:1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001
111. Pferschy-Wenzig E-M, Bauer R (2015) The relevance of pharmacognosy in pharmacological research on herbal medicinal products. Epilepsy Behav 52:344–362. https://doi.org/10.1016/j.yebeh.2015.05.037
112. Lage M, Cantrell CL (2009) Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. Sci Hortic (Amsterdam) 121:366–373. https://doi.org/10.1016/j.scienta.2009.02.017
113. Aschoff S (1818) Beiträge sur kenntnis des safrans. Berl Jb Pharm 19:142–157
114. Speranza G, Dada G, Manitto P et al (1984) 13-cis-Crocin-a new Crocinoid of saffron. Gazz Chim Ital 114:189–192. ISSN: 0016-5603
115. Tarantilis PA, Tsoupras G, Polissiou M (1995) Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. J Chromatogr A 699:107–118. https://doi.org/10.1016/0021-9673(95)00044-N
116. Carmona M, Zalacain A, Sánchez AM et al (2006) Crocetin esters, picrocrocin and its related compounds present in *Crocus sativus* stigmas and *Gardenia jasminoides* fruits. Tentative identification of seven new compounds by LC-ESI-MS. J Agric Food Chem 54:973–979. https://doi.org/10.1021/jf052297w
117. Sánchez AM, Carmona M, Ordoudi SA et al (2008) Kinetics of individual Crocetin Ester degradation in aqueous extracts of saffron (*Crocus sativus* L.) upon thermal treatment in the dark. J Agric Food Chem 56:1627–1637. https://doi.org/10.1021/jf0730993
118. Ordoudi SA, de los Mozos Pascual M, Tsimidou MZ (2014) On the quality control of traded saffron by means of transmission Fourier-transform mid-infrared (FT-MIR) spectroscopy and chemometrics. Food Chem 150:414–421. https://doi.org/10.1016/j.foodchem.2013.11.014
119. Anastasaki E, Kanakis C, Pappas C et al (2010) Differentiation of saffron from four countries by mid-infrared spectroscopy and multivariate analysis. Eur Food Res Technol 230:571–577. https://doi.org/10.1007/s00217-009-1197-7
120. Breukers S, Øpstad CL, Sliwka H, Partali V (2009) Hydrophilic carotenoids: surface properties and aggregation behavior of the potassium salt of the highly unsaturated diacid norbixin. Helv Chim Acta 92:1741–1747. ISSN: 0018-019X
121. Serrano-Díaz J, Sánchez AM, Maggi L et al (2011) Synergic effect of water-soluble components on the coloring strength of saffron spice. J Food Compos Anal 24:873–879. https://doi.org/10.1016/j.jfca.2011.03.014

122. Castellar MR, Montijano H, Manjón A, Iborra JL (1993) Preparative high-performance liquid chromatographic purification of saffron secondary metabolites. J Chromatogr A 648:187–190. https://doi.org/10.1016/0021-9673(93)83301-8
123. PITSIKAS N, ZISOPOULOU S, TARANTILIS P et al (2007) Effects of the active constituents of *Crocus sativus* L., crocins on recognition and spatial rats' memory. Behav Brain Res 183:141–146. https://doi.org/10.1016/j.bbr.2007.06.001
124. Li N, Lin G, Kwan Y-W, Min Z-D (1999) Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. J Chromatogr A 849:349–355. https://doi.org/10.1016/S0021-9673(99)00600-7
125. Winterstein E, Teleczky J (1922) Constituents of the saffron. I. Picrocrocin. Helv Chim Acta 5:376–400
126. Straubinger M, Jezussek M, Waibel R, Winterhalter P (1997) Novel Glycosidic constituents from saffron. J Agric Food Chem 45:1678–1681. https://doi.org/10.1021/jf960861k
127. Straubinger M, Bau B, Eckstein S et al (1998) Identification of novel glycosidic aroma precursors in Saffron (*Crocus sativus* L.). J Agric Food Chem 46:3238–3243. https://doi.org/10.1021/jf980119f
128. Carmona M, Zalacain A, Alonso GL (2006) The aroma, in the chemical composition of saffron: color, taste and aroma, ed. by. Editorial Bomarzo SL, Albacete, pp. 123–124
129. Sánchez AM, Carmona M, Jarén-Galán M et al (2011) Picrocrocin kinetics in aqueous saffron spice extracts (*Crocus sativus* L.) upon thermal treatment. J Agric Food Chem 59:249–255. https://doi.org/10.1021/jf102828v
130. Orfanou O, Tsimidou M (1996) Evaluation of the colouring strength of saffron spice by UV – vis spectrometry. Food Chem 57:463–469
131. Lozano P, Castellar M, Simancas M, Iborra J (1999) A quantitative high-performance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L.) products. J Chromatogr A 830:477–483. https://doi.org/10.1016/S0021-9673(98)00938-8
132. Hamid B, Sam S, Islam T et al (2009) The free radical scavenging and the lipid peroxidation inhibition of Crocin isolated from Kashmiri saffron (*Crocus sativus*) occurring in northern part of India. Int J Pharm Tech Res 1:1317–1321. ISSN: 0974-4304
133. Hosseinzadeh H, Sadeghnia HR, Ghaeni FA et al (2011) Effects of saffron (*Crocus sativus* L.) and its active constituent, Crocin, on recognition and spatial memory after chronic cerebral Hypoperfusion in rats. Phyther Res 26:381. https://doi.org/10.1002/ptr.3566
134. Nilakshi N, Gadiya RV, Abhyankar M, Champalal KD (2011) Detailed profile of *Crocus sativus*. Int J Pharma Bio Sci 2:530–540. ISSN: 0975-6299
135. Gismondi A, Serio M, Canuti L, Canini A (2012) Biochemical, antioxidant and antineoplastic properties of Italian saffron (<i>*Crocus sativus* L<i>.). Am J Plant Sci 3:1573–1580. https://doi.org/10.4236/ajps.2012.311190
136. Bolhasani A, Bathaie SZ, Yavari I et al (2005) Separation and purification of some components of Iranian saffron. Asian J Chem 17:725. ISSN: 0970-7077
137. Madan CL, Kapur BM, Gupta US (1966) Saffron. Econ Bot 20:377–385
138. Cossignani L, Urbani E, Simonetti MS et al (2014) Characterisation of secondary metabolites in saffron from Central Italy (Cascia, Umbria). Food Chem 143:446–451. https://doi.org/10.1016/j.foodchem.2013.08.020
139. Zougagh M, Ríos A, Valcárcel M (2005) An automated screening method for the fast, simple discrimination between natural and artificial colorants in commercial saffron products. Anal Chim Acta 535:133–138. https://doi.org/10.1016/j.aca.2004.11.060
140. Corti P, Mazzei E, Ferri S et al (1996) High performance thin layer chromatographic quantitative analysis of Picrocrocin and Crocetin, active principles of saffron (*Crocus sativus* L.-Iridaceae): a new method. Phytochem Anal 7:201–203. https://doi.org/10.1002/(SICI)1099-1565(199607)7:4<201::AID-PCA304>3.0.CO;2-4
141. Lech K, Witowska-Jarosz J, Jarosz M (2009) Saffron yellow: characterization of carotenoids by high performance liquid chromatography with electrospray mass spectrometric detection. J Mass Spectrom 44:1661. https://doi.org/10.1002/jms.1631

142. Pfister S, Meyer P, Steck A, Pfander H (1996) Isolation and structure elucidation of carotenoid−Glycosyl esters in Gardenia fruits (*Gardenia jasminoides* Ellis) and saffron (*Crocus sativus* Linne). J Agric Food Chem 44:2612–2615. https://doi.org/10.1021/jf950713e
143. Sugiura M, Shoyama Y, Saito H, Abe K (1994) Crocin (crocetin di-gentiobiose ester) prevents the inhibitory effect of ethanol on long-term potentiation in the dentate gyrus in vivo. J Pharmacol Exp Ther 271:703–707. ISSN: 0022-3565
144. Zalacain A, Ordoudi SA, Díaz-Plaza EM et al (2005) Near-infrared spectroscopy in saffron quality control: determination of chemical composition and geographical origin. J Agric Food Chem 53:9337–9341. https://doi.org/10.1021/jf050846s
145. García-Rodríguez MV, López-Córcoles H, Alonso GL et al (2017) Comparative evaluation of an ISO 3632 method and an HPLC-DAD method for safranal quantity determination in saffron. Food Chem 221:838–843. https://doi.org/10.1016/j.foodchem.2016.11.089
146. D'Archivio AA, Giannitto A, Maggi MA, Ruggieri F (2016) Geographical classification of Italian saffron (*Crocus sativus* L.) based on chemical constituents determined by high-performance liquid-chromatography and by using linear discriminant analysis. Food Chem 212:110–116. https://doi.org/10.1016/j.foodchem.2016.05.149
147. Rodríguez-Neira L, Lage-Yusty MA, López-Hernández J (2014) Influence of culinary processing time on Saffron's bioactive compounds (*Crocus sativus* L.). Plant Foods Hum Nutr 69:291–296. https://doi.org/10.1007/s11130-014-0447-4
148. Valle García-Rodríguez M, Serrano-Díaz J, Tarantilis PA et al (2014) Determination of saffron quality by high-performance liquid chromatography. J Agric Food Chem 62:8068–8074. https://doi.org/10.1021/jf5019356
149. Karimi E, Oskoueian E, Hendra R, Jaafar HZE (2010) Evaluation of *Crocus sativus* L. stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules 15:6244–6256. https://doi.org/10.3390/molecules15096244
150. Martinez-Tome M, Jimenez AM, Ruggieri S et al (2001) Antioxidant properties of Mediterranean spices compared with common food additives. J Food Prot 64:1412–1419. ISSN: 0362-028X
151. NORBAK R, KONDO T (1999) Flavonol glycosides from flowers of Crocus speciosus and *C. antalyensis*. Phytochemistry 51:1113–1119. https://doi.org/10.1016/S0031-9422(99)00109-0
152. Makhlouf H, Saksouk M, Habib J, Chahine R (2011) Determination of antioxidant activity of saffron taken from the flower of *Crocus sativus* grown in Lebanon. Afr J Biotechnol 10:8093–8100. ISSN: 1684-5315
153. Asdaq SMB, Inamdar MN (2010) Potential of *Crocus sativus* (saffron) and its constituent, Crocin, as Hypolipidemic and antioxidant in rats. Appl Biochem Biotechnol 162:358–372. https://doi.org/10.1007/s12010-009-8740-7
154. Straubinger M, Jezussek M, Waibel R, Winterhalter P (1997) Two Kaempferol Sophorosides from *Crocus Sativus*. Nat Prod Lett 10:213–216. https://doi.org/10.1080/10575639708041197
155. Forkmann G, Martens S (2001) Metabolic engineering and applications of flavonoids. Curr Opin Biotechnol 12:155–160. https://doi.org/10.1016/S0958-1669(00)00192-0
156. Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. Phytochemistry 55:481–504. https://doi.org/10.1016/S0031-9422(00)00235-1
157. Hosseinzadeh H, Karimi G, Niapoor M (2004) Antidepressant effect of *Crocus sativus* L. stigma extracts and their constituents, crocin and safranal, in mice. Acta Hortic 650:435–445. https://doi.org/10.17660/ActaHortic.2004.650.54
158. Bouhsain Z, Garrigues S, de la Guardia M (1997) PLS-UV spectrophotometric method for the simultaneous determination of paracetamol, acetylsalicylic acid and caffeine in pharmaceutical formulations. Fresenius J Anal Chem 357:973–976. https://doi.org/10.1007/s002160050284
159. Espinosa-Mansilla A, Salinas F, Zamoro A (1994) Simultaneous determination of chlorpyrifos and carbaryl by differential degradation using diode-array spectrophotometry optimized by partial least squares. Analyst 119:1183. https://doi.org/10.1039/an9941901183
160. de la Peña AM, Durán-Merás I, Moreno MD et al (1995) Resolution of ternary mixtures of salicylic, salicyluric and gentisic acids by partial least squares and principal component

regression: optimization of the scanning path in the excitation-emission matrices. Fresenius J Anal Chem 351:571–576. ISSN: 0937-0633
161. Berzas NJJ, Gomez LMA, Murillo PJA, Amador-Hernandez J (1998) Simultaneous fluorimetric determination of pyridoxal, pyridoxamine and pyridoxic acid by partial least squares using non-linear variable angle synchronous spectra. Analyst 123:483–488. ISSN: 0003-2654
162. Haghighi B, Feizy J, Kakhki AH (2007) LC determination of adulterated saffron prepared by adding styles colored with some natural colorants. Chromatographia 66:325–332. https://doi.org/10.1365/s10337-007-0321-8
163. Carmona M, Zalacaín A, Rodríguez MI et al (2003) Comparison of different extraction procedures and HPLC methods to detect crocins in saffron. I Int Symp Saffron Biol Biotechnol 650:303–306
164. Loskutov A, Beninger C, Hosfield G, Sink K (2000) Development of an improved procedure for extraction and quantitation of safranal in stigmas of *Crocus sativus* L. using high performance liquid chromatography. Food Chem 69:87–95. https://doi.org/10.1016/S0308-8146(99)00246-0
165. Rambla FJ, Garrigues S, de la Guardia M (1997) PLS-NIR determination of total sugar, glucose, fructose and sucrose in aqueous solutions of fruit juices. Anal Chim Acta 344:41–53. https://doi.org/10.1016/S0003-2670(97)00032-9
166. Verma RS, Middha D (2010) Analysis of saffron (*Crocus sativus* L. Stigma) components by LC–MS–MS. Chromatographia 71:117–123. https://doi.org/10.1365/s10337-009-1398-z
167. Tarantilis PA, Beljebbar A, Manfait M, Polissiou M (1998) FT-IR, FT-Raman spectroscopic study of carotenoids from saffron (*Crocus sativus* L.) and some derivatives. Spectrochim Acta A Mol Biomol Spectrosc 54:651–657. https://doi.org/10.1016/S1386-1425(98)00024-9
168. Anastasaki E, Kanakis C, Pappas C et al (2009) Geographical differentiation of saffron by GC–MS/FID and chemometrics. Eur Food Res Technol 229:899–905. https://doi.org/10.1007/s00217-009-1125-x

Use of Nanotechnology for Immobilization and Entrapment of Food Applicable Enzymes

69

Milad Fathi, Mehri Karim, Soroush Rahimi Khoigani, and Vahid Mosayebi

Contents

Abstract

Enzyme immobilization is defined as artificially restriction of enzyme mobility that leads to change of its structurer, properties, and activity. Since enzymes are very sensitive, their classical immobilization methods are still limited mostly as a result of reduction of enzyme activity. Because of unique and tunable properties of nanomaterials, they have been increasingly applied as carrier for enzyme immobi-

M. Fathi (✉) · M. Karim · S. R. Khoigani · V. Mosayebi
Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
e-mail: mfathi@cc.iut.ac.ir; mehri.karim@ag.iut.ac.ir; s.rahimikhoigani@ag.iut.ac.ir; v.mosayebi@iut.ac.ir

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_52

lization. Using nanotechnology some features, such as multifunctionality, addressability, stability, and multi-compartmentalization, could be improved. In this chapter enzyme immobilization using some nanostructure materials such as nanoparticles, magnetic nanoparticles, nanofibers, nanoflowers, nanoliposome, and solgel silica has been reviewed, and their advantages and disadvantages were reviewed.

Keywords
Nanotechnology · Enzymes · Nanofibers · Nanoflowers · Magnetic nanoparticles · Nanoliposomes · Solgel

1 Introduction

Enzymes are biological catalysts which accelerate chemical reactions by lowering activation energy [1]. Nowadays enzymes have a wide range of applications in food productions such as cheese, bread, yoghurt, and fermented beverages [2]. These biological compounds have excellent properties such as high activity, selectivity, and specificity that may permit to implement the most complex chemical processes [3, 4]. But use of enzymes, purely in food, will be associated with problems such as (1) the need of a lot of enzymes, (2) high costs, (3) increased residual enzyme left in the product, (4) low stability of the enzyme, (5) higher labor costs, (6) incompatible of the continuous process, and (7) low activity [5]. Therefore a new method called immobilization emerged to overcome these limitations which is the powerful tool to improve almost all enzyme properties [3]. During immobilization, enzymes are attached to an inert and insoluble material such as porous glass, polystyrene, agarose beads, and zeolites by physical (hydrophobic and van der Waals), ionic, or covalent binding [4]. The structures of supports have important effect on performance of immobilized enzymes. Thus, the characteristics and reactive groups of material and immobilization conditions are almost considered in development of new supports [3]. Commonly used methods of enzyme immobilization can be classified into (i) physical methods like adsorption, encapsulation, and entrapment and (ii) chemical methods including electrostatic interactions, chelating with a metal, and covalent coupling. Encapsulation and entrapment techniques are commonly used, but the main problem with these methods is the enzyme leakage and slow diffusional mass transfer of substrates and products within the support material. Immobilization using physical adsorption and electrostatic interactions is easy to run, but there are risks regarding non-specific protein binding and loss of enzymes during operation [6]. The main advantage of using physical immobilization methods is their compatibility with mass production. Chemical coupling methods, on the other hand, are more efficient than physical adsorption and entrapment methods with regard to retention of enzyme activity, while they are more complex [7]. Additionally, these methods suffer from the drawback of potential denaturation and deactivation caused by the distortion of the three-dimensional conformation of the protein as a result of multipoint binding [6]. Altogether, the practical applications of the classic immobilization methods are

still limited mostly as a result of the reduction of enzyme activity after immobilization [8]. The reason for that is the change of enzyme conformational integrity which in turn leads to the loss of dynamic properties of enzyme [9].

Application of nanotechnology as a new method leads to some improvement in material properties such as multifunctionality, remote addressability, stability, and multi-compartmentalization [10]. Size reduction of enzyme carrier materials to nanoscale can ameliorate the enzyme features, because this particle size provides minimum diffusional limitation, maximum surface area per unit mass, and high enzyme loading [11]. Enhancement of enzyme stability and activity in some nanostructures such as nanoparticles, nanoflower, nanofibers, mesoporous materials, solgel silica, cross-linked enzyme aggregates (CLEAs)/crystals (CLECs), and single-enzyme nanoparticles have recently been reported [12, 13]. Several studies on the enzyme immobilization on nanosized material of polysulfone nanofibers for extracellular lipase from *Penicillium notatum* [14], PEGylated poly(urea-urethane) nanoparticles for *Candida antarctica* lipase [15], carrageenan hydrogel beads for b-galactosidase [16] and lactase [17], single-enzyme nanoparticles (SENs) for α-chymotrypsin and trypsin [18], nanogel for phenoxazinone synthase [19], and silica gel for laccase [20] have been reported.

2 Enzyme Immobilization

Enzyme immobilization is defined as artificial restriction of enzyme mobility [21]. During immobilization, enzymes are attached to an inert and insoluble material such as porous glass, polystyrene, agarose beads, and zeolites [22]. As shown in Fig. 1, enzymes can be immobilized on the support by different methods, namely, physical adsorption, covalent bonding, entrapment or encapsulation, and cross-linking [4, 23].

2.1 Physical Adsorption

During physical adsorption, enzyme is attached to the support by the interaction of reversible or noncovalent bonding (Fig. 1a) which included ionic, hydrogen bonding, hydrophobic, and van der Waals interactions. Ionic and hydrogen bonding are stronger than hydrophobic and van der Waals interactions. Physical adsorption has several advantages such as simplicity in process that is not affected by the enzyme structure and inexpensive process. Because it is composed of reversible bonding, enzyme can be easily leached [21].

2.2 Covalent Bonding

This immobilization method is composed of covalent attachment between amino acid groups such as histidine, thiol, threonine, and glutamic acid and active groups of solid carrier such as indolyl and imidazole [20] (Fig. 1b). Properties of immobilized

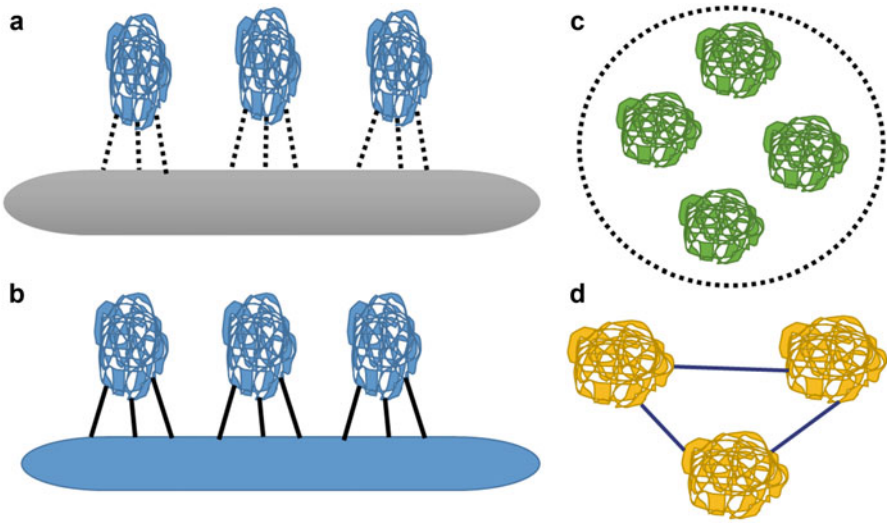


Fig. 1 Physical adsorption (**a**), covalent bonding (**b**), entrapment or encapsulation (**c**), cross-linking (**d**)

enzyme is dependent on the type of active groups and their distribution over the solid carrier [21]. Enzyme leaching in this method is low but structure of enzyme can be changed [4].

2.3 Entrapment and Encapsulation

In this method enzymes are physically confined in the limited space (Fig. 1c) by polymeric matrices, cross-linked arrays, hollow fibers, ultrafilter membranes, and liposomes. Entrapment method has advantages, such as its simplicity in process and reduction of chemical changes of enzymes, while low stability of enzymes, being sensitive to environmental changes which can disrupt capsule, and enzyme leaching are its disadvantages [21].

2.4 Cross-Linking

Because selection of support or carrier and designing immobilization process conditions are time-consuming and more than 50% enzyme activity is lost when it is immobilized on the support, the use of cross-linking is increased. Cross-linking is a carrier-free method in which enzymes are chemically cross-linked together without a solid carrier; therefore its volumetric activity is not decreased [4, 21] (Fig. 1d). Direct cross-linking of enzymes resulted in low activity and weak mechanical stability; therefore this method usually is not used [21]. But recently two methods of

cross-linking (cross-linked enzyme crystals and cross-linked enzyme aggregates) emerged to overcome these deficiencies [4, 24].

2.5 Limitations of Enzyme Immobilization

It is necessary to mention that enzyme immobilization has practical limitations such as the cost of support which in some cases is more expensive than enzyme, the amount of loaded enzyme on the support being low, and structural changes of enzyme [21].

2.6 Selection of Support

The structure and morphology of supports have a large effect on the performance of immobilized enzymes. Thus, the characteristics of the support, functional groups, and immobilization conditions are important [3]. Different nanostructures such as nanofibers, nanoflowers, nanoparticles, nanoliposome, and solgel were used successfully as support because they have high surface to volume ratio [25]. One of the important supports or carriers for enzymes is nanofibers. Nanofibers are defined as long fibers with fine diameters which range from several micrometer to 10 nm and are fabricated by electrospinning process [26].

3 Enzyme Immobilization on Nanofibers

The term electrospinning is derived from "electrostatic spinning" [27] and is an electrohydrodynamic process which converts a polymeric solution to fibrous material. Electrospinning process is composed of different parts such as power supply, syringe pump, and grounded collector. Polymer solution is ejected from needle syringe with constant rate, and then the polymer is stretched by electrostatic force which exists between the needle (as positive pole) and collector (as negative pole). This phenomenon causes decrease in fiber diameter and solvent evaporation. Figure 2 shows schematic of electrospinning instruments [22, 28].

Electrospinning has powerful ability to produce fibers in the range of micrometers to 10 nm. Fibers produced by this method have unique properties such as high surface area, alterable surface properties, and high porosity which directly correlate with solvent vapor pressure. On the other hand, when vapor pressure of solvent is high, the solvent vigorously evaporate from fibers and create many holes or pores on the surface of fibers [23, 29]. Moreover some studies prepared nanofibers with specific secondary structure, which contained core-shell (fabricated by two polymers and specific needle), hollow (fibers with hollow interior), and porous (fibers having a porous surface which is created by two methods: selective removal of a component and application of phase separation) forms [28]. The unique properties of nanofibers

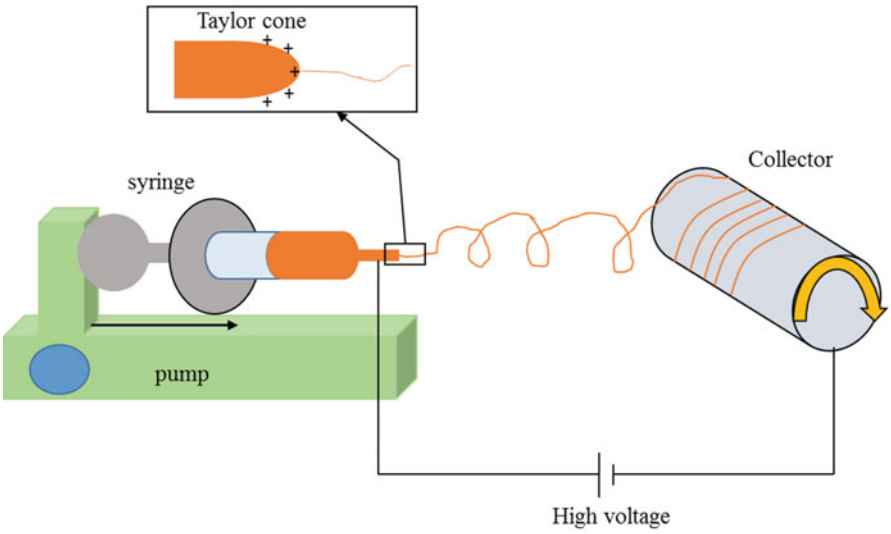


Fig. 2 Schematic electrospinning process

enable them to utilize in a wide range of applications such as tissue engineering applications, dressings for wound healing, energy generation applications, drug delivery, filtration, and enzyme immobilization [27]. Many studies have immobilized enzymes on nanofibers; investigated their properties such as stability, activity, and enzyme loading; and optimized conditions by modifying surface of fibers or selecting the best fibers with special enzyme to improve these properties. Activity of enzymes is calculated by dividing micromole of substrate to minute per gram of catalyst in defined condition [21]. Quantity of loaded enzyme is obtained by subtracting the final concentration (after washing fibers) from the initial concentration [30]. Table 1 shows the results of some studies which worked on the immobilization of enzyme using nanofibers.

Generally, enzyme activity in immobilized state is lower than in free state because immobilization process has limited mass transfer. On the other hand immobilization reduces connection of enzyme and substrate. Moreover when enzymes are immobilized by covalent bonding, it is possible that its structure changed, and therefore its activity decreases [30, 35, 40]. Several factors have influence on activity, stability, and quantity of loaded enzyme.

(i) Mass transfer is an effective factor on the immobilized enzyme activity which is classified in two categories, namely, external mass transfer that is defined as transportation of substrate from environment to the surface of immobilized enzyme and internal mass transfer which is expressed as diffusion of substrate into the support or carrier media [21]. Nanofibers caused higher mass transfer and higher enzyme activity by increasing surface to volume ratio [30, 35, 36].

Table 1 The result of some studies on the enzyme immobilization by nanofibers

Nanofibers	Immobilized enzyme	Fiber diameter	Enzyme loaded (mg/g fibers)	Activity retention %	Immobilization method	Reference
Polystyrene	α-Chymotrypsin	120 nm–1 μm	14.0	65	Chemical	[31]
Silk fibroin	α-Chymotrypsin	205–320 nm	56.6	66.78	Chemical	[32]
Polystyrene and polystyrene-co-maleic anhydride	Esterase	200–1,000 nm	–	80	Chemical	[33]
Polystyrene and polystyrene-co-maleic anhydride	β-Glucosidase	200–1,000 nm	–	91	Chemical	[34]
Poly-acrylonitrile-*co*-maleic acid	Lipase	100 nm	21.2 ± 0.71	37.6 ± 1.8	Chemical	[30]
Polyacrylonitrile	Lipase	150–300 nm	21.2 ± 1.3	81.3 ± 1.1	Chemical	[35]
Polyacrylonitrile	Lipase	100 ± 20 nm	23.2 ± 1.6	56.4 ± 0.7	Physical	[36]
Poly[acrylonitrile-co-(2-methacryloyloxyethyl phosphorylcholine)]	Lipase	90 ± 20 nm	22.9 ± 1.5	76.8 ± 0.6	Physical	[36]
Cellulose	Lipase	200 nm	–	–	Chemical	[37]
Poly(styrene-co-maleic anhydride)	α-Chymotrypsin	444 ± 106 nm	–	–	Chemical	[29]
Poly(ε-caprolactone) and poly(D,L-lactic-*co*-glycolic acid)-poly(ethylene glycol)-NH2 (PLGA-*b*-PEG-NH2)	Lysozyme	352 ± 168 nm	–	–	Chemical	[38]
Poly (acrylonitrile-*co*-acrylic acid)	Catalase	181.49 ± 15.04 nm	23.9 ± 0.62	33.11	Chemical	[39]
Poly (acrylonitrile-*co*-acrylic acid) multi-walled carbon nanotubes	Catalase	286.72 ± 9.32 nm	31.1 ± 4.54	47.90	Chemical	[39]
Polyvinyl alcohol	Cellulase	200 nm	–	65	Chemical	[40]
As-spun polystyrene/poly styrene-*co*-maleic anhydride	Lipase	619 ± 175 nm	5.6 ± 2.2	16.5	Chemical	[41]

(*continued*)

Table 1 (continued)

Nanofibers	Immobilized enzyme	Fiber diameter	Enzyme loaded (mg/g fibers)	Activity retention %	Immobilization method	Reference
Alcohol-pretreated polystyrene/poly styrene-*co*-maleic anhydride	Lipase	575 ± 202 nm	42.4 ± 18.5	16.5	Chemical	[41]
Polyaniline	Lipase	–	–	80	Chemical	[42]
Polyacrylonitrile	Catalases	–	24.45 ± 2.33	32.4 ± 3.2	Chemical	[43]
Polyacrylonitrile with multi-walled carbon nanotubes	Catalases	–	29.81 ± 3.76	45.3 ± 3.5	Chemical	[43]
Poly(acrylonitrile-*co*-*N*-vinyl-2-pyrrolidone)	Catalases	–	18.39 ± 3.29	37.5 ± 2.7	Chemical	[43]
Poly(acrylonitrile-*co*-*N*-vinyl-2-pyrrolidone) with multi-walled carbon nanotubes	Catalases	–	25.77 ± 4.10	48.7 ± 3.6	Chemical	[43]
Polysulfone	Lipase	–	0.8 ± 0.12	6.2 ± 0.32	Physical	[14]
Polysulfone and poly(*N*-vinyl-2-pyrrolidone)	Lipase	–	0.59 ± 0.09	26.7 ± 0.42	Physical	[14]
Polysulfone poly(ethylene glycol)	Lipase	–	1.24 ± 0.15	18.7 ± 0.23	Physical	[14]

(ii) Diameter of fibers, by decreasing fiber diameter, and surface of fibers increased, so the amount of loaded enzyme increased which leads to higher activity of nanofibers [30–32, 36, 42].
(iii) Type of enzyme interaction with nanofibers is another factor affecting its activity and stability. Lee et al. [34] find that stability and activity of immobilized β-glucosidase by aggregation method is more than the immobilized enzyme by covalent method because loaded enzyme in aggregation is higher than covalent method [34].
(iv) Autolysis of enzyme during the immobilization resulted in production of amino acids, and then these compounds compete on the active sites of nanofibers therefore decreasing enzyme loading, stability, and activity.
(v) Availability of active group on the nanofibers. Sometimes active groups are covered by other nanofibers and so are not available for enzyme, reducing enzyme loading [31].
(vi) Fibers' compatibility with immobilization conditions.

There are some limitations with immobilized enzyme which can be solved by some modifications of nanofibers' surface. The modification methods can be mentioned:

(i) Modification of surface by biomimetics methodology which creates a condition for immobilized enzyme same as living cells. For example, nanofibers with carboxyl groups were modified by chitosan or gelatin [25].
(ii) Modification of surface in order to increase enzyme mobility. In this method, spacer arms (such as poly(ethylene glycol)) are added to the nanofibers to increase enzyme mobility (Fig. 3a) [25].
(iii) Modification of surface in order to increase electrical conductivity. This method is applied to redox enzymes which can be used for polymers with high electrical conductivity.
(iv) Modification of surface by adding or grafting polymers on the nanofibers. As shown in Fig. 4b, c, some polymers, for example, poly acrylic acid, can be grafted on cellulose nanofibers by different methods which included gel-like or brushlike structure. Gel-like structures have high enzyme loading capacity but low mass transfer, and brushlike structures have low enzyme loading capacity [25].

4 Immobilization Using Magnetic Nanoparticles

By enzyme immobilization, its structure, properties, and activity can be changed. Since enzymes are very sensitive, their immobilization needs a proper methodology for maintaining or finally improving their properties such as stability and activity. Immobilization can be reversible and irreversible. Irreversible immobilizations (covalent attachment of the enzyme to the surface or entrapment in a matrix or encapsulation) prevent enzyme from leaching, but reactions happen. This system

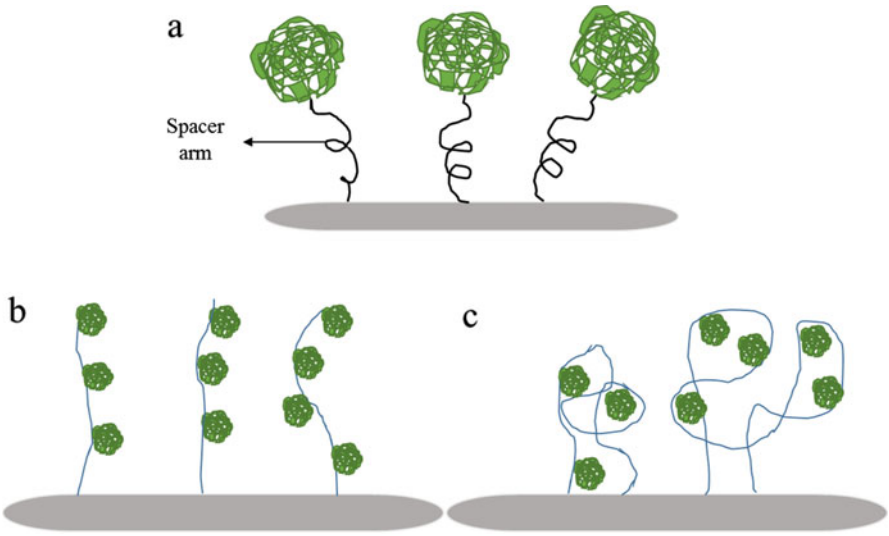


Fig. 3 Schematic representation of spacer arm (**a**), brushlike (**b**), and gel-like (**c**) structures

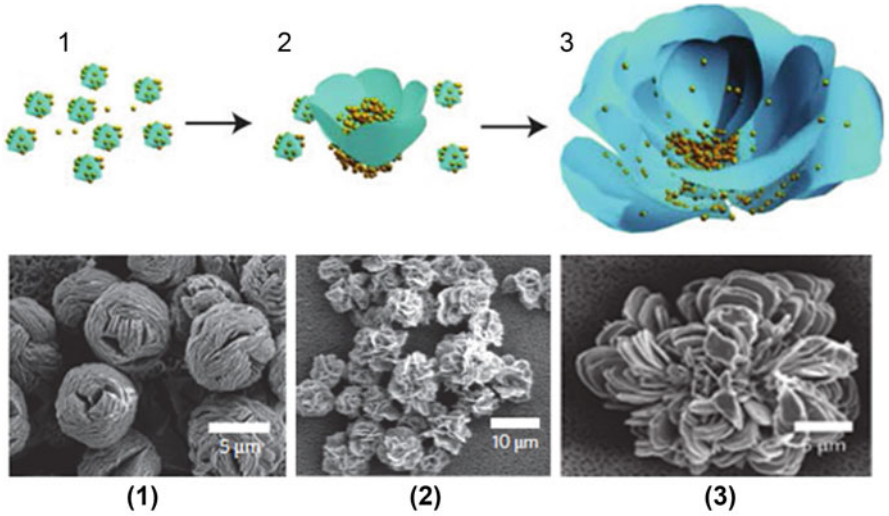


Fig. 4 Up, proposed mechanism, comprising three steps: (*1*) nucleation and formation of primary crystals, (*2*) growth of crystals, and (*3*) formation of nanoflowers. Down: SEM images of carbonic anhydrase hybrid nanoflowers at protein concentrations of (*1*) 0.5 mg ml^{-1}, (*2*) 0.1 mg ml^{-1}, and (*3*) 0.02 mg ml^{-1} [83] (with permission)

permits recovery of support after enzyme inactivation. In reversible immobilization (e.g., in immobilization using magnetic nanoparticles), the simplest method is direct adsorption over the surface, and the interactions between nanoparticle surface and

enzyme surface can be hydrogen bonding, van der Waals forces, and hydrophobic interactions. This system can be affected by pH, ionic strength, temperature, or polarity of the solvent [44].

In recent years, nanoparticles as solid carriers are employed in various immobilization methods [45]. While their limitation is removing them from solution and environment. For this reason, researchers introduced magnetic nanoparticles that would be easily recovered for reuse [46]. Magnetic nanoparticles among the rest of nanomaterials are suitable carriers for bioactive materials such as peptides, enzymes, antibodies, and nucleic acids [47]. Their benefits are tailored surface chemistry, low toxicity, low cost, improved enzyme activity and stability, high surface area, easy separation, and recyclability [46]. Various magnetic particles and magnetic supports have been applied [48]. Among different types of iron oxide, the most popular magnetic nanoparticles are nanoscale zerovalent iron, Fe_3O_4, and Fe_2O_3, with the former being used more frequently since Fe^{+2} state has good potential for acting as an electron donor [49].

Various studies have been performed on magnetic nanoparticle application for immobilization of enzymes like cholesterol oxidase by Fe_3O_4 nanoparticles for analysis of total cholesterol in serum [50]; laccase on chitosan-magnetic nanoparticle for bioremediation of environmental pollutants [51]; α-amylase on cellulose-coated magnetite nanoparticles for starch degradation [52]; pectinase over the silica-coated magnetic (Fe_3O_4-SiO_2) nanoparticles for apple juice clarification [53]; peroxidase on gold-chitosan nanoparticle for rapid deterioration of H_2O_2, water treatment, and pharmaceutical and biomedical applications [54]; cyclodextrin glycosyl transferase on cellulose-silver nanoparticle for biosensors [55]; alcohol oxidase on cellulose-silver nanoparticle for determination of methanol and methyl ester content of pectin [56]; co-immobilized alpha amylase and glucoamylase on magnetic chitosan beads for complete hydrolysis of starch into glucose [57]; and multiple immobilized enzymes of maltodextrin phosphorylase, glucose-1-phosphate thymidylytransferase, and pyrophosphatase on amino-functionalized magnetic nanoparticles for production of uridine diphosphate glucose [58].

There are three methods to prepare magnetic nanoparticles [59]:

(i) Physical methods such as (a) size reduction to the nanometer scale and dispersing in an aqueous solution and (b) condensation of precursors from a liquid and gaseous phase [60]. The difficulty of producing the desired particle size and shape is the main drawback of these methods [61]. However, magnetic nanoparticles with an average particle size of 20–50 nm and narrow particle size distribution can be produced by a new laser ablation or evaporation synthesis method [62].

(ii) Wet chemical preparation methods such as chemical coprecipitation, hydrothermal, solgel reactions, flow injection syntheses, electrochemical, aerosol/vapor methods [63], sonolysis [64], and thermal decomposition [65].

(iii) Microbial methods that magnetic nanoparticles are produced by a biomineralization process [66]. The advantages of this method are high efficiency, good reproducibility, scalability, and control over the particle size [67].

During enzyme immobilization, cross-linking agent has an important role in enzyme activity, recovery, and stability [68]. Among cross-linking agents for protein, glutaraldehyde is the best choice. It is inexpensive and readily available and has ability to make covalent bonds with most of enzymes [69]. While due to some drawbacks, glutaraldehyde cannot be commonly used as a cross-linker in all cases. Small size of glutaraldehyde causes to be placed in enzyme active sites, resulting in inactivation of enzyme in some cases [70]. In addition, its toxic nature has harmful effect on human and aquatic life, if leaching of glutaraldehyde from prepared materials occurred [71]. For this reason, recently, cross-linker agents based on polysaccharides for proteins have been noticed [72]. Newly, pectin-based cross-linker has been used to immobilize glucoamylase onto 3-aminopropyl triethoxysilane-modified magnetic nanoparticles. Results show that pectin is more effective than traditional glutaraldehyde [73].

When magnetic nanoparticles covered with chitosan are employed as support for β-galactosidase, the same or even higher activity in a wider range of temperature and pH, than the free form of enzyme, was observed, and 92% of its activity after 15 successive cycles was maintained. Immobilization of protease on magnetic nanoparticle for reducing autolysis of products showed significant resistance to thermal inactivation, and about 59% of its initial activity after 2 h at 65 °C was retained [44]. Sojitra and Nadar [74] reported that when pectinase was immobilized on chitosan-magnetic nanoparticles by dextran polyaldehyde as a macromolecular cross-linking agent, its V_{max} and K_m values were nearly similar to native form. The residual activity of immobilized pectinase was 85% after seven successive cycles of reuse, and it maintained up to 89% of residual activity on storage of 15 days which displayed great stability and durability. Eventually, magnetic pectinase was applied for apple juice clarification and showed turbidity reduction up to 74% after 150 min of treatment. The effect of particle size on activity and recycling capabilities of glucose oxidase immobilized onto magnetic nanoparticles had been studied by Park and McConnell [75]. Three different sizes of magnetic nanoparticles (5 nm, 26 nm, and 51 nm) had been used, and the results showed about 20% of activity was lost for the large-sized (51 nm) and medium-sized (26 nm) glucose oxidase-magnetic nanoparticle and approximately 96% activity was lost for the smallest one (5 nm) after ten cycles. Table 2 showed enzyme-recovered activities (the activity of the immobilized enzyme comparing to the activity of its free form). Apparently most of them are less than the activity of free enzymes. Magnetic silica nanocomposite materials due to coating of the surface of magnetite nanoparticles with functionalized silica will not be aggregated in liquid, and their chemical stability and biocompatibility will be improved. The binding of enzymes on the hydrophilic surface of silica nanoparticles in aqueous solution will be performed better [44].

5 Immobilization on Organic-Inorganic Hybrid Nanoflowers

Organic-inorganic hybrid nanoflowers (HNFs) are novel promising materials for immobilization of enzymes that demonstrate mostly much greater activities and stabilities than free and conventionally immobilized enzymes. They attracted

Table 2 Examples of enzymes immobilized on magnetic nanoparticles and their recovered activity

Enzyme	Coupling agent	Magnetic carrier	Recovered activity (%)	Reference
Cellulase	Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride	Fe_3O_4	30.2	[76]
Glucose oxidase	Glutaraldehyde	$CoFe_2O_4$, SiO_2	80	[77]
Cholesterol oxidase	Glutaraldehyde	eFe_2O_3, SiO_2	60	[78]
Candida rugosa lipase	Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride	Fe_3O_4, lauric acid	180	[79]
Esterase	Glutaraldehyde	Fe_3O_4	63	[80]
Alkaline phosphatase	Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride	Fe_3O_4	44	[81]

growing attention in recent years due to their simple, eco-friendly, and low energy-intensive synthesis procedure [82]. The production method was discovered for the first time by accidental addition of $CuSO_4$ solution to phosphate-buffered saline (PBS) containing protein (bovine serum albumin (BSA)) [83]. The typical BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ HNFs was synthesized as follows: 3 mL of 10 mM phosphate-buffered saline (pH = 7.4) containing 0.1 mg of BSA and 20 μL of $CuSO_4$ aqueous solution were added at room temperature (25 °C). After incubating for 3 days at room temperature, a blue color precipitate was formed at the bottom of test tube. Analyzing the precipitate with scanning electron microscopy (SEM) revealed a porous and flowerlike structure for HNFs, shown schematically in Fig. 4 [83].

The enhanced activity of HNFs was thought to be due to (1) greater surface area of nanopetals and as a result less limitations against mass transfer (2) cooperative effects of immobilized enzyme molecules on each other and (3) better confinement of enzymes inside the nanoflowers [84]. Following Zare group, many other researchers utilized this novel immobilization technique for the synthesis of various new HNFs using several other enzymes and metal ions. As examples, laccase [85], lipase [86], horseradish peroxidase [87], soybean peroxidase [88], lactoperoxidase [89], glucose oxidase [90], urease [91], trypsin [87], chymotrypsin [92], papain [93], his-tagged enzymes [94], and α-acetolactate decarboxylase [95] have already been immobilized in the form of HNFs. In most cases, literature data shows that enzyme-incorporated HNFs have higher activities compared to free or conventionally immobilized enzymes.

These enzyme-based HNFs have been used as biosensors, bioanalytical devices, biofuel cells, and biocatalysts. For example, colorimetric sensors for detection of hydrogen peroxide and phenol were developed by Sun et al. [90]. Wang et al. synthesized α-amylase-$CaHPO_4$ HNFs for digestion of protein [96]. Recent reviews by Altinkaynak and Tavlasoglu [84] discussed in more detail the types, structural characteristics, potential mechanism of formation, and futuristic bio-based applications of some enzyme-inorganic hybrid nanoflowers. Co-immobilization of two or more different proteins or enzymes simultaneously in a single unit is an interesting idea for extending functionality, and applications of hybrid nanoflowers attracted

much attention recently. Co-immobilized glucose oxidase and horseradish peroxidase hybrid nanoflowers (GOx-HRP-$CU_3(PO_4)_2$) enabled the two-step reaction in only one-step cascade catalytic reaction [90]. Ye and Zhu [97] developed concanavalin A-GOx-$CaHPO_4$ (CGC) hybrid nanoflowers with a facile and eco-friendly method which was used as probes for on-site detection of *E. coli* O157:H7. They reported that the enzymatic activity of GOx decreased by 27% probably due to the limited mass transfer in CGC configuration. By contrast, GOx immobilized in CGC nanoflowers showed more storage stability with more than 90% residual activity after 30 days at room temperature. Table 3 shows some recent works concerning with enzyme immobilization using (HNFs) technique. Cui et al. developed a new strategy to increase further the activity and stability of lipase-embedded hybrid nanoflowers. Normally, lipases have a concealing polypeptide chain named "lid" on their surface, blocking the access of substrate to active site of enzyme which in this case enzyme is in its inactive form [98]. But, surfactants cause the dislocation of lid and as a result lipase change to its open and active form [98].

6 Enzyme Entrapment in Nanoliposomes

Encapsulation of enzyme in a polymeric network by covalent or noncovalent bonds is named entrapment. Liposomes are spherically aggregated amphiphilic molecules in water and can be produced by natural ingredients. Liposome sizes are in the range of 10–100 nm for monolayer liposomes to several micrometers for multilayer liposomes. Nanoliposomes are liposomes in nanosize and used in encapsulation and controlled release systems. Compared to liposomes, nanoliposomes have more surface area and potential to increase solubility, improve bioavailability, and meliorate controlled release [106]. They can be applied to encapsulate protein such as enzymes owing to their ability to contain water-soluble enzymes within [106]. Liposomes and nanoliposomes separate encapsulated enzymes from the surroundings and maintain them in conditions that would hinder their activity or cause denaturation. In addition, they can be applied as a means of controlled release. Enzymes can deliver their content in specific parts of the food system or in an appropriate time [107]. When amphiphilic molecules such as phospholipids are put in an aqueous environment and sufficient amount of energy is supplied, phospholipids aggregate; they can arrange themselves in the form of liposomes. During this process, hydrophilic materials in solution can be entrapped in liposomes, and lipid-soluble molecules can be entrapped in liposomal bilayers by dissolving them with the lipids [107]. During liposome preparation, enzyme can be entrapped in the aqueous interlaminar spaces [108]. Preparation method and its condition (such as lipid concentration, enzyme purity, and phospholipid type) have important effects on enzyme encapsulation efficiency [108].

There are several methods to produce nanosized liposomes such as mechanical (extrusion, sonification, high-pressure homogenization, microfluidization, and colloid mill) and nonmechanical (reversed-phase evaporation and depletion of mixed detergent-lipid micelles). Also, many methods for liposome stabilization

Table 3 Enzyme immobilization with hybrid organic-inorganic nanoflowers

Enzyme	Hybrid nanoflower	Application	Relative activity (%)	Storage stability (days)	Reusability (cycles)	Specific advantage	Reference
α-Acetolactate decarboxylase (ALDC)	Alginate coated ALDC-$Ca_3(PO_4)_2$	Prevention of diacetyl (off-flavor compound) formation in beer	98	–	80% (6)	Improved stability against broad range of pH and temperature, improved recyclability	[95]
Peroxidase from Turkish black radish (TBR)	TBR-$Cu_3(PO_4)_2$	Decolorization of Victoria blue dye	450	–	–	Excellent stability and reusability	[99]
Peroxidase from horseradish (HRP)	Streptavidin-HRP-$Cu_3(PO4)_2$	Combined with ELISA, used as colorimetric sensor for detection of disease-related biomarker	–	>80% (30, RT)	75% (6)	Improved catalytic activity	[100]
Lipase from *Candida rugosa*	Glutaraldehyde-treated lipase-$CuSO_4$	–	95	–	>70% (4)	Improved reusability	[101]
Glucose oxidase (GOx) from *Aspergillus niger*	Concanavalin A-GOx-$CaHPO_4$	On-site detection of *Escherichia coli* O157:H7	73	>90% (30, RT)	–	A simple but potentially powerful amplification biosensing technology with excellent simplicity, portability, sensitivity, and adaptability	[97]
Horseradish peroxidase (HRP)	Antibody-HRP-$Cu_3(PO_4)_2$ three-in-one nanoflower	As a novel enzyme-labeled antibody (ELISA) for *Escherichia coli* O157:H7 detection	–	93% (10, RT)	–	Easier preparation, high antigen capture capability, enhanced enzymatic activity, potential alternative to conventional ELISA	[102]

(*continued*)

Table 3 (continued)

Enzyme	Hybrid nanoflower	Application	Relative activity (%)	Storage stability (days)	Reusability (cycles)	Specific advantage	Reference
Lipase from porcine pancreas	Lipase-$Cu_3(PO4)_2{\cdot}3H_2O$	–	878	–	–	–	[82]
Papain	Papain-$Cu_3(PO4)_2{\cdot}3H_2O$		7260	–	–	–	
Lipase from bovine pancreas	Surfactant-activated lipase-$CuSO_4{\cdot}5H_2O$	–	460	95% (20, 25 °C)	>90% (8)	Improved kinetic parameters, higher temperature and pH resistance, good mechanical stability	[98]
Lipase from *Burkholderia cepacia* (BCL)	BCL-$Ca_3(PO_4)_2{\cdot}nH_2O$	–	308	>75% (15,	80% (5)	–	[103]
Glucoamylase	Glucoamylase-$Cu_3(PO_4)_2{\cdot}3H_2O$	Liquefaction of oligosaccharides and starch	204	91% (25, RT)	70% (8)	Improved thermostability	[104]
L-arabinose isomerase from *Paenibacillus polymyxa*	PPAI-$Ca_3(PO_4)_2$	Production of L-ribulose and D-tagatose	–	–	–	Improved thermal and acid stability	[105]
Papain	Papain-$Zn_3(PO_4)_2{\cdot}4H_2O$	–	–	82.3% (36, 4 °C)	88.8% (10)	Improved operational stability, improved thermostability, long storage life	[93]
Urease from bovine serum	Lipase-$Cu_3(PO_4)_2{\cdot}3H_2O$	–	2000	96.3% (30, 4 °C)	–	–	[91]
Soybean peroxidase (SBP)	SBP-$Cu_3(PO_4)_2{\cdot}3H_2O$	Simultaneously purification and immobilization of SBP	446	–	–	Excellent reusability	[88]

such as lyophilization, freezing, spray-drying, and supercritical fluid (SCF) technology have been studied. Lyophilization is the preferred method to prolongate the liposome shelf life, especially for liposomes containing thermosensitive materials like enzyme [109].

Most common methods for nanoliposome production and their advantages and disadvantages are listed in Table 4. Large-scale production of liposome and nanoliposome for food application is always easy.

Physical stability of nanoliposomes depends on their size, number of layers, phospholipid structure, and manufacturing method. Temperature, ionic concentration, pH, and presence of phospholipase enzymes affect the release of the encapsulated enzyme. When the enzymes are put into the bilayer position of nanoliposomes, their release became faster than when they are located in the center [110]. It should be noted that leaking of entrapped enzymes from liposomes may occur during storage. Nanoliposomes are thermodynamically unstable and during their storage they aggregate, precipitate, and flocculate. Increasing interparticle repulsion by coating the vesicle surfaces with special polymers, like polyethylene glycol or chitosan and using stabilizing agents like cholesterol or glycerol, can improve their stability [107].

Application of entrapped enzyme using nanoliposome has been reported for proteolysis enhancement of cheese for better texture and flavor in half normal time and lower amount of enzyme. Some advantages of lipid vesicles applied for cheese production are as follows: (1) they can be produced from naturally present ingredients in cheese, (2) can protect casein from early hydrolysis during cheese manufacturing, (3) and can properly separate in curd milk [106]. Encapsulations of β-galactosidase to induce the slow digestion of lactose for lactose intolerants and various cheese-ripening enzymes like lipase, nutrease, chymotrypsin, chymosin, neutral protease, cyprosine, flavourzyme, and palatase in liposomes and

Table 4 Most common methods for nanoliposome production: advantages and disadvantages

Method	Advantages	Disadvantages	Reference
Probe sonication	Rapid method	Possible degradation of active material because of high-energy sonication	[110]
Homogenization	Efficient in producing dispersed nanoparticles	Possible degradation of active material because of high-energy input	[110]
Microfluidization	No organic solvent needed	Possible degradation of active material because of high energy and pressure input	[108]
Heating method	No organic solvent needed	Multistep technique; possibility of enzyme denaturing	[108]
Mozafari's method	Very simple and rapid method; no organic solvent needed	Possibility of enzyme denaturing	[109]

nanoliposomes have been reported earlier and were reviewed by Mohammadi and Mahmoudzade [110].

7 Enzyme Entrapment in Solgel

Solgels are inorganic gel in the form of films, fibers, or nanospheres that are stable in chemical, thermal, and photochemical properties. Oxide mixtures of silica, alumina, alumnosilica, titanium, or zirconium are generally used for solgel preparation. For encapsulation of biomaterials like enzymes, silica solgels are often good choice. Because of using relatively mild conditions in their production, activity of the entrapped enzymes is almost retained [111]. Silicon dioxide (SiO_2) and silicon tetroxide (SiO_4) are commonly used for enzyme immobilization. The former is flexible and the latter is rigid, while both of them are in 3D polymers. They have adsorptive properties because of hydrophilic and hydrophobic sites [112]. Silica (or silica gel) is nontoxic and biocompatible and does not swell very much, and its porosity remains unchanged [113]. Since silica carriers are chemically inert, their modification is required for activation. Commonly for introducing amino group, they are modified by treating aminoalkyl triethoxysilanes and a variety of methods for enzyme immobilization [112]. Their functionalities can be changed by decorating the surface of the silica with free silanols through chemical derivatization (silanization reactions), electrostatic interactions (adsorption of polycations), and hydrogen bonding [113]. In a typical manufacturing, tetramethyl orthosilicate or tetraethyl orthosilicate is hydrolyzed into "sol" and then enzyme solution added to the "sol." It initiates condensation reaction and makes "gel," and encapsulation of enzyme in silicate matrices is performed. Various pores and channels in the final silicate matrices in the range of 0.1–500 nm are formed [12]. If obtained solgels are dried before use, gels with different pore sizes (xerogels, aerogels, or ambigels) are obtained. When hydrophilic solgel at room temperature is dried, xerogels with pores of 1–10 nm in diameter are obtained. Aerogels can be made by drying the solgel with supercritical CO_2 and have near unaltered porosity. By gelation at pH 7 and drying with supercritical CO_2, aerogels with large distribution in pore diameters (2–80 nm), mostly between 12 and 40 nm, are formed. When hydrophobic gels are dried at room temperature, ambigels with the same size and porosity as wet gels are produced. If hydrophobic gels are dried with supercritical CO_2, aerogels with hydrophobic properties are obtained [111].

Recently aerogels have been applied for encapsulation of several enzymes, like tyrosinase for phenol removal from aqueous solution and cellulase for hydrolysis of carboxymethyl cellulose [111]. Entrapped enzymes in solgel can be used in electrochemical and optical biosensors to determine the concentration of various analytes. Solgel-encapsulated creatine kinase was resistant to heat and has maintained 50% of its activity ten times longer than the free enzyme. Phosphatases in silica solgel matrices are resistant to harsh pH conditions. For example, alkaline phosphatase with optimum activity at pH 9.5 can be active at a pH as low as 0.9. Probably, this was due to space limitation in the pores [114]. For preventing the leaching of

encapsulated enzymes during storage, careful optimization process is required. However, if leaching is prevented, solgel method leads to a relatively stable form of enzyme immobilization and prevents from unfolding and denaturation of encapsulated enzymes [12]. The nature of the encapsulated protein and the gel are the most major factors in enzyme retention. If molecular mass of protein is as low as 8000 Da, it can be efficiently retained, while larger molecules with molecular mass over 100,000 Da, like polylysine, can diffuse inside the same gel [115]. It should be noted that solgel-immobilized enzymes generally have lower activity than the free enzymes because of small pore size and non-open pore obstacle. Moreover, the dissimilar pore sizes of most silica gel made less reproducible processes. Unlike solgel silica, mesoporous silica materials supply tunable and similar pore system, functionalizable surfaces, and closed nanospaces for enzyme immobilization [116].

8 Conclusions

Over the last few years, some interesting new developments in enzyme immobilization have entered an exciting new phase. Apart from retention, recovery, and stabilization, other advantages to enzyme immobilization such as enhanced enzyme activity, modification of substrate selectivity, and multienzyme reactions have emerged. In this chapter, some enzyme stabilization methods and their properties were discussed. Application of magnetic nanoparticles can improve the stability of the immobilized enzymes as well as reduce the need for downstream separation processes to recover the biocatalyst. Nanofibers with hollow and porous structure have unique properties to be utilized in a wide range of applications for enzyme encapsulation. Immobilized enzymes in solgel retain their bioactivity and are even protected by the silica cage. Such inorganic matrices offer several advantages compared with organic polymers such as mechanical strength or chemical inertia. Moreover, they do not swell in water and can be easily shaped into microcapsules, films, or fibers. The enhanced activity of nanoflowers was due to greater surface area of nanopetals, cooperative effects of immobilized enzyme molecules on each other, and better confinement of enzymes inside their structure. These technologies were initially developed in the pharmaceutical world and now expanding in other fields like food processing. Cost and scale-up are the main issues which are limiting the application of these types of technologies. It is clear that these areas of researches still have a very big opportunity for researchers in order to develop new ideas concerning modeling and scale-up of immobilized enzymes.

References

1. Whitehurst RJ, Van Oort M (2009) Enzymes in food technology. Wiley, New Delhi
2. Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications. Curr Opin Biotechnol 13:345–351

3. Mateo C, Palomo JM, Fernandez-Lorente G, Guisan JM, Fernandez-Lafuente R (2007) Improvement of enzyme activity, stability and selectivity via immobilization techniques. Enzyme Microb Technol 40:1451–1463
4. Sheldon RA (2007) Enzyme immobilization: the quest for optimum performance. Adv Synth Catal 349:1289–1307
5. James J, Simpson BK, Marshall MR (1996) Application of enzymes in food processing. Crit Rev Food Sci Nutr 36:437–463
6. Cao Y, Wen L, Svec F, Tan T, Lv Y (2016) Magnetic AuNP@Fe_3O_4 nanoparticles as reusable carriers for reversible enzyme immobilization. Chem Eng J 286:272–281
7. Savolainen A, Zhang Y, Rochefort D, Holopainen U, Erho T, Virtanen J et al (2011) Printing of polymer microcapsules for enzyme immobilization on paper substrate. Biomacromolecules 12:2008–2015
8. Ding C, Sun H, Ren J, Qu X (2017) Immobilization of enzyme on chiral polyelectrolyte surface. Anal Chim Acta 952:88–95
9. Secundo F (2013) Conformational changes of enzymes upon immobilisation. Chem Soc Rev 42:6250–6261
10. Delcea M, Yashchenok A, Videnova K, Kreft O, Möhwald H, Skirtach AG (2010) Multicompartmental micro-and nanocapsules: hierarchy and applications in biosciences. Macromol Biosci 10:465–474
11. Jia H, Zhu G, Wang P (2003) Catalytic behaviors of enzymes attached to nanoparticles: the effect of particle mobility. Biotechnol Bioeng 84:406–414
12. Kim J, Grate JW, Wang P (2006) Nanostructures for enzyme stabilization. Chem Eng Sci 61:1017–1026
13. Hyeon JE, Shin SK, Han SO (2016) Design of nanoscale enzyme complexes based on various scaffolding materials for biomass conversion and immobilization. Biotechnol J 11:1386
14. Wang Z-G, Wang J-Q, Xu Z-K (2006) Immobilization of lipase from *Candida rugosa* on electrospun polysulfone nanofibrous membranes by adsorption. J Mol Catal B Enzym 42:45–51
15. Cipolatti EP, Valério A, Nicoletti G, Theilacker E, Araújo PH, Sayer C et al (2014) Immobilization of Candida antarctica lipase B on PEGylated poly (urea-urethane) nanoparticles by step miniemulsion polymerization. J Mol Catal B Enzym 109:116–121
16. Zhang Z, Zhang R, Chen L, McClements DJ (2016) Encapsulation of lactase (β-galactosidase) into κ-carrageenan-based hydrogel beads: impact of environmental conditions on enzyme activity. Food Chem 200:69–75
17. Zhang Z, Zhang R, McClements DJ (2017) Lactase (β-galactosidase) encapsulation in hydrogel beads with controlled internal pH microenvironments: impact of bead characteristics on enzyme activity. Food Hydrocoll 67:85
18. Kim J, Grate JW (2003) Single-enzyme nanoparticles armored by a nanometer-scale organic/inorganic network. Nano Lett 3:1219–1222
19. Jia H, Gao Z, Ma Y, Zhong C, Wang C, Zhou H et al (2016) Preparation and characterization of a highly stable phenoxazinone synthase nanogel. Chem Cent J 10:1–7
20. Datta S, Christena LR, Rajaram YRS (2013) Enzyme immobilization: an overview on techniques and support materials. 3 Biotech 3:1–9
21. Drauz K (2012) Enzyme catalysis in organic synthesis: a comprehensive handbook. Wiley, New Delhi
22. Tran DN, Balkus KJ (2012) Enzyme immobilization via electrospinning. Top Catal 55:1057–1069
23. Hwang ET, Gu MB (2013) Enzyme stabilization by nano/microsized hybrid materials. Eng Life Sci 13:49–61
24. Sheldon R, Schoevaart R, Van Langen L (2005) Cross-linked enzyme aggregates (CLEAs): a novel and versatile method for enzyme immobilization (a review). Biocatal Biotransformation 23:141–147
25. Wang Z-G, Wan L-S, Liu Z-M, Huang X-J, Xu Z-K (2009) Enzyme immobilization on electrospun polymer nanofibers: an overview. J Mol Catal B Enzym 56:189–195

26. Wang H, Shao H, Hu X (2006) Structure of silk fibroin fibers made by an electrospinning process from a silk fibroin aqueous solution. J Appl Polym Sci 101:961–968
27. Bhardwaj N, Kundu SC (2010) Electrospinning: a fascinating fiber fabrication technique. Biotechnol Adv 28:325–347
28. Li D, Xia Y (2004) Electrospinning of nanofibers: reinventing the wheel? Adv Mater 16:1151–1170
29. Kim BC, Nair S, Kim J, Kwak JH, Grate JW, Kim SH et al (2005) Preparation of biocatalytic nanofibres with high activity and stability via enzyme aggregate coating on polymer nanofibres. Nanotechnology 16:S382
30. Ye P, Xu Z-K, Wu J, Innocent C, Seta P (2006) Nanofibrous membranes containing reactive groups: electrospinning from poly (acrylonitrile-co-maleic acid) for lipase immobilization. Macromolecules 39:1041–1045
31. Jia H, Zhu G, Vugrinovich B, Kataphinan W, Reneker DH, Wang P (2002) Enzyme-carrying polymeric nanofibers prepared via electrospinning for use as unique biocatalysts. Biotechnol Prog 18:1027–1032
32. Lee KH, Ki CS, Baek DH, Kang GD, Ihm D-W, Park YH (2005) Application of electrospun silk fibroin nanofibers as an immobilization support of enzyme. Fibers Polym 6:181–185
33. Lee JH, Hwang ET, Kim BC, Lee S-M, Sang B-I, Choi YS et al (2007) Stable and continuous long-term enzymatic reaction using an enzyme–nanofiber composite. Appl Microbiol Biotechnol 75:1301–1307
34. Lee S-M, Jin LH, Kim JH, Han SO, Na HB, Hyeon T et al (2010) β-Glucosidase coating on polymer nanofibers for improved cellulosic ethanol production. Bioprocess Biosyst Eng 33:141
35. Li S-F, Chen J-P, Wu W-T (2007) Electrospun polyacrylonitrile nanofibrous membranes for lipase immobilization. J Mol Catal B Enzym 47:117–124
36. Huang XJ, Xu ZK, Wan LS, Innocent C, Seta P (2006) Electrospun nanofibers modified with phospholipid moieties for enzyme immobilization. Macromol Rapid Commun 27:1341–1345
37. Huang X-J, Chen P-C, Huang F, Ou Y, Chen M-R, Xu Z-K (2011) Immobilization of Candida rugosa lipase on electrospun cellulose nanofiber membrane. J Mol Catal B Enzym 70:95–100
38. Kim TG, Park TG (2006) Surface functionalized electrospun biodegradable nanofibers for immobilization of bioactive molecules. Biotechnol Prog 22:1108–1113
39. Wang ZG, Xu ZK, Wan LS, Wu J, Innocent C, Seta P (2006) Nanofibrous membranes containing carbon nanotubes: electrospun for redox enzyme immobilization. Macromol Rapid Commun 27:516–521
40. Wu L, Yuan X, Sheng J (2005) Immobilization of cellulase in nanofibrous PVA membranes by electrospinning. J Membr Sci 250:167–173
41. Nair S, Kim J, Crawford B, Kim SH (2007) Improving biocatalytic activity of enzyme-loaded nanofibers by dispersing entangled nanofiber structure. Biomacromolecules 8:1266–1270
42. Lee G, Kim J, Lee J-h (2008) Development of magnetically separable polyaniline nanofibers for enzyme immobilization and recovery. Enzyme Microb Technol 42:466–472
43. Wan L-S, Ke B-B, Xu Z-K (2008) Electrospun nanofibrous membranes filled with carbon nanotubes for redox enzyme immobilization. Enzyme Microb Technol 42:332–339
44. Netto CG, Toma HE, Andrade LH (2013) Superparamagnetic nanoparticles as versatile carriers and supporting materials for enzymes. J Mol Catal B Enzym 85:71–92
45. Ansari SA, Husain Q (2012) Potential applications of enzymes immobilized on/in nano materials: a review. Biotechnol Adv 30:512–523
46. Ranjbakhsh E, Bordbar A, Abbasi M, Khosropour A, Shams E (2012) Enhancement of stability and catalytic activity of immobilized lipase on silica-coated modified magnetite nanoparticles. Chem Eng J 179:272–276
47. Huang SH, Liao MH, Chen DH (2003) Direct binding and characterization of lipase onto magnetic nanoparticles. Biotechnol Prog 19:1095–1100
48. Dyal A, Loos K, Noto M, Chang SW, Spagnoli C, Shafi KV et al (2003) Activity of Candida rugosa lipase immobilized on γ-Fe_2O_3 magnetic nanoparticles. J Am Chem Soc 125:1684–1685

49. Kodama R (1999) Magnetic nanoparticles. J Magn Magn Mater 200:359–372
50. Kouassi GK, Irudayaraj J, McCarty G (2005) Examination of cholesterol oxidase attachment to magnetic nanoparticles. J Nanobiotechnol 3:1
51. Kalkan NA, Aksoy S, Aksoy EA, Hasirci N (2012) Preparation of chitosan-coated magnetite nanoparticles and application for immobilization of laccase. J Appl Polym Sci 123:707–716
52. Namdeo M, Bajpai S (2009) Immobilization of α-amylase onto cellulose-coated magnetite (CCM) nanoparticles and preliminary starch degradation study. J Mol Catal B Enzym 59:134–139
53. Seenuvasan M, Malar CG, Preethi S, Balaji N, Iyyappan J, Kumar MA et al (2013) Fabrication, characterization and application of pectin degrading Fe_3O_4–SiO_2 nanobiocatalyst. Mater Sci Eng C 33:2273–2279
54. Lin J, Qu W, Zhang S (2007) Disposable biosensor based on enzyme immobilized on Au–chitosan-modified indium tin oxide electrode with flow injection amperometric analysis. Anal Biochem 360:288–293
55. Ma Z, Ding T (2009) Bioconjugates of glucose oxidase and gold nanorods based on electrostatic interaction with enhanced thermostability. Nanoscale Res Lett 4:1236
56. Ling YP, Heng LY (2010) A potentiometric formaldehyde biosensor based on immobilization of alcohol oxidase on acryloxysuccinimide-modified acrylic microspheres. Sensors 10:9963–9981
57. Yang K, Xu N-S, Su WW (2010) Co-immobilized enzymes in magnetic chitosan beads for improved hydrolysis of macromolecular substrates under a time-varying magnetic field. J Biotechnol 148:119–127
58. Dong Q, Ouyang L-M, Yu H-L, Xu J-H (2010) Efficient biosynthesis of uridine diphosphate glucose from maltodextrin by multiple enzymes immobilized on magnetic nanoparticles. Carbohydr Res 345:1622–1626
59. Reddy LH, Arias JL, Nicolas J, Couvreur P (2012) Magnetic nanoparticles: design and characterization, toxicity and biocompatibility, pharmaceutical and biomedical applications. Chem Rev 112:5818–5878
60. Charles SW (2002) The preparation of magnetic fluids. In: Ferrofluids. Springer, New York, pp 3–18
61. DeCastro CL, Mitchell BS (2002) Nanoparticles from mechanical attrition. In: Synthesis, functionalization, and surface treatment of nanoparticles. American Scientific Publishers, California, pp 1–15
62. Amendola V, Riello P, Meneghetti M (2010) Magnetic nanoparticles of iron carbide, iron oxide, iron@ iron oxide, and metal iron synthesized by laser ablation in organic solvents. J Phys Chem C 115:5140–5146
63. Gupta AK, Gupta M (2005) Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 26:3995–4021
64. Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L et al (2008) Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chem Rev 108:2064–2110
65. Oh JK, Park JM (2011) Iron oxide-based superparamagnetic polymeric nanomaterials: design, preparation, and biomedical application. Prog Polym Sci 36:168–189
66. Timko M, Molcan M, Hashim A, Skumiel A, Muller M, Gojzewski H et al (2013) Hyperthermic effect in suspension of magnetosomes prepared by various methods. IEEE Trans Magn 49:250–254
67. Baumgartner J, Bertinetti L, Widdrat M, Hirt AM, Faivre D (2013) Formation of magnetite nanoparticles at low temperature: from superparamagnetic to stable single domain particles. PLoS One 8:e57070
68. Talekar S, Joshi A, Joshi G, Kamat P, Haripurkar R, Kambale S (2013) Parameters in preparation and characterization of cross linked enzyme aggregates (CLEAs). RSC Adv 3:12485–12511
69. Barbosa O, Ortiz C, Berenguer-Murcia Á, Torres R, Rodrigues RC, Fernandez-Lafuente R (2014) Glutaraldehyde in bio-catalysts design: a useful crosslinker and a versatile tool in enzyme immobilization. RSC Adv 4:1583–1600

70. Mateo C, Palomo JM, Van Langen LM, Van Rantwijk F, Sheldon RA (2004) A new, mild cross-linking methodology to prepare cross-linked enzyme aggregates. Biotechnol Bioeng 86:273–276
71. Arsenault A, Cabana H, Jones JP (2011) Laccase-based CLEAs: chitosan as a novel cross-linking agent. Enzym Res 2011:1–11
72. Talekar S, Nadar S, Joshi A, Joshi G (2014) Pectin cross-linked enzyme aggregates (pectin-CLEAs) of glucoamylase. RSC Adv 4:59444–59453
73. Nadar SS, Rathod VK (2016) Magnetic macromolecular cross linked enzyme aggregates (CLEAs) of glucoamylase. Enzyme Microb Technol 83:78–87
74. Sojitra UV, Nadar SS, Rathod VK (2017) Immobilization of pectinase onto chitosan magnetic nanoparticles by macromolecular cross-linker. Carbohydr Polym 157:677–685
75. Park HJ, McConnell JT, Boddohi S, Kipper MJ, Johnson PA (2011) Synthesis and characterization of enzyme–magnetic nanoparticle complexes: effect of size on activity and recovery. Colloids Surf B Biointerfaces 83:198–203
76. Jordan J, Kumar CS, Theegala C (2011) Preparation and characterization of cellulase-bound magnetite nanoparticles. J Mol Catal B Enzym 68:139–146
77. Wang H, Huang J, Wang C, Li D, Ding L, Han Y (2011) Immobilization of glucose oxidase using $CoFe_2O_4/SiO_2$ nanoparticles as carrier. Appl Surf Sci 257:5739–5745
78. Šulek F, Knez Ž, Habulin M (2010) Immobilization of cholesterol oxidase to finely dispersed silica-coated maghemite nanoparticles based magnetic fluid. Appl Surf Sci 256:4596–4600
79. Liu W, Bai S, Sun Y (2004) Preparation of magnetic nanoparticles and its application to enzyme immobilization. Chin J Process Eng 4:362–366
80. Shaw S-Y, Chen Y-J, Ou J-J, Ho L (2006) Preparation and characterization of Pseudomonas putida esterase immobilized on magnetic nanoparticles. Enzyme Microb Technol 39:1089–1095
81. Saiyed Z, Sharma S, Godawat R, Telang S, Ramchand C (2007) Activity and stability of alkaline phosphatase (ALP) immobilized onto magnetic nanoparticles (Fe_3O_4). J Biotechnol 131:240–244
82. Li Y, Fei X, Liang L, Tian J, Xu L, Wang X et al (2016) The influence of synthesis conditions on enzymatic activity of enzyme-inorganic hybrid nanoflowers. J Mol Catal B Enzym 133:92–97
83. Ge J, Lei J, Zare RN (2012) Protein-inorganic hybrid nanoflowers. Nat Nanotechnol 7:428–432
84. Altinkaynak C, Tavlasoglu S, Ocsoy I (2016) A new generation approach in enzyme immobilization: organic-inorganic hybrid nanoflowers with enhanced catalytic activity and stability. Enzyme Microb Technol 93:105–112
85. Zhu L, Gong L, Zhang Y, Wang R, Ge J, Liu Z et al (2013) Rapid detection of phenol using a membrane containing laccase nanoflowers. Chem Asian J 8:2358–2360
86. Zhang B, Li P, Zhang H, Wang H, Li X, Tian L et al (2016) Preparation of lipase/$Zn_3(PO_4)_2$ hybrid nanoflower and its catalytic performance as an immobilized enzyme. Chem Eng J 291:287–297
87. Lin Z, Xiao Y, Wang L, Yin Y, Zheng J, Yang H et al (2014) Facile synthesis of enzyme–inorganic hybrid nanoflowers and their application as an immobilized trypsin reactor for highly efficient protein digestion. RSC Adv 4:13888–13891
88. Yu Y, Fei X, Tian J, Xu L, Wang X, Wang Y (2015) Self-assembled enzyme–inorganic hybrid nanoflowers and their application to enzyme purification. Colloids Surf B Biointerfaces 130:299–304
89. Altinkaynak C, Yilmaz I, Koksal Z, Özdemir H, Ocsoy I, Özdemir N (2016) Preparation of lactoperoxidase incorporated hybrid nanoflower and its excellent activity and stability. Int J Biol Macromol 84:402–409
90. Sun J, Ge J, Liu W, Lan M, Zhang H, Wang P et al (2014) Multi-enzyme co-embedded organic–inorganic hybrid nanoflowers: synthesis and application as a colorimetric sensor. Nanoscale 6:255–262

91. Somturk B, Yilmaz I, Altinkaynak C, Karatepe A, Özdemir N, Ocsoy I (2016) Synthesis of urease hybrid nanoflowers and their enhanced catalytic properties. Enzyme Microb Technol 86:134–142
92. Yin Y, Xiao Y, Lin G, Xiao Q, Lin Z, Cai Z (2015) An enzyme–inorganic hybrid nanoflower based immobilized enzyme reactor with enhanced enzymatic activity. J Mater Chem B 3:2295–2300
93. Zhang B, Li P, Zhang H, Fan L, Wang H, Li X et al (2016) Papain/$Zn_3(PO_4)_2$ hybrid nanoflower: preparation, characterization and its enhanced catalytic activity as an immobilized enzyme. RSC Adv 6:46702–46710
94. Patel SK, Otari SV, Kang YC, Lee J-K (2017) Protein–inorganic hybrid system for efficient his-tagged enzymes immobilization and its application in L-xylulose production. RSC Adv 7:3488–3494
95. Zhao F, Wang Q, Dong J, Xian M, Yu J, Yin H et al (2017) Enzyme-inorganic nanoflowers/alginate microbeads: an enzyme immobilization system and its potential application. Process Biochem 57:87–94
96. Wang L-B, Wang Y-C, He R, Zhuang A, Wang X, Zeng J et al (2013) A new nanobiocatalytic system based on allosteric effect with dramatically enhanced enzymatic performance. J Am Chem Soc 135:1272–1275
97. Ye R, Zhu C, Song Y, Lu Q, Ge X, Yang X et al (2016) Bioinspired synthesis of all-in-one organic–inorganic hybrid nanoflowers combined with a handheld pH meter for on-site detection of food pathogen. Small 12:3094–3100
98. Cui J, Zhao Y, Liu R, Zhong C, Jia S (2016) Surfactant-activated lipase hybrid nanoflowers with enhanced enzymatic performance. Sci Rep 6:27928
99. Altinkaynak C, Tavlasoglu S, Kalin R, Sadeghian N, Ozdemir H, Ocsoy I et al (2017) A hierarchical assembly of flower-like hybrid Turkish black radish peroxidase-Cu^{2+} nanobiocatalyst and its effective use in dye decolorization. Chemosphere 182:122–128
100. Liu Y, Chen J, Du M, Wang X, Ji X, He Z (2017) The preparation of dual-functional hybrid nanoflower and its application in the ultrasensitive detection of disease-related biomarker. Biosens Bioelectron 92:68–73
101. Lee HR, Chung M, Kim MI, Ha SH (2017) Preparation of glutaraldehyde-treated lipase-inorganic hybrid nanoflowers and their catalytic performance as immobilized enzymes. Enzyme Microb Technol 105:24
102. Wei T, Du D, Zhu M-J, Lin Y, Dai Z (2016) An improved ultrasensitive enzyme-linked immunosorbent assay using hydrangea-like antibody–enzyme–inorganic three-in-one nanocomposites. CS Applied Materials & Interfaces 8(10):6329–6335
103. Ke C, Fan Y, Chen Y, Xu L, Yan Y (2016) A new lipase–inorganic hybrid nanoflower with enhanced enzyme activity. RSC Adv 6:19413–19416
104. Nadar SS, Gawas SD, Rathod VK (2016) Self-assembled organic inorganic hybrid glucoamylase nanoflowers with enhanced activity and stability. Int J Biol Macromol 92:660–669
105. Xu Z, Wang R, Liu C, Chi B, Gao J, Chen B et al (2016) A new L-arabinose isomerase with copper ion tolerance is suitable for creating protein–inorganic hybrid nanoflowers with enhanced enzyme activity and stability. RSC Adv 6:30791–30794
106. Mozafari MR, Khosravi-Darani K, Borazan GG, Cui J, Pardakhty A, Yurdugul S (2008) Encapsulation of food ingredients using nanoliposome technology. Int J Food Prop 11:833–844
107. Reza Mozafari M, Johnson C, Hatziantoniou S, Demetzos C (2008) Nanoliposomes and their applications in food nanotechnology. J Liposome Res 18:309–327
108. Meesters GM (2010) Encapsulation of enzymes and peptides. In: Encapsulation technologies for active food ingredients and food processing. Springer, New York, pp 253–268
109. Fathi M, Mozafari M-R, Mohebbi M (2012) Nanoencapsulation of food ingredients using lipid based delivery systems. Trends Food Sci Technol 23:13–27

110. Mohammadi R, Mahmoudzade M, Atefi M, Khosravi-Darani K, Mozafari M (2015) Applications of nanoliposomes in cheese technology. Int J Dairy Technol 68:11–23
111. Rother C, Nidetzky B (2009) Enzyme immobilization by microencapsulation: methods, materials, and technological applications. In: Encyclopedia of industrial biotechnology. Weily, New Delhi
112. Sirisha V, Jain A, Jain A (2016) Chapter 9: Enzyme immobilization: an overview on methods, support material, and applications of immobilized enzymes. Adv Food Nutr Res 79:179–211
113. Cellesi F, Tirelli N (2006) Sol–gel synthesis at neutral pH in W/O microemulsion: a method for enzyme nanoencapsulation in silica gel nanoparticles. Colloids Surf A Physicochem Eng Asp 288:52–61
114. Avnir D, Coradin T, Lev O, Livage J (2006) Recent bio-applications of sol–gel materials. J Mater Chem 16:1013–1030
115. Pierre A (2004) The sol-gel encapsulation of enzymes. Biocatal Biotransformation 22:145–170
116. Lee C-H, Lin T-S, Mou C-Y (2009) Mesoporous materials for encapsulating enzymes. Nano Today 4:165–179

Methods for Seafood Authenticity Testing in Europe

70

Véronique Verrez-Bagnis, Carmen G. Sotelo, Rogério Mendes, Helena Silva, Kristina Kappel, and Ute Schröder

Contents

V. Verrez-Bagnis (✉)
Ifremer, Nantes, France
e-mail: vverrez@ifremer.fr

C. G. Sotelo
Instituto de Investigaciones Marinas (CSIC), Vigo, Spain
e-mail: carmen@iim.csic.es

R. Mendes · H. Silva
Portuguese Institute for the Sea and Atmosphere, Lisbon, Portugal
e-mail: rogerio@ipma.pt; hsilva@ipma.pt

K. Kappel · U. Schröder
Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel, Germany
e-mail: kristina.kappel@mri.bund.de; ute.schroeder@mri.bund.de

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_69

Abstract

Seafood authenticity is a key parameter for seafood quality, particularly in Europe where regulations provide a strict framework for seafood labeling. A wide variety of methods are commonly used in control laboratories (private or public) to identify seafood species, but emergent approaches for the development of new and fast DNA- and protein-based methods for species differentiation are also considered. To address the challenges in controlling further labeling requirements in the latest European legislation on seafood product traceability and labeling (Regulation (EU) 1379/2013), a review of the development of methods to identify fishing areas and to distinguish between wild and farmed fish, as well as an overview of the advanced methods that could be used for differentiation of fresh and frozen-thawed fish, is given. These methods will become increasingly important in the near future as the risk-based control of food authenticity is prescribed by the new EU control regulation (Regulation (EU) 2017/625).

Keywords

Seafood identification · Traceability · Seafood geographical discrimination · Seafood authentication

1 Introduction

The consumption of fish and seafood products has increased considerably worldwide, from an average of 9.9 kg per capita in the 1960s to 19.7 kg in 2013 (26.8 kg per capita in industrialized countries) [1]. Fish and seafood products are among the most traded food commodities in the world and represent about 9% of total agricultural exports and 1% of world merchandise trade in value [1]. Trade in fish and seafood products has expanded considerably in recent decades, rising by more than 245% in terms of quantity (live weight equivalent) from 1976 to 2014 and by 515% if trade in fish for human consumption alone is taken into consideration. This trade is driven by high demand and is fueled by growing complexity in commodity flows, with the fisheries sector operating in an increasingly globalized environment. In addition, there is significant trade in fisheries services, with some products crossing multiple national borders during the seafood supply chain. The European Union is a

net importer of fisheries and aquaculture products, importing 5,947,708 tons and exporting 2,145,169 tons in 2014 [2].

Fisheries and aquaculture production are very heterogeneous in terms of species and food products. Fifty percent of the catches traded are processed (i.e., fillets, portions, elaborated products) because of their high perishability and also so as to prepare convenient products. In this way, by losing the morphological characteristics, they generate more opportunities for fraudulent operations [1].

Mislabeling has been identified by numerous studies for different species and types of seafood [3, 4]. In addition to being an economic problem, it is thought to contribute to fish decline because it frequently hides illegal, unreported, and unregulated (IUU) fishing, accounting for approximately one fifth of the global catch and, thus, posing a major threat to sustainable fisheries [5].

For fishery or aquaculture products sold to the final consumer, the European EU 1379/2013 regulation requires that commercial designation and scientific names are shown on the labels. It is also established that different member states can legally (i) designate official commercial names for fish species which can be different from one country to another, (ii) give a common commercial name for a specific genus, and/or (iii) group some fish species together for commercial purposes (e.g., the European regulation stipulates that canned products labeled as tuna should be prepared either from the *Thunnus* genus species or from skipjack tuna (*Katsuwonus pelamis*)).

International trade generates an increased need for international harmonization of the conventions for naming fish species and requires standardized species identification tools, methods for the easy identification of fishing grounds or farming areas, and production methods. These measures will contribute to improving controls by customs officers, fishery inspectors, and fish industries, ultimately preventing fraud and increasing consumers' trust in seafood labeling and traceability. Validated and harmonized methods are the prerequisite for realizing the new EU control regulation (Regulation (EU) 2017/625) which dictates the risk-based control of food authenticity.

This chapter first reviews the methods commonly used for seafood authentication which are mainly based on DNA. Second, different approaches to the development of quick DNA-based methods and new identification procedures are presented. Finally, there is a review of the development of methods for identifying fishing areas and distinguishing between wild and farmed fish. An overview of the advanced methods that could be used for differentiating fresh and frozen-thawed fish addresses some of the challenges for controlling new labeling requirements in the latest European legislation on seafood product traceability and labeling.

2 Common Methods Used for Seafood Surveillance

2.1 Protein-Based Methods: Isoelectric Focusing (IEF) and Other Electrophoresis Techniques

Although many different DNA methods have been developed and successfully implemented in the last 25 years, isoelectric focusing (IEF) technology has not yet disappeared because users appreciate its benefits in terms of simplicity and cost and

time efficiency. For that reason, official laboratories still continue to use IEF to identify certain fish species [6]. Recent publications by Kappel and Schröder (2016) [7] and Wang and Hsieh (2016) [8] have demonstrated how reliable this technology is as a screening method for proving labeling compliance in the case of common sole, cod, and striped catfish.

While self-drawn gels were used in the early days, commercial gels facilitated and accelerated the task, making it possible to standardize a method indicated in national legislations for many years [9, 10].

The principle of the method is based on the separating water-soluble sarcoplasmic proteins, predominantly found in the white muscle tissue of fish, in a polyacrylamide precast gel with a pH gradient (mostly used in a wide range of pH 3–10 and 0.3 mm thick) connected to an electric field with specific settings [11]. Proteins are electrically charged macromolecules whose total charge is dependent on the negative and positive charges of the amino acid side chains and therefore on the pH of the environment. During the IEF separation procedure, proteins migrate within the gel with a pH gradient depending on their charge until they reach the position where their total charge is neutral. These protein-specific positions are called isoelectric points (pI), and the resulting protein patterns are comparable with a barcode, forming important parameters for identifying species in unknown samples.

After the protein bands have been visualized, for instance, with a Coomassie dye SERVA Violet and fixed with acid, the samples must be verified by comparing the banding patterns with those of reference individuals that were run in the same gel. Only intense protein bands are used for evaluation. The pI values of the characteristic bands are calculated by comparing the positions of the marker proteins (commercial calibration pI kit, adjusted to the pH gradient applied) with the sample bands using a visualization system and analysis software [12]. Piñeiro et al. (2000) [13] demonstrated the high reproducibility of characteristic protein profiles because the IEF technique seems to be less affected by polymorphism factors such as intraspecies variability.

Many fish species and products have been identified using IEF technology, for example, catfish, tilapia, and snapper [14]; Sparidae species [15]; barramundi and tilapia [16]; different Aegean and Atlantic species [12, 17]; swordfish and spearfish [18]; puffer fish [19]; red snapper [20]; tunas, bonitos, and mackerels [21]; perch species and flat fish species in combination with two-dimensional electrophoresis [22, 23]; or gadoid species using auxiliary detection of specific enzymes [24] to detect food fraud practices. In particular, the acidic proteins in proximity to the anode (pI values from 3.5 to 5.5) can be applied for species identification. These characteristic, small-sized Ca^{2+}-binding proteins (<14 kDa) are identified as parvalbumins [25], which are heat stable and give species-specific patterns, not only in raw but also in processed fish such as cooked [26] or smoked fish fillets [27]. Kappel and Schröder (2016) [7] and Wang and Hsieh (2016) [8] were also able to show that prepared restaurant samples of common sole and striped catfish were easy to differentiate by means of acidic protein patterns.

Different application modalities were developed and standardized to extend the scope of IEF. Processing methods such as cooking, frying, and smoking lead to more

or less pronounced denaturation as well as degradation of the proteins and result in loss of characteristic protein bands in IEF [28]. Due to specific heat stable parvalbumins, applying native IEF to heated products is still possible with a few fish species [28]. Nevertheless, other electrophoresis techniques as denaturing urea IEF or sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) have shown better results for species identification in high pressure-treated products [29] and may be alternative approaches in cooked fish species depending on the type of product [30, 31]. A collaborative study revealed that urea IEF has a greater species-discriminating power than SDS-PAGE for smoked and gravad salmonid products, as well as smoked eels [32]. In general, the research group responsible recommended using native IEF, urea IEF, and SDS-PAGE in combination depending on the product and species.

In some cases, closely related species could not be differentiated by IEF alone, because of nearly identical protein band patterns.

Overall, the different examples of applications for IEF, urea IEF, and SDS-PAGE highlight their broad range of applications and show their possibilities for saving time and costs in case of species identification.

2.2 Immunological Methods

Immunological methods are based on the specificity of the antigen-antibody (Ag-Ab) reaction. Generally, studies on the use of these methods for fish species identification are rare, partly because of the high number of commercialized fish species [33]. Muscle proteins are generally used as antigens, and polyclonal antibodies (pAbs) or monoclonal antibodies (mAbs) are generated specially.

pAbs directed against muscle proteins have thus been developed to distinguish fish species such as halibut, sole, cod, eel, sardine, salmon, trout, haddock, grouper, and Nile perch mainly using the enzyme-linked immunosorbent assay (ELISA)-based methodology [34–37] or tuna and less-valued scombrid species [38]. Fish-specific polyclonal antisera have also been generated against a 36 kDa thermostable protein from a mix of different fish species (Atlantic salmon, yellowfin tuna, swordfish, black grouper, tilapia, red snapper, amberjack, basa, catfish, and perch) to detect the presence of fish muscle in food products for the protection of fish-allergic children or adults [39]. To specifically detect possible adulteration of surimi-based products, ELISA and dot-blot methods have also been used to check for the presence of crab meat using pAbs raised against crustacean arginine kinase [40]. A competitive ELISA was also developed to quantify Alaska pollock surimi in crabsticks using pAbs generated against a 15-amino-acid peptide from the myosin light chain 1 [41]. Likewise, a polyclonal anti-mackerel myosin light chain 1 antiserum was used to detect different fish species in boiled and dried fish products by immunoblotting partially purified fractions of myosin light chains [42]. Even though the production of pAbs is simple, their use is very limited in routine analysis because of their low specificity, resulting in cross-reactivity problems and the need to continuously require new immunizations in animals.

Monoclonal antibodies have thus been used because of their high specificity and absence of cross-reactivity against other species, as well as their unlimited production. These mAbs have been used to identify red snapper [43], grouper [44–46], catfish [47–49], wreck fish [46], and three species of clam [50].

Although immunoassays, once developed, are easy to use and have high sensitivity and throughput, their use is limited because of a number of disadvantages: (i) they require analysis in parallel reference samples to verify their cross-reactivity with other or similar species, and (ii) they are not suitable for forensic purposes in the analysis of processed food products given that processing may alter the three-dimensional structure of proteins, thus losing and/or modifying the targeted protein recognition site(s).

2.3 Main DNA-Based Methods

2.3.1 DNA Sequencing: BLAST and FINS

DNA sequencing makes it possible to directly retrieve genetic information from the DNA molecule: the order of nucleotidic bases is fundamental to genetics, evolution, and molecular taxonomy, and a great deal of scientific effort has thus been devoted to finding suitable methods for achieving this goal. In 1977, Frederick Sanger [51] proposed the chain termination method, and it has been the basis for all sequencing methods for the last 40 years. This methodology requires the use of DNA polymerases and chemically modified deoxynucleotides, so-called dideoxynucleotides. Briefly, the method consists of using a DNA template, often a PCR amplicon, which, after denaturation and reaction with a matching primer, is exposed to a mixture of deoxynucleotides (dATP, dCTP, dGTP, dTTP), dideoxynucleotides (ddATP, ddCTP, ddGTP, ddTTP), and a polymerase. For several cycles, the DNA polymerase synthesizes the complementary strand by incorporating dNTPs and stopping when a ddNTP is randomly incorporated. This strategy generates a series of overlapping DNA fragments in which the last NTP of each newly synthesized oligonucleotide is known because it is a ddNTP (earlier these ddNTP were radioactively labeled, and four simultaneous reactions were prepared with each of them). These fragments, which differ in size by one nucleotide, could be separated by electrophoresis, and the sequence can easily be retrieved by putting the size of the fragments in order [52].

Using DNA sequencing to identify fish species was first reported by Bartlett and Davidson [53]. They combined the capacity provided by PCR to produce a substantial amount of a specific DNA fragment, mitochondrial cytochrome b (*cytb*), with the relative easiness of DNA sequencing, thanks to the early sequencing kits and the capacity to analyze DNA sequences with a phylogenetic approach named FINS: forensically informative nucleotide sequencing [54].

Therefore, one important aspect to be considered when applying this technique is the target DNA marker to be used. Early developments (1990s) considered *cytb* to be an adequate marker for species identification as it codes for a fully functional monomer protein, a component of the electron transport chain, whose structure was well-known for having both conserved and variable regions. These features

made it possible to design universal primers for amplifying fragments which contained species-specific nucleotides [55]. Although some *cytb* DNA sequences for certain fish species were available at that time in public databases such as the NCBI (National Center for Biotechnology Information), it was still necessary to collect most commercial ones to sequence their *cytb* gene. It was nevertheless a very useful starting point and is still used nowadays as a reliable marker [56–62].

In 2009, Rasmussen and Morrisey [63] reviewed the use of DNA markers in seafood products from 1995 until 2008, in particular in each of the main seafood species groups. Their compilation showed that mitochondrial *cytb* was used in 39% of the studies, whereas other mitochondrial markers altogether were used in 30% of the cases. At that time, COI was used in only 4% of studies (Fig. 1).

In 2014, Griffith et al. [6], on the basis of a survey of authenticity testing laboratories across six European countries, reported that most laboratories were using *cytb* (73%), COI (28%), and *16S* rRNA (18%).

However, the use of a universal barcode such as one fragment of the COI gene has gained much attention in recent years. This approach was proposed by Herbert et al. in 2003 [64, 65], and it is based in a relatively long fragment of 650 bp of the COI gene with universal primers. Although it is an interesting approach for standardization of fish species identification, it presents a certain number of problems for the identification of some fish species groups (i.e., tunas [66]), as some degeneration of the primers is needed in order to make possible the amplification of certain fish species [67], and the target amplicon has to be reduced if thermally processed seafood products are to be analyzed (minibarcodes [68, 69]). In addition, for other major seafood groups, such as crustaceans and mollusks, *16S* rRNA markers are usually preferred [63, 70].

Another important aspect that should be considered when working with DNA sequences is the DNA sequence reference database, necessary for comparing the sequence obtained from the sample. There are two main approaches: having a proprietary database or relying on public databases. Although some laboratories

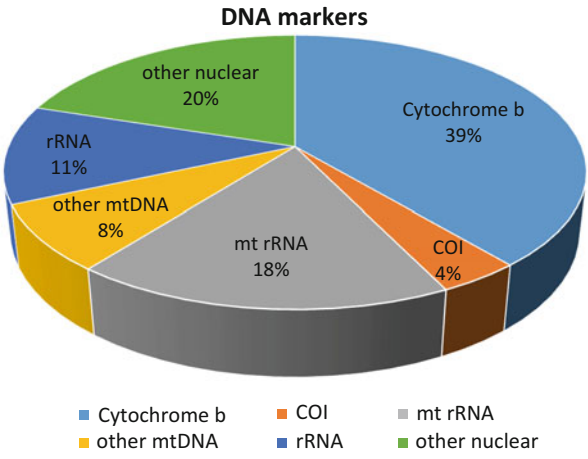


Fig. 1 Analysis of the use of DNA markers for seafood identification in research publications for the period 1995–2008 compiled by Rasmussen and Morrisey [63]

can afford to have proprietary databases, these usually involve considerable efforts in sampling and sequencing. Public databases are a perfect solution, as the effort is divided between many different laboratories, both private and public, around the world, and a large number of sequences of species and specimens can be collected. Probably the largest of all of them, hosting 260,000 formally described species, is the NCBI DNA sequence database GenBank [71], where it is possible to retrieve sequence from many different fish DNA fragments from around the world. Some authors have reported that GenBank is not specifically designed for species identification, and inconsistencies have been found in the terminology and sequence data for particular species, reflecting taxonomical issues in particular groups or taxons. There is also no guarantee that only authenticated voucher specimens are used [72]. The other major public database containing reference sequences is the Fish Barcode of Life Initiative (FISH-BOL; http://www.fishbol.org). In this case, the main objective of the database is to identify fish species around the world using genetic information from voucher specimens with taxonomic identifications and specifically a 650 bp COI marker. This initiative was launched in 2005, and since then many publications have shown the importance and convenience of having a standard barcode [73, 74] for fish identification.

Finally, once the sample DNA sequences have been obtained and the appropriate sequence database is available, in order to identify the species present in the sample, the use of a robust method is needed to analyze the level of similarity between the sample and reference sequences. The most commonly used approach is forensically informative nucleotide sequencing, which involves genetic distance methods for estimating the similarity and a phylogenetic tree to represent the distances obtained. Several genetic distance methods have been used for species identification, including Kimura-2 (takes into account nucleotide substitution and type, transitions, and transversions), Tamura-Nei (takes into account nucleotide substitution and type and also nucleotide frequencies), and Jukes-Cantor (equal rate of nucleotide substitution) [72]. However, models which take into account the complexity of type of nucleotide substitution and frequency might be more appropriate for species identification than simpler models [70]. Once the genetic distance matrix has been calculated, the topography can be visualized either with Neighbor-Joining or Unweighted Pair Group Method with Arithmetic Mean (UPGMA) methods, and in both cases a phylogenetic tree is produced (Fig. 2).

Another approach for species identification is to use particular nucleotides in a DNA fragment to identify the species in a sample. This has been referred to as character-based identification and requires prior identification of diagnostic nucleotides [75]. This approach is similar to PCR-RFLP, where the presence of certain diagnostic characters is evaluated by means of a restriction enzyme [76], and will be needed for the future development of microarray-based identification systems, where only particular nucleotide positions will be interrogated [75].

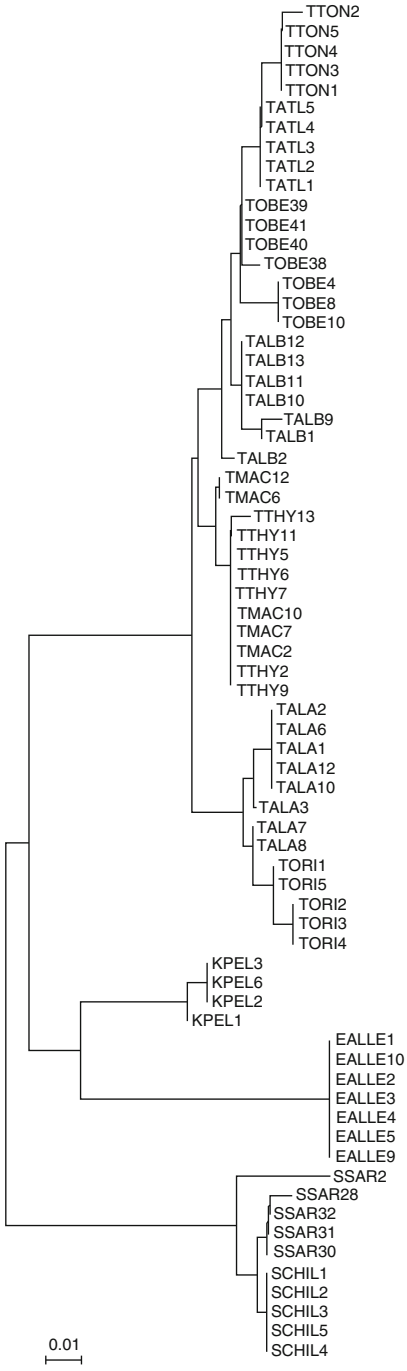


Fig. 2 Phylogenetic tree based on the cytochrome b DNA sequences of different species in the Scombridae family

2.3.2 Real-Time PCR (qPCR)

The first real-time or qPCR systems were introduced into the market in the middle of the 1990s, and the success of this technology could be seen in the increasing number of publications at the beginning of 2000. In contrast to the endpoint PCR, qPCR systems benefit from amplification progress that is recorded in real time using special fluorescent technology without any further analysis. Furthermore, qPCR enables the user to quantify the target DNA of interest in the exponential amplification phase. The fact that post-PCR proceedings, such as gel electrophoresis, enzyme digestion, or sequencing, are no longer necessary shortens the reaction time enormously and prevents any "carryover" with foreign DNA. Therefore, qPCR is suitable as a high-throughput method using specific primers and different fluorophores and probes for detecting and quantifying specific target DNA in a simple or multiplex approach in approximately 1 h. In terms of different fluorescent technologies, the simplest choice is SYBR® green I which binds to the minor groove of dsDNA. Much higher specificity is achieved by working with specifically designed hydrolysis probes (5′nuclease probes) that hybridize with one target DNA strand if there is complementarity. The most commonly used probes are TaqMan® probes, which rely on the fluorescence resonance energy transfer (FRET) mechanism. Numerous reporter dyes, such as FAM™, HEX™, ROX™, or Cy5, and reporters such as TAMRA are used, for example. Besides TaqMan® probes, other approaches with hybridization probes based on FRET, like molecular beacons and Scorpion® probes, can also be used [77]. In order to improve the affinity of probes for the target DNA, incorporating minor groove binder (MGB) groups or locked nucleic acids (LNA) has led to an increase in the melting temperature, thus making it possible to shorten the probes with a simultaneous increase in sequence specificity, especially when there are only one or two mismatches, for instance, in allele genotyping assays or identification of closely related species [78, 79]. In addition, great attention should be paid to optimizing and validating during the development of a qPCR method [80, 81] because very frequently key essentials such as positive controls or testing of robustness are missing in many applications.

Although qPCR has been widely used in numerous fields, in this chapter only works related to species identification in questions of seafood and allergens will be discussed. One of the first research works on fish species differentiation using qPCR was published in 2002 by Taylor et al. [82]. In this work, a simultaneous identification method using specific primers (ATPase6 gene) and species-specific MGB TaqMan® probes was developed to identify fish or fish eggs in three gadoid species (cod, haddock, and whiting) providing a tool for stock assessment, spawning site mapping, and detection of commercial fraud. Further examples clearly show that only certain questions justify the considerable effort required to develop a qPCR method for species identification of fishery products.

Justified by the increasing mislabeling of specific fish products in certain markets, research groups have presented qPCR methods for species authentication that mainly target mitochondrial genes. Although *16S* rDNA is known to have highly conserved regions, some research groups have used it for fish species or genus differentiation. Using the *16S* rDNA gene, qPCR methods were developed to

differentiate Nile perch and wreck fish from grouper species [83] or to identify the European eel [84] and species such as gilthead sea bream, among others [85]. Particularly worthy of mentioning is that Lopez and Pardo (2005) tried to develop a semi-quantitative qPCR method to identify albacore and yellowfin tuna on the basis of *cytb* and by performing relative quantification of these species by estimating their Cq values in relation to the Cq value of the unspecific PCR products of a scombroid species [86].

More often than *16S* rDNA, the mitochondrial gene COI is used for fish species identification by means of qPCR: cod [87], swordfish [88], two anglerfish species [89], seven commercially important salmon and trout species [90], grouper species of four genera [91], as well as escolar and oil fish [92].

Depending on the species of fish and the experiences of certain research groups, the mitochondrial gene *cytb* is also used in qPCR approaches for species identification. Examples of applications are tunas. They are a highly valued fish species and in some cases overexploited. Methods were therefore developed to identify yellowfin tuna, Atlantic bluefin tuna, and albacore in cans [93] as well as yellowfin tuna, bigeye tuna, southern bluefin tuna, and Pacific bluefin tuna [94] in separate simplex qPCR systems. The applicability of qPCR to tuna cans has only recently been demonstrated by Bojolly et al. (2017) [95] for identifying and quantifying (absolute as well as relative) of bigeye tuna and yellowfin tuna. Due in particular to the fact that qPCR is based on the amplification of short fragments (approx. from 60 to 180 bp), it is best suited to determining species in highly processed products such as sterilized tuna cans.

Although qPCR technology makes possible multiplex approaches, few users have taken this advantage. In this respect, Giusti et al. (2015) was one of the few research groups to develop a multiplex approach to identifying the gemfish species *R. pretiosus* alongside *L. flavobrunneum* and differentiating them from potentially replaced species such as cod, tunas, and sablefish using all three of the mitochondrial genes mentioned: *16S* rDNA, COI, and *cytb* [96].

Also suitable for fish species differentiation is the nuclear and ribosomal gene region *ITS* (internal transcribed spacer) which is applied to simplex qPCR of Atlantic salmon [97] and European sole [98].

Only a few qPCR studies address the problem of quantifying fish species by means of nuclear genes, as in the work on haddock in commercial products using transferrin [99] or the study on Atlantic cod, Atlantic salmon, and European plaice on the basis of nuclear sequences such as pantophysin, growth hormone, and parvalbumin [100].

In addition to fish identification, a few qPCR methods concerning mollusks have been presented in the last 10 years. In terms of gastropods and bivalves, management or ecological studies were the reasons for developing such high-throughput assays. Target bivalve species such as abalone [101], the *Mytilus* species [102], oysters [103], octopus and the main substitute species [104], and squid [105, 106] have been the focus of research groups using mitochondrial and nuclear gene regions.

As qPCR technology is highly versatile in terms of its applications, a variety of methods that were submitted in the framework of food safety, especially for

detecting food allergens related to fish, shrimps, and mussels, need to be mentioned. The challenge of these methods is that detecting allergens requires high sensitivity in order to detect trace amounts of the special allergen against an abundance of different food ingredients. In contrast to protein-based methods like enzyme-linked immunosorbent assays (ELISA), qPCR analysis is not usually affected to any extent by high temperatures. In addition, gene targets for qPCR applications are not limited to the genes coded for allergenic proteins. They can be chosen on the basis of other characteristics, such as sensitivity or species specificity, for instance. A specific gene sequence for parvalbumin identified as the main allergenic protein in fish [107] was the target of Sun et al. (2009) when developing a potential tool for detecting and labeling management of fish in food [108]. Other target gene sequences of *18S* rDNA [109] or *Hoxc13* [110] were selected to determine allergenic fish proteins in foods. In the case of crustaceans, one of the main allergenic proteins is tropomyosin. However, the qPCR assays for detecting crustacean allergens were developed on *12S* rDNA, *16S* rDNA, and *cytb* or COI [111–113]. Commercial test kits for allergen detection of crustaceans, fish, and mollusks are already available, for example, from R-Biopharm®, Germany.

Finally, universal qPCR methods for detecting a broad spectrum of fish in highly processed matrices such as animal feed should also be mentioned. Only a few research groups have published fish-related issues in this context. In particular, Martin et al. (2010, 2013) [114, 115] and Benedetto et al. (2011) [116] described qPCR applications using conserved gene regions of mitochondrial *12S* rDNA for detecting fish in feed.

All these qPCR applications show more or less potential for seafood authenticity issues, but to the best of our knowledge, no qPCR has been standardized for any official method so far.

3 Emergent Methods for Species Identification

3.1 Microarrays

PCR products can be analyzed by hybridization to species-specific oligonucleotide probes arrayed on so-called ‘DNA chips’ or ‘DNA microarrays’. These DNA microarrays can contain hundreds to several thousand or even millions of DNA probes attached like small spots on the array surface. Upon hybridization of labeled PCR products, species can be identified directly, based on the pattern of positive probes.

DNA microarrays are mainly used for gene expression profiling today, but there are various other applications. However, species authentication using DNA microarrays is still a niche application, and only a few studies targeting marine species have been published so far. One of the pioneer works on DNA microarrays for identifying marine species was the EU-funded Fish & Chips project [117], carried out from 2004 to 2007. This project aimed to move “Towards DNA chip technology as a standard analytical tool for the identification of marine organisms in biodiversity

and ecosystem science." Two DNA microarrays were developed capable of identifying 30 European marine fish species [118] and 15 marine invertebrates, respectively, with oligonucleotide probes binding to fluorescently labeled PCR products of mitochondrial DNA markers [119]. Another DNA microarray targeting 30 fish species among 79 different vertebrates was developed in France [120] to analyze food and forensic samples. Several studies on DNA microarrays for detecting various fish (or even whale) species have also been published by a South Korean group [121–126]. In addition, a DNA microarray to authenticate American catfish products was developed by the American Food and Drug Administration (FDA) in order to protect domestic aquaculture against adulteration by imported species [127].

A commercial DNA chip for species differentiation of food products was launched in France in 2004. The "FoodExpert-ID" (bioMérieux, France) could identify 33 vertebrate species including 15 fish species with an Affymetrix high-density DNA chip comprising 80,000 *16S* rDNA oligonucleotide probes [128]. However, the product line was discontinued, probably because it did not find a strong market [129]. In contrast to this, two commercial DNA microarray systems targeting exclusively land vertebrate species in food products, the "CarnoCheck DNA-chip" (Greiner Bio-One, Austria) and the "LCD Array Kit MEAT 5.0" (Chipron, Germany) [130], seem to have achieved market success with fast and easy-to-handle formats and are already used by some official food surveillance laboratories in Germany.

DNA microarray technology with its early nature requires extensive technical equipment such as microarray spotting robots and fluorescence laser scanners. This has hampered implementation of DNA microarrays in routine applications for species differentiation. Recent developments, like the CarnoCheck DNA-chip and the LCD Array, have led to smaller and cheaper devices and may introduce the technique into official food surveillance laboratories with wider distribution. Furthermore, a current research project in Germany is working on developing a DNA microarray for seafood species which is more suited to small laboratories or even on-site applications in the fish-processing industry [131].

3.2 High-Resolution Melting (HRM)

High-resolution melting (HRM) is based on the fact that double-stranded DNA will dissociate at different temperatures (Tm, melting temperature) [132] when the size and/or the DNA sequence is different, especially when there is different GC content. This approach has been used in qPCR to differentiate between amplicons and primer dimers; however it did not make possible fine differentiation among amplicons with only small sequence differences [133], and therefore a number of improvements in the dyes and equipment were made to make it possible to detect SNPs or just a few differences.

It is thus necessary that the appropriate chemistry be used to detect dissociation (saturation dyes which strongly emit fluorescence only with double-stranded DNA), thermocyclers with the capacity to perform temperature ramps

precisely and reproducibly and with a system for collecting fluorescence data, and that the appropriate analysis software be used. The technique involves three steps: (1) amplification of the DNA in the presence of fluorophores, considering that HRM performs better if amplicons are shorter than 300 bp; (2) melting of the amplicons by increasing the temperature; and (3) data analysis requiring registering the changes in fluorescence (F) with temperature (T) in order to obtain graphs where, after normalization, it is possible to observe shape and Tm differences [133].

This approach has been used to successfully identify a variety of vegetables [134], milk [135], GMOs [136], meat animal species [137], species of gadoids [138, 139], and mussels [140]. One of the main advantages of HRM compared with other techniques such as RFLP is the higher accuracy, lower price, and reduced analysis time (only 1.5 h). The cost is also lower compared with similar techniques such as TaqMan® assays [141], so it is becoming an emergent technique for identification of seafood species.

3.3 Digital PCR

Digital PCR (dPCR) is another emerging technique which was developed in the 90s, with the first reported application in 1999 [142]. Quantification of target DNA molecules by qPCR presented some issues, such as amplification efficiency, the requirement of standard curves, and nonspecific amplification, among others. Digital PCR overcomes some of these, and it is claimed to be a better approach for absolute quantification of specific DNA molecules. The principle underlying the technique is to amplify individually template DNA molecules so that after amplification the resulting products can also be individually identified. In fact, in dPCR, target DNA molecules are randomly partitioned in individual PCR reactions, some of these reactions will not contain any target DNA molecules, and others will contain one or more. These individual PCR will be performed until endpoint and positive reactions are recorded versus negative ones, and a concentration of target DNA molecules is calculated as the ratio of positive reactions and number of reactions analyzed [143].

Different platforms have been developed and are commercialized nowadays. Roughly speaking, they can be divided grosso modo in those using microfluidic chip technology [144] (Biomark HD dPCR system by Fluidigm) and those using droplet generators coupled with chips (QuantStudio® family by Life Technologies, RainDrop™ instrument by RainDance Technologies) or microfluidic approaches [145, 146] (BioRad QX200TM Droplet Digital™ PCR system). Although no publications have been found for quantification of fish species in seafood, they are expected to appear soon, as in other food categories, some applications have been published using this technique, especially when issues regarding the presence of particular species, such as the horse meat scandal [145], need a reliable species identification and quantification methodology.

3.4 Next-Generation Sequencing (NGS)

Classic Sanger sequencing of PCR products is not suited to analyzing products with mixed species content and can easily overlook admixtures of species in low concentrations. "next-generation sequencing" (NGS) techniques, also known as "high-throughput sequencing" (HTS), can overcome this problem by massively parallel sequencing of different target sequences in one reaction. "Metabarcoding", the targeted parallel sequencing of preamplified genetic markers, has already been used in many marine ecological studies such as eDNA surveys, in which the presence of fish or invertebrate species is recorded indirectly via the DNA shed by the organisms into the water column [147, 148]. However, few studies have been published on metabarcoding for species detection in food (e.g., meat [149, 150], candy [151]), traditional Chinese medicine [152, 153], herbal supplements [154], or seafood products.

In an early study, the suitability of pyrosequencing was explored for detecting species in fish cakes [155] and was compared to microarray-based species differentiation. However, few details are given about the nature of the target DNA or the outcome of the comparison. Another pyrosequencing study was carried out to identify the species of individual fish samples targeting *16S* rDNA. In cases of ambiguous results, segments of the NADH dehydrogenase subunit II gene (ND2) or *cytb* gene, respectively, also had to be sequenced for exact species assignment [156]. A similar pyrosequencing approach targeting short stretches of *16S* rDNA, as well as the COI gene, was used to identify individual bivalve mollusks as a "simple, rapid, and cost-effective alternative" for species differentiation [157].

Identification of species in mixed seafood samples or products by means of metabarcoding has been published recently by two groups. The first study analyzed the reliability of metabarcoding two short *cytb* segments on the MiSeq platform (Illumina, USA) with prepared mixtures of different tuna species as a prerequisite for metabarcoding-based species authentication in tinned tuna [158]. Although all the species from the mixtures could be detected with this approach, substantial bias was identified in read recovery, i.e., skipjack tuna sequences were always much more prominent than those of *Thunnus* species. In the second publication, the application of a commercially available metabarcoding system (Biopremier, Portugal) with the Ion Torrent Personal Genome Machine (Life Technologies, USA) was described to authenticate Brazilian cod products [159]. Despite the small amount of scientific literature on metabarcoding for food products and still unresolved problems such as PCR bias or incomplete primer universality, targeted metabarcoding is already being offered by European service providers at ever lower costs.

As PCR-based bias in sequence read recovery is a common phenomenon of NGS [160] and considerably hampers quantification of species ingredients in complex food products, the development of a PCR-independent NGS approach is very promising as described in two publications [161, 162]. The "All-Food-Seq" (AFS) software pipeline for quantification of species composition in food uses metagenomics shotgun sequencing and sequence read counting to infer species

proportions. However, reference genomes are needed to map sequences and species assignment. In view of the vast diversity of fish and seafood species, this approach remains a significant challenge for seafood authentication. In light of this, another approach for targeted NGS may gain significant importance: combining droplet PCR with NGS techniques [163, 164] might be able to overcome the problem of PCR bias and lead to more reliable or even quantitative metabarcoding results.

3.5 Isothermal Amplification

Thanks to the existence of reference DNA sequence databases, and in addition to metabarcoding using NGS technologies, development of "handheld" systems based on DNA identification would facilitate the authentication processes and reduce the time needed to identify species [165]. This type of "handheld" device is mainly based on isothermal amplifications, but so far very few systems have been developed.

Nucleic acid sequence-based amplification (NASBA) was first developed by Compton in 1991 [166]. This is primer-dependent technology to amplify nucleic acids in a single mixture at constant temperature. Immediately after its first development, NASBA was used for the rapid diagnosis and quantification of HIV-1 in patient sera [167]. Nevertheless, this technique was only used for the first time for fish authenticity purposes in 2013. Ulrich et al. [168] developed a 90-min multiplex RT-NASBA assay targeting a portion of the *16S* rRNA gene for accurate identification of most commercially important grouper species present on the FDA seafood list. The same authors have further developed a handheld device based on RT-NASBA assays [169]. They have demonstrated that the field sensor (80 min for assay completion) is only slightly less sensitive than the benchtop instrument and could discern 49 of the 61 FDA allowable species (80.3% of groupers – no target sequence available for 3 species).

Loop-mediated isothermal amplification (LAMP), a method based on isothermal amplification of DNA, was first developed by Notomi et al. (2000) [170]. The method requires a DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA.

The first report of an application for LAMP in fish authentication was recently published by Saull et al. [171]. They designed a LAMP assay based on the *cytb* gene as targeted DNA to discriminate Atlantic cod (*Gadus morhua*) from two closely related species, Pacific cod (*Gadus macrocephalus*) and Greenland cod (*Gadus ogac*). Reactions were obtained in a heating block at 63 °C for 60 min and were concluded by heating up to 80 °C for 1 min in order to denature the Bst polymerase. The method they developed was able to detect 0.1% w/w *Gadus morhua* in a homogenized raw fish mix. The LAMP assay showed a higher tolerance of amplification inhibitors than PCR and was shown to be highly specific as it did not generate positive results when challenged with a range of nontarget species, including *Gadus macrocephalus* and *Gadus chalcogrammus.* Nevertheless, validation of this method is required, involving greater numbers of samples (in particular

processed products) and fish species, before the LAMP assay can be used as a routine test.

Ye et al. [172] have also recently developed LAMP-based assays for identification of jumbo flying squid (*Dosidicus gigas*) using the COI gene to design species-specific primers. Only *D. gigas* DNA was detected by real-time fluorescence detection (RealAmp) or by visual detection using SYBR® Green I staining (Visual-LAMP), after incubation at 65 °C for 30 min. The authors reported that the detection limits were 10 pg for purified *D. gigas* DNA and 0.01% w/w *D. gigas* in homogenized cephalopod mixtures. They also specified that these methods can be applied to all kinds of processed squid and squid-containing products.

A third isothermal nucleic acid amplification technique, recombinase polymerase amplification (RPA), was developed by Piepenburg et al. [173]. Their innovative approach coupled isothermal recombinase-driven primer targeting of template material with strand-displacement DNA synthesis. It achieved exponential amplification with no need for pretreatment of the DNA sample. Reactions were sensitive, specific, fast, and operated at a constant low temperature. RPA reaction products can be detected in a simple sandwich assay, thereby establishing an instrument-free DNA testing system. This was the technology used by TwistDx Ltd. (a biotechnology company based in Cambridge, UK) to identify red snapper (*Lutjanus campechanus*) (a highly prized fish species) in 20 min using a dedicated analysis kit (TwistFlow® Red Snapper not currently available).

Helicase-dependent amplification (HDA) is another DNA amplification method at a constant temperature, but, although the simplicity of the reaction procedure makes HDA a very interesting alternative [174], to our knowledge it is not yet used in seafood identification.

3.6 PCR-ELISA and Dipstick

DNA methods for species identification based on DNA probes have gained attention in the last decade as they are a convenient alternative to sequencing methods. One example of these approaches is the immunodetection of DNA [175] by an ELISA (enzyme-linked immunosorbent assay) to probe the presence of a specific DNA fragment in PCR products [176]. The method involves three main steps: (i) amplification and labeling of the sample DNA digoxigenin (DIG-dUTP), (ii) hybridization of the labeled and amplified DNA with a specific probe which is attached to a microplate well, and (iii) detection of hybridization with an antibody conjugated with an enzyme (Fig. 3) [177].

Some publications have shown this technique in applications to detect allergenic components in food [178] and detect and quantify viruses [179, 180].

PCR-ELISA has also been used to identify fish species, such as Nile perch [181], tunas [182], and gadoids [183]. One of the advantages of using PCR-ELISA is that there is now wider access to conventional PCR and extended use of ELISA equipment and reagents in many food control laboratories for different applications (such as detecting allergenic substances, pathogens, etc.) making this approach available to

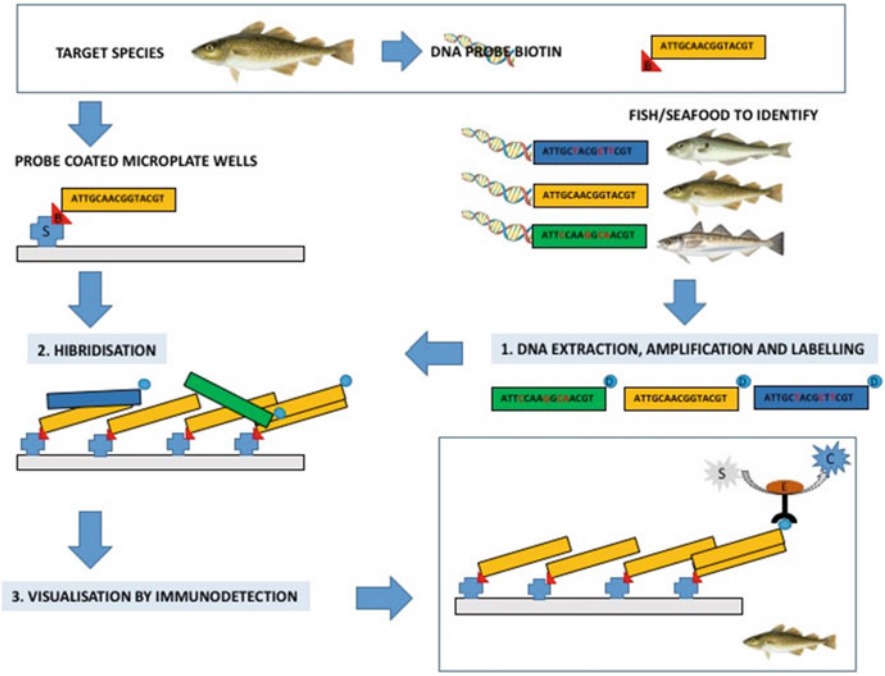


Fig. 3 Schematic representation of a PCR-ELISA design for species identification

many laboratories. In addition, it simplifies and reduces the analysis time as it makes possible a quick and visual inspection of both positives and negatives (Fig. 4). Another advantage is that it also makes it possible to detect mixed species present in a food product [183].

Another type of DNA visualization technique is known as lateral flow dipstick assay (LFDA). This methodology can be applied to a number of the assays mentioned above for obtaining DNA, such as PCR or isothermal amplification. The aim of this technique is to simplify as much as possible the process of probing the presence of a specific genotype in a sample, involving only visual inspection of the result [184]. Only two steps are needed to perform the assay: (1) PCR amplification of a specific DNA fragment and (2) detection of PCR products with the naked eye using the dipstick [185].

The universal dipsticks are available commercially to design different tests (http://milenia-biotec.de). Basically, these kits are composed of a membrane dipstick with three zones, the sample application zone, the test zone, and the control zone [185], and reagents to perform the visualization experiment.

DNA should be labeled beforehand by PCR with, for example, biotin and 6-FAM as this will be required for the visualization step. In the sample application zone of the dipstick, gold nanoparticles linked to an antibody which recognizes 6-FAM will react with DNA labeled with this molecule. When the complex labeled DNA-gold

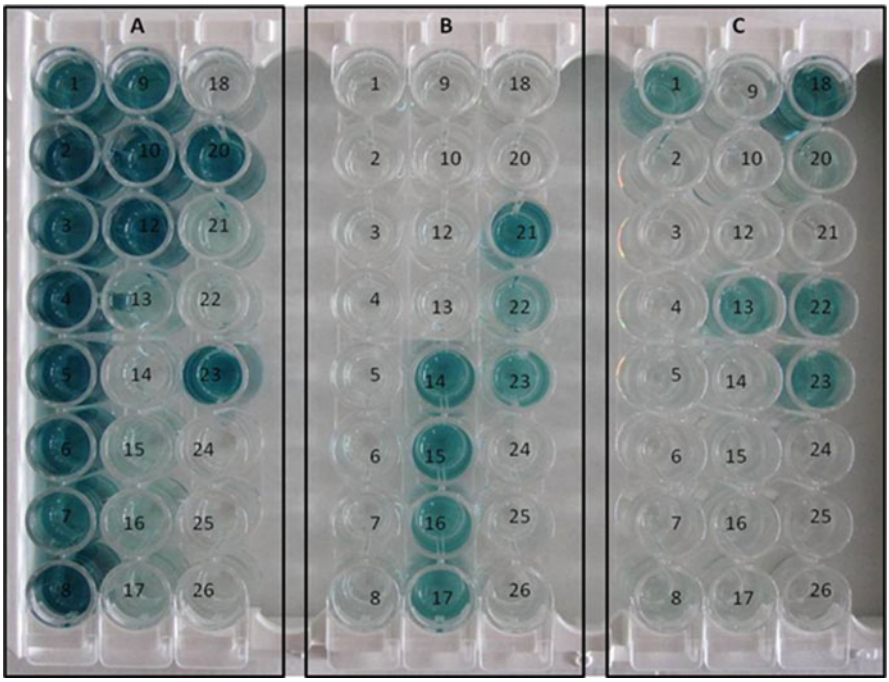


Fig. 4 PCR-ELISA plate results for three species probes tested with commercial samples: (**a**) *Gadus morhua* probe, (**b**) *G. chalcogrammus* probe, (**c**) *Molva molva* probe. Blue-colored wells correspond to positive signals, in some samples a mixture of species was detected (1 and 18), while sample 23 is a deliberate mixture of the three species [183]. Adapted with permission from Taboada et al. (2014) [183]. Copyright (2014) American Chemical Society

nanoparticles reaches the test zone, it is captured by the biotin ligand. Finally, the control zone has antibodies attached that recognize the anti-6-FAM present in the nanoparticle; therefore it makes it possible to test the correct flow of reagents through the dipstick [185] (see Fig. 5). Positive and negative results are easily seen within 5 min after loading the labeled PCR. Figure 5 shows the resulting dipsticks for positive and negative results. This technique has been applied to the detection of viruses [186], bacteria [187], GMOs [188], coffee [185], and seafood [185].

3.7 Mass Spectrometry Methods (MALDI-TOF, LC/MS/MS)

Progress in recent years in proteomic tools and particularly in new techniques based on mass spectrometry (MS), mainly matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) and electrospray ionization-ion trap (ESI-IT) MS, brings potential for developing new methods in the particular case of seafood authentication. Proteomic tools were first developed to investigate the proteome which is defined as the large-scale analysis of proteins present in a particular biological

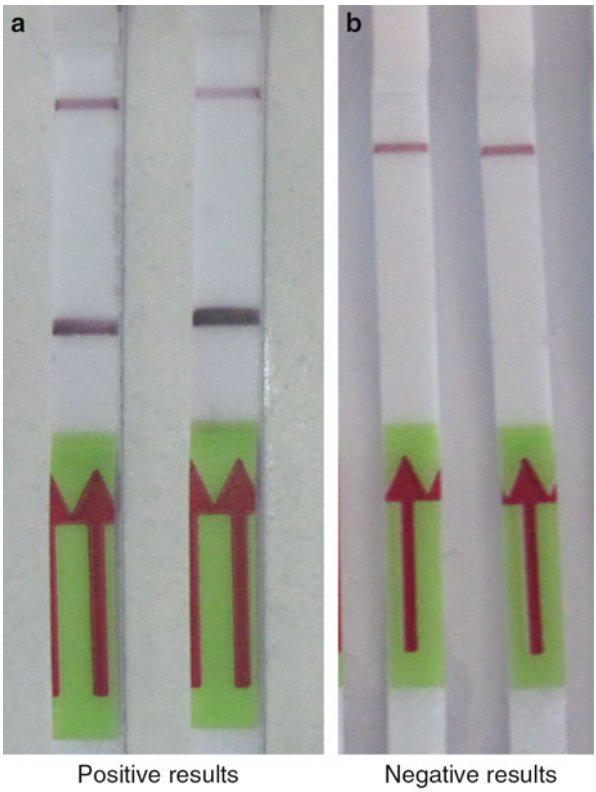


Fig. 5 Dipsticks showing positive results (species identification, in this case the positive shows the presence of *Gadus morhua*; negative shows the absence of *G. morhua*)

system at a given moment. Nowadays, automated data acquisition and powerful bioinformatics processing have made possible high-throughput protein and peptide identification and quantification. In this context, using proteomic tools to investigate seafood products (authentication, food quality, and safety) is steadily increasing. These "molecular profiling" approaches in food science and particularly in seafood products based on MS methodologies have been thoroughly reviewed in recent papers [189–196]).

As pointed out by Ortea et al. (2016) [194], proteomics methodologies can be applied to both global (whole protein) and specific (peptide-based) approaches or top-down and bottom-up workflows for food authentication.

In the bottom-up approach [197], peptides were generated after protein digestion, most commonly using trypsin. The bottom-up approaches are divided into two groups depending on whether the fractionation step, for reducing sample complexity, is performed at the protein level (before enzymatic digestion) or at the peptide level (after enzymatic digestion). In the first strategy, proteins were first isolated based on their isoelectric point and their molecular weight using a two-dimensional electrophoresis (2-DE) gel-based approach. After excision from the gel and digestion into peptides, the peptides are analyzed by MS for protein identification. In the second

strategy (called "shotgun" proteomics), the resulting mixtures of peptides are generally separated by high-performance liquid chromatography (HPLC) or ultrahigh-performance liquid chromatography (UHPLC) before being analyzed using MS.

The top-down approach [198] characterizes the fragments produced by dissociating the proteins directly inside the spectrometer. Ortea et al. (2016) [194] pointed out that even if this analysis is currently available thanks to new dissociation mechanisms and new high-resolution MS equipment, instrumental constraints limit its use.

3.7.1 Application to Shellfish Identification

The first application of a complete bottom-up proteomic approach to species identification was made by López et al. [199] on three species of European marine mussel. They characterized species-specific peptides by comparing MALDI-TOF maps generated from prominent protein spots (after 2-DE protein isolation). These species-specific peptides were found to be tropomyosin peptides.

Arginine kinase (a sarcoplasmic protein) was also used as a biomarker to identify shrimp species thanks to its variability found in 2-DE profiles. The peptide mass fingerprinting (PMF) spectra of arginine kinase were firstly used to differentiate six commercially important shrimps [200]. This study describes the first application of MS-based proteomics in species authentication. Two subsequent studies [201, 202], based on 2-DE, tryptic in-gel digestion, MALDI-TOF, and electrospray ionization-ion trap (ESI-IT) mass spectrometry, were carried out to identify species-specific arginine kinase peptides in the identification of the seven most commercially important species of shrimps and prawns. Using these characterized peptides, a shotgun proteomic approach [203] proved to be useful for penaeid shrimps. The approach was based on using high-intensity focused ultrasound-assisted trypsin digestion for ultrafast sample preparation, peptide separation, and identification by reversed-phase capillary LC (RP-HPLC) coupled with an ion trap working in the ion monitoring scanning mode (SIM) and applied to commercial shrimp samples.

A final study [204] on shrimp identification based on MALDI-TOF mass spectrometry used commercial mass spectral fingerprint matching software (Bruker BioTyper). A mass spectrum reference database was first constructed from the analysis of six shrimp species of commercial importance. This in-house generated database was further tested using 74 unknown shrimp samples from the 6 reference species. Seventy-two samples were identified at the species level and two samples were identified at the genus level.

3.7.2 Applications in Fish Species Authentication

Before the development of proteomic tools including MS analysis, differences in 1-D electrophoresis (SDS-PAGE and IEF) and 2-DE sarcoplasmic protein profiles were used to distinguish between fish species. Combining 2-DE and peptide mass fingerprinting (PMF) spectra, analyzing parvalbumin fractions from nine commercial, closely related species from the genus *Merluccius* and one grenadier species, demonstrated that the isoform patterns were species-specific [205]. MALDI-TOF

mass fingerprints of the peptides produced by tryptic digestion made genus differentiation possible, and some specific masses made it possible to identify certain hake to the species level. This work was completed with the development of a new proteomics workflow using a particular combination of 11 peptides, resulting from the use of accelerated in-solution trypsin digestion of thermostable protein parvalbumins under an ultrasonic field provided by high-intensity focused ultrasound (HIFU) and the monitoring of several peptides by selected MS/MS ion monitoring in a linear ion trap mass spectrometer [206]. This strategy made it unequivocally possible to identify all commercial fish species from the Merlucciidae family. Using the classical proteomic approach, the same authors also used fragmentation spectra to characterize by de novo peptide sequencing the different isoforms of nucleoside diphosphate kinase B (NDK B) from all the commercial hake from the family Merlucciidae and grenadiers [207]. Nevertheless, even though the authors showed that specific NDK tryptic peptides can be useful for differentiating between the two genera and classifying the hake according to their geographical origin (European-African or American), the strategy clearly identified only one species of hake (*M. bilinearis*) from the others.

Using a similar approach (a combination of 2-DE and PMF), differences in sarcoplasmic proteins from two closely related fish species, *Sperata seenghala* and *Sperata aor*, were also investigated [208]. One of the proteins identified by MALDI-TOF and liquid chromatography-tandem mass spectrometry (LC-MS), triosephosphate isomerase (TPI), was found to have three isoforms, of which two were specific to *S. aor* and one was specific to *S. seenghala*.

Mazzeo et al. [209] have developed an innovative method based on MALDI-TOF mass spectrometry on muscular protein extracts from 25 different fish species. Highly specific mass spectrometric profiles were obtained, and signals generated from about 11 kDa proteins, later identified as parvalbumins, were selected as specific biomarkers for unambiguous discrimination of species subjected to fraudulent substitutions, such as those belonging to Gadidae and Pleuronectiformes. In 2016, the author's group also published [210] their previously published data and other data that clearly demonstrated the reliability of MALDI-TOF MS, showing that it can be upgraded by making use of modern mass spectrometers, thus guaranteeing faster analysis and performing completely automated data acquisition and processing.

Wulff et al. [211, 212] demonstrated the suitability of a new proteomics method based on large-scale pairwise comparison of tandem mass spectra (proteome-wide comparison without peptide or protein identification) of fresh samples of different fish species. They established an automated and standardized workflow using existing, well-tested software for species identification and comparing raw and differentially processed samples using a database of spectral libraries of individual species. They showed that it was possible to identify the unknown origin of unknown species, even in processed products [213]. Furthermore, in a recent study [214] in the context of an interlaboratory comparison using data from different types of mass spectrometer and in an attempt to distinguish closely related flatfish by analyzing heavily contaminated samples, they clearly demonstrated that when using

untargeted data acquisition and spectral library matching, 94–97% of the samples were correctly identified.

In the same approach, Ulrich et al. [215] used a MALDI-TOF MS-based method on the vitreous fluid of fish eyes, and all the mass spectra obtained were processed by means of MALDI Biotyper OC 3.1 and ClinProTools 3.0 software. This methodology clearly distinguished between the two closely related species brown and rainbow trout. But further studies are needed to validate this approach for identifying other fish species and establishing a database with reference mass spectra. In Germany, some groups (competent authorities and private companies) are establishing reference databases for MALDI-TOF profiles for fish species identification [216, 217]. In addition, this approach showed limited applicability in the identification of time of storage.

4 Advanced Methods for Determining Geographic Origin in Seafood

Rapid identification techniques for species and geographical origin are a current requirement for the purposes of fraudulent labeling prevention and food regulation control [218]. In many countries (i.e., EU and US regulations), place of origin labeling for processed foods is required for imported marine seafood products as well as for products sold to the final consumer. In addition, there is growing awareness and concern among consumers regarding what they are buying, a need to know where their seafood was harvested and/or produced [219], and the sustainability of the products they purchase. Fraud concerning fish origin can also reduce the effectiveness of conservation and management programs that help to protect overexploited and endangered species [220]. According to El Sheikha and Montet (2016) [221], methods for determining geographical origin depend on the substrate studied and can be categorized into physicochemical and biological techniques. The former test components such as fatty acids [222], trace elements [223], or chemical constituents (i.e., antioxidants) and assume that these components characterize that particular origin of fish. However, modifications in diet or environment may change their actual profiles [224]. The biological techniques use analysis of isoenzymes and different molecular-based techniques [225, 226]. Each method has a number of advantages or disadvantages depending on the state of the product, as summarized in Leal et al. (2015) [219]. Trace element analysis is a simple methodology, but it is not applicable to processed products or those with no mineral structures such as fillets. Fatty acid analysis is also a fast, inexpensive, and simple methodology but cannot be applied to all processed products. Also, lipids are susceptible to oxidation. Finally, analyzing DNA is used in an increasing number of fishery products, because it is species-specific, highly sensitive, and accurate, but includes a number of complex and time-consuming methodologies. In addition, it cannot be applied to all processed products or to geographically close populations. Depending on whether or not there are sound baseline databases and quality reference material of known origin, many authors prefer to use a

combined analysis, such as trace elements and DNA markers or even the shape of the otoliths and molecular markers [227].

4.1 Analyzing Trace Elements

Trace element fingerprints (TEF) of fish and shellfish mineral structures (shells, otoliths, and bones) have been successfully used to distinguish specimens of different geographical origin [228]. In hard, inert mineral structures, trace elements depend on their availability in seawater, which varies in each ecosystem and reflects the geographical surroundings of a given specimen throughout its life. TEF can be analyzed by means of inductively coupled plasma-mass spectrometry (ICP-MS) or laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). A wide range of element ratios can be measured in these calcified structures, although the most common are Ba/Ca, Cd/Ca, Cu/Ca, Cr/Ca, Mg/Ca, Mn/Ca, Pb/Ca, Sr/Ca, U/Ca, and Zn/Ca. Distinguishing cockles from geographically close populations – only one km apart – was carried out successfully in the work of Ricardo et al. (2015) [229] using ratios of Ba and Mn as the most important for differentiating locations. Yamashita et al. (2008) [218] used multivariate analysis of trace elements in muscle and bone to distinguish tuna species and skipjack tuna meat in canned products; Japanese eel from Japan, Taiwan, and China from fish bone; littleneck clam from Japan, Korea, and China using shells; and chinook salmon from Japan, Canada, Chile, and Russia with fresh and frozen muscle. Tanner et al. (2012) [230] used the ratios Mg/Ca, Cu/Ca, and Ba/Ca on the otoliths of *Solea solea* and found that these correlated well with different water salinities. They later used otolith chemistry to delineate different stocks for this species [231]. Wells, Thorrold, and Jones (2000) [232] used scales to distinguish weakfish from five estuarine locations and found high correlations between Mn/Ca, Sr/Ca, and Ba/Ca ratios, in both scales and otoliths. Their study favored the use of scales as they are a nonlethal alternative to otoliths and are easier to collect and prepare. To succeed in using trace elements on the geographic identification of fish and shellfish, it is generally agreed that there is a need to define reference ranges of trace elements in foods from animals, along with information on geographic origin, genetic background, production time, and type of production system. Danezis et al. (2016) [233] stressed that these reference ranges are critical both for defining the quality control standards for measurements and for identifying signatures that characterize food origin be it geographic, production system, or any other indicator of interest together with new algorithms for analyzing all the data.

4.2 Stable Isotope Analysis

Stable isotopes are different forms of the same element, differing only in the number of neutrons in the nucleus of the atom resulting in different weight atoms and diverse distribution in the environment. These atoms are incorporated into animal tissues

through ingestion of water and feed and their ratio can vary according to environmental conditions [234]. The most commonly used isotope ratios are $^{2}H/^{1}H$ (or D/H), $^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{15}N/^{14}N$, $^{34}S/^{32}S$, and $^{87}Sr/^{86}Sr$ of the elements H, C, N, O, S, and Sr. They can differentiate foods based on their geographical origin and are measured reliably in routine work in different matrices, with their results comparable between different laboratories [235]. Stable isotope analysis is thus a promising approach for authenticating the origin of different organisms. Krivachy et al. (2015) [236] stated that C, N, H, O, S, B, and Sr isotopes are adequate for determining the geographic origin of several plants, as related to the soil and their fixation in the plant. Li et al. (2016) [237] indicated that stable isotope and multielement analysis were two of the most widely used methods in traceability of aquaculture products and related fish to production methods (wild or farmed, organic or intensive), due to their distinctively different food sources. Nevertheless, in fish and shellfish, stable isotopes are affected by more complicated factors: isotope fractionation in animals, geographical origin of the sample, physiological and anatomic properties, and even their positions in the food chains.

Stable isotopes are usually analyzed in an isotope ratio mass spectrometry (IRMS) instrument, and the ratio is obtained by comparing the value of the isotopes with its natural form. Generally, the analysis in fish is made using otoliths [238–240], which provide a record of fish life. Tissues such as fillets, which integrate diet over months and within the same species, can be very different from site to site [241]. Evaporation, condensation, and precipitation of water in nature affect the $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios [234]. Changes in diet can also affect $\delta^{15}N$ in the organs and muscles of fish, and those changes need a certain period of time to remain steady, as Olsen et al. (2015) [242] showed in cod after only 40 days of a dietary shift. Isotopes also vary between tissues within the same fish, so many authors use scales and otoliths, which reflect the events that occurred during the fish's growth [243]. Curtis et al. (2014) [244] suggest using both stable isotope and trace element analysis depending on the situation, because the first is more accurate, for instance, for distinguishing hatchery-reared and wild spotted trout, while the latter seems to have greater discriminatory capacities for studies over a period of time. This has also been confirmed by Moreno-Rojas et al. (2008) [245].

Morrison et al. (2007) [246] used isotopes to authenticate the production origin of gilthead sea bream (*Sparus aurata*) and $\delta^{18}O$ as a discriminatory factor. Wolff et al. (2012) [247] used the strontium isotope ratio ($^{87}Sr/^{86}Sr$) of otoliths as markers for reservoir fish and for tracking the movements of invasive fish in river-reservoir systems. They found that these markers are site-specific and temporally stable over time. Recently, Carrera and Gallardo (2017) [248] succeeded in using the stable isotope ratio (SIR) to determine the geographical origin of all commercial fish species in the Merlucciidae family using SIR analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). It is rare for a single element to provide full information for food authenticity, and usually several stable isotopes need to be combined to determine the geographic origin of aquaculture products [249]. Other isotope ratios, such as $\delta^{13}C$ and $\delta^{15}N$, do not offer a consensus on their use. Some authors, like Kim et al. (2015) [250], find the former to be discriminatory for geographic origins, and others,

like Chaguri et al. (2015) [251], suggest that $\delta^{15}N$ is a better indicator for wild versus farmed fish. Menard et al. (2007) also used $\delta^{15}N$ and found that latitude affected this ratio in yellowfin tuna and swordfish [252].

As stated above, isotope ratio analysis has been used with success to verify geographical origin in various cases in relation to legal or other enforcement activities. However, to determine the geographical origin of a certain food item, the stable isotopic data of the test samples must be compared with a reliable database or databank containing some information about the basic element profile and multivariate data evaluation [235]. In 2009, after a major case of fraud, the first European caviar database appeared based on the isotopes of C, O, H, N, and S in the water and the roe protein fractions of 29 authentic caviar samples.

When using isotope ratios, a sound database should be used with representative samples, different tissues, and structures, with previous isotope values to be compared with the intended organisms especially if the items were subjected to any kind of processing, such as freezing or ice storage [253].

4.3 DNA-Based Techniques

Molecular-based analysis has greatly improved in the last few years thanks to technological progress in equipment. Its performances have also increased in terms of speed. Markers such as allozymes, AFLP, whole mitochondrial genomes, RAPD and RFLP on mitochondrial DNA (mtDNA), and nuclear DNA have frequently been used in fishery research. Generically, many studies reveal that it is possible to differentiate fish populations using microsatellites, highly variable genetic markers. Mitochondrial DNA has also been used to detect the causes of population divergence by means of historical isolation in certain marine fish.

Microsatellites are a set of short repeated DNA sequences (2–13 base pairs) at a particular locus on a chromosome, which vary in number in different individuals and have been used for genetic fingerprinting since the early 1980s [254]. They are analyzed by conventional PCR amplification and amplicon size determination, followed by Sanger DNA sequencing, and are a cheap and easy methodology, with several applications in population genetics. In the field of fisheries and aquaculture, microsatellites are useful for characterizing genetic stocks and dense linkage maps [255]. Lane, Symonds, and Ritchie (2016) [256] used nine polymorphic microsatellite DNA loci to investigate the phylogeography of hapuku wreckfish around New Zealand and to distinguish its populations. Sanchez et al. (2016) [257] divided jumbo squid into two population groups using an innovative nuclear microsatellite locus and a fragment of 675 bp from the mtDNA ND2. In another study on the distribution of *Octopus vulgaris* in the NE Atlantic and Mediterranean, Cabranes et al. (2008) [258] found significant differences in samples separated by up to 200 km using five polymorphic microsatellite DNA markers. A similar work by De Luca et al. (2016) [259] using 13 microsatellite loci was carried out to study genetic variations and population structures in *Octopus vulgaris* in seven sampling sites in the Mediterranean and southern Portugal. The results showed some distinct

populations but no clear separation of all of them. The authors also used the DNA barcoding approach, analyzing COI gene fragments to understand the phylogenetic relationships within populations. They concluded, however, that it is not a good marker for detecting genetic structure. Some authors have used a combination of microsatellites, allozymes, and mtDNA [260] microsatellites and mtDNA markers to separate the effect of migration, genetic drift, and local adaptation of populations [261]. Zhigileva et al. (2013) [262] used nuclear DNA inter-simple sequence repeat (ISSR) markers and compared them with allozymes, having found that these provide more precise geographical differences in population groups of fish. A different hypothesis for tracing the source of a product involves analyzing the microbial communities of the food and statistically linking this analysis to their origin. Le Nguyen et al. (2008) [263] tested the bacterial community structures of *Pangasius* fish from South Vietnam and found that the band pattern of the bacterial communities isolated from the fish, obtained by PCR-DGGE, was linked to the microbial environment of the fish. The *16S* and *26S* rDNA profiles generated by PCR-DGGE (PCR-denaturing gradient gel electrophoresis analysis) were used by Montet et al. (2008) [264] to detect variations in the structures of bacteria and yeast communities in fish and fruit.

In a recent review, Cuellar-Pinzon et al. (2016) [265] suggest that microsatellite and mitochondrial DNA-based markers, though rarely used in recent years, will probably be replaced in fishery research by increasingly accessible techniques such as gene sequencing and next-generation sequencing (NGS)-derived markers. Davey and Blaxter (2010) [266] proposed the use of restriction site-associated DNA sequencing (RADSeq) that can identify and score thousands of genetic markers, randomly distributed across the target genome, from a group of individuals. This has the advantage that it can be used on species with no, or limited, existing sequence data. Within the FishPopTrace European-funded project, forensically validated panels of SNP (single nucleotide polymorphism) markers were proposed for geographic origin assignment of four commercially important fish species, cod (*Gadus morhua*), hake (*Merluccius merluccius*), herring (*Clupea harengus*), and common sole (*Solea solea*) [267–269].

Whether using TEF, stable isotopes, or any of the molecular biology techniques, determining the geographic origin of fish and other fishery products needs multivariate analysis methods and specific genetic software to support the processing of the results.

5 Methods for Distinguishing Wild Seafood from Farmed

Different approaches have been used over the years in the methods proposed for differentiating wild/farmed fish. The studies published have compared genetics, chemical characteristics, fatty acid composition, concentration of certain trace elements, levels of certain pollutants, stable isotope concentrations, morphology, and organoleptic characteristics [270]. A recent comprehensive review of tools for differentiating the wild and farmed origins of Mediterranean fish [270] showed that analyzing fatty acids and/or lipids includes the most frequently used method

(63% of all the reviewed works). Other, less studied, methods involved the morphological differences between wild and farmed fish (37%) and differences in the proximate composition (27%). The techniques using stable isotopes and pollutants were the least frequently used methods (12% each). SNP-assays have also been developed by Bylemans et al. (2016) [271] to trace farmed fish. At present, there are no known official standardized methods. Nonetheless, on account of the accuracy of the results, stable isotope fingerprinting and fatty acid analysis seem to be the most promising and suitable methods for differentiating wild and farmed fish and are discussed below.

5.1 Analyzing Stable Isotopes

Of all the isotopes, carbon (^{13}C), nitrogen (^{15}N), and oxygen (^{18}O), being major constituents of organic matter, transfer from one organism to another through the food cycle and increase the isotope fingerprint as a result of assimilation and growth. On account of this, determining stable isotope ratios is an important analytical tool in trophic studies, as they show the relative levels of different isotopes in a particular atom determined by isotope ratio mass spectrometry (IRMS). Depending on the atom selected, the isotope ratios may reflect, in the case of animals, the isotopic makeup of the diet or feed which has been used [272–274].

Food availability and origin in wild fish differ significantly from that of farmed fish. Farmed fish feed is poorer in raw materials of marine origin and has an increasingly large component of vegetable meals and oils, characterized by a higher terrestrial carbon input and a lighter isotope content. This can be exploited to determine seafood authenticity, and most research has investigated the use of these isotopes, namely, $^{13}C/^{12}C$ and $^{15}N/^{14}N$ [275], to distinguish farmed from wild fish or organic from conventionally farmed fish.

Several studies have shown the existence of distinct differences in the isotope ratios between wild and farmed fish [242, 246, 276–281] and among wild, conventionally and organically farmed fish [282]. Farmed fish could be distinguished from wild fish mostly because of a constantly lighter isotopic composition of $\delta^{13}C$, thus revealing the land-based origin of some of the feed raw materials and their lower ^{13}C content. The higher lipid content of farmed fish seems to induce greater isotopic fractionation of ^{13}C than in wild fish on account of the higher metabolic turnover and a lower fat content, both resulting from food shortage [270].

For $\delta^{15}N$, significant differences were also found between wild and farmed sea bass [276, 277] and seabream [246] with the wild fish muscle characterized by a higher ^{15}N isotopic content than the farmed fish. These differences were attributed to the higher trophic level of wild fish marine feed and also to the different inputs of terrestrial N sources in the farmed feed mixtures.

Most works published on fish authenticity and stable isotopes have a relatively reduced sample size. Nevertheless, using stable isotope ratios or any other marker for authenticity needs to address all the variables affecting the marker with comprehensive sampling and sufficient data. A study of this nature performed in a large sample

(171 fish) of wild and farmed Atlantic salmon (*Salmo salar*) of 32 different origins within Europe, North America, and Tasmania was presented by Thomas et al. [283]. In order to maximize the range of variation present, sampling was extended to all seasons over two consecutive years and included fish raised using different practices. The results showed that $\delta^{15}N$ measured on choline and $\delta^{18}O$ measured on total oil could be used to successfully distinguish between fish of authentic wild and farmed origin. However, the authors also added that to strengthen the certainty of identifying mislabeling in market-derived fish, analyzing the percentage of linoleic acid (LA, 18:2n-6) in the lipidic fraction should be included.

Published data has shown however that though isotope analysis can be accurate standardization of methodologies is necessary in order to be able to obtain responses over an entire range of farmed and wild species. Although a cost- and time-effective alternative to other analytical techniques for identifying wild and farmed fish, comparison with databases of known isotope values or ranges of wild fish species is also necessary to determine authenticity, and, as different factors (e.g., food source, availability) affect isotope ratios, these need to be comprehensive and frequently updated.

5.2 Analyzing Fatty Acids and/or Lipids

Farmed fish has been shown to have in general a higher lipid content than wild fish [284–287] mostly as an expression of the high fat content of their diet and intensive feeding regime. As finfish and crustacean farming depends on scarce marine resources that provide key dietary nutrients, such as fish meal and fish oil, aquaculture diets are diversified to contain a wide variety of alternative plant-based ingredients such as legume seeds, oilseed cakes, leaf meals, and an increasing proportion of vegetable oils [288]. As the fatty acid (FA) profile of the fish tissue is the expression of the incorporation and accumulation of the FAs present in the diet [289], differences in the lipid composition and dietary FA profiles of the feed have been used to distinguish between wild and farmed fish. The presence of high levels of FAs of terrestrial origin such as oleic acid (OA, 18:1n-9), α-linolenic acid (LNA, 18:3n-3), or LA, which are usually found at low levels in marine fish, is generally evidence of these differences [277, 290]. The prevalence of LA, augmented LNA, and the generally increased n-6/n-3 ratio in the flesh of farmed fish therefore seems to be the result of incorporating increased levels of plant oils as an alternative raw material for fish feeds [291]. Contrary to what happens in freshwater fish, the deficiency in marine fish of the enzymes (δ-5-6-desaturase) needed to elongate the carbonic chain of LA and LNA from the fish feed and transform them into n-6 and n-3 FAs [292] may justify the increased levels of these FAs.

The vast majority of studies report significant differences between wild and farmed fish mostly in terms of LA, arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) [286, 293–296]. For example, LA and ARA were generally found in higher and lower levels, respectively, in farmed sea bass and sea bream [277, 297, 298].

Nevertheless diverse patterns of FA distribution (e.g., EPA and DHA) and FA indexes (e.g., n-3/n-6) were determined between wild and farmed fish in other fish species. Major factors affecting this heterogeneity are dietary related and include high variability in terms of the FAs in the feeds available on the market, which is a consequence of the fish oil used as a source of long-chain n-3 polyunsaturated fatty acids [299, 300]. Likewise, wild fish also show considerable FA profile variation depending on season, food availability, and fishing ground [301, 302] all of which affect the usability of FAs for authenticity purposes. Nevertheless, in some cases, this variation – and in particular the distinctive FAs accumulated in farmed fish from feed produced with raw materials from different marine environments – has been used as biomarkers. A clear example is the monounsaturated fatty acid, 22:1n-11, which originates in dietary fish oils produced from Northern Atlantic fish and is not a normal constituent of the body lipids of warm water fish such as sea bream. It is nonetheless found in farmed fish of this species but is almost absent in wild specimens [303].

Though terrestrial fatty acids in fish feeds are considered to be strong biomarkers and distinct FA signatures and different stable isotope ratios may characterize wild and farmed fish species, using multivariate analysis has been proposed as a method for analyzing in a more robust way the effects of all the variables analyzed [277, 304]. This technique was used successfully in the farmed/wild differentiation of several species, namely, bogue [222], saithe and cod [305], Mediterranean horse mackerel [306], sea bass [277], salmon [307], and turbot [304].

In terms of analytical methods, the most common testing protocols involve gas chromatography (GC) to determine the origin of fish using fatty acid profiles [308], although other fat-related analytical methods such as near-infrared spectroscopy [296] and nuclear magnetic resonance [309, 310] have also been used. Comprehensive two-dimensional gas chromatography-mass spectrometry (GC × GC–MS) was also used to determine fatty acid fingerprints in fish (*Sparus aurata* and *Dicentrarchus labrax*) from Southern Italy [311] in order to highlight differences in the fatty acid profiles of farmed and wild fish samples. GC × GC-MS analysis showed that omega-3 fatty acids were naturally more concentrated in wild fish, whereas their presence in cultivated fish was lower and in favor of n-6 and oleic acids, as a distinct consequence of food supplementation. In this study, some fatty acids were detected only in one type of fish (wild or farm-raised) or were determined at very different levels, such as the biological mediator ARA, which was almost 50 times more concentrated in wild-caught fish.

Despite the reported usability of these methodologies for farmed/wild fish differentiation, problems with procedure standardization have hindered wider use in seafood authentication [312]. Variability in laboratory results has mostly been attributed to equipment characteristics, differences arising from lipid extraction and diverse methodologies of fatty acid esterification. To ensure the consistency and accuracy of these methods and ultimately convert them into regulations, not only continuous monitoring of fatty acid/stable isotopic composition of both cultivated and wild fish is recommended but also necessary standardization of the methodologies through collaborative work.

6 Advanced Methods for Determining Previously Frozen Seafood

Fresh fish and seafood are highly perishable foodstuffs that deteriorate rapidly at high storage temperatures, and this limits their shelf life and safety. Even though numerous preservation methods have been used to extend the shelf life of fish, freezing is still the most frequently used preservation technology. The aim of freezing is to decrease microbiological growth and the activity of deteriorating enzymes by converting the available water into ice crystals [313]. Nevertheless, extended textural and functional changes can occur on account of cell rupture by enlarged ice crystals and also due to inadequate freezing procedures, post-freezing, and thawing processes [314]. As a result, frozen fish usually has a much lower price than fresh fish, and the substitution of frozen-thawed for fresh fish is a significant authenticity issue [315]. In fact, even though changes occur during freezing, consumers do not have the ability to differentiate between fresh and frozen-thawed fish because of the similarity of the sensorial characteristics of the two products.

The ability to differentiate between fresh and frozen-thawed fish is all the most important for preventing fraudulent practices as (i) freezing-thawing has been a management process for fresh fish and expedites supplies of "fresh products" to consumers based on demand; (ii) fish that is consumed raw, such as sushi and sashimi, is obligatorily a frozen-thawed product and needs to be checked, on account of special regulations that include compulsory freezing at −20 °C for at least 24 h for parasite control (Regulations (EC) No. 178/2002, 853/2004, and 2074/20 05); and also (iii) safety issues can arise, as thawed meat is more susceptible to microbial growth.

Different analytical methods have been proposed, and these can be classified into three main categories: enzymatic, physiological, and physical. Enzymatic methods are based on determining increases in the enzymatic activity of specific enzymes that leak into tissue exudate following cellular disruption that occurs during the freezing/thawing process. These methods can involve assays of enzymes originally bound to (i) mitochondria (β-hydroxyacyl-CoA-dehydrogenase [316, 317], glutamate oxaloacetate transaminase [318], lipoamide reductase and 5' AMP deaminase [319], cytochrome C oxidase [320, 321], aspartate aminotransferase [322], lactate dehydrogenase [319, 323], succinate dehydrogenase [324], fumarase and glutamate dehydrogenase [323], L-malate dehydrogenase [325], L-malate-NADP oxidoreductase [326]), (ii) red blood cells (β-N-acetylglucosaminidase [327, 328]), and (iii) lysosomes (α-glucosidase, β-galactosidase, and β-N-acetylglucosaminidase [329–331], acid phosphatase [330, 331], β-galactosidase and β-glucuronidase [323]). The disadvantages of using enzymes as freezing indicators have however been reported, namely, in relation to the existence of corresponding mitochondrial isoenzymes in the cytoplasm and also because the activity of differentiating enzymes can be highly sensitive to other stresses imposed on the product (e.g., previous ice storage) [332]. Which type of enzyme (mitochondrial, lysosomal, or extracted from blood cells) is the most suitable for authentication purposes depends also to some extent on the fish species [315, 331] and is also a limiting factor.

Physiological methods may involve examining the opacity of the fish eye lens, namely, change in the transparency of the medulla (central part) of the eye which becomes turbid or opaque after freezing [315, 333, 334]. The robustness of this method must, however, be checked as differences have been reported as not being evident in species like plaice and eel [335, 336]. Determining the hematocrit value as an index of blood cell destruction or as judgment of the integrity of red blood cells by microscopy has also been proposed for differentiating freeze-thawed fish from fresh fish [329, 337, 338].

In an attempt to overcome the tedious and destructive nature of physiological methods, which are also time-consuming and require highly trained operators, attention has recently been focused on the development of advanced noninvasive and nondestructive instrumental techniques such as front-face fluorescence spectroscopy (FFFS). Limitations in some methods because of fish or fillet samples with no blood, eye lens, or skin are also overcome with these new methods. A study of the potential of FFFS combined with chemometric tools to differentiate frozen-thawed from fresh whiting fillets (*Merlangius merlangus*) showed that nicotinamide adenine dinucleotide (NADH) fluorescence spectra recorded directly on fillets have the potential to differentiate frozen-thawed from fresh fish and may dramatically reduce analytical time and costs when looking at enzymatic and biochemical measurements [339]. The NADH emission spectra (excitation 340 nm) of fresh fish showed a maximum at 455 nm, while frozen-thawed fish was characterized by a maximum at 379 nm. Similarly, FFFS made possible reliable discrimination between fresh and frozen/thawed sea bass (*Dicentrarchus labrax*) fillets (72 out of 78 samples) and was therefore considered a promising tool in routine inspections [340]. NADH fluorescence spectra and Fisher linear discriminant analysis was also considered a potential method for reliable differentiation between frozen-thawed and fresh fish, as a 100% correct classification of large yellow croaker (*Larimichthys crocea*) fresh/frozen-thawed samples was achieved using this technique [341].

Physical methods can be based on changes in the electric resistance of tissues, as used by the FISCHTESTER, the RT Fish Freshness Tester, or the Torrymeter [315, 342–345] to account for the loss of impedance in fish induced by the freezing/thawing cycle [346]. As membranes are destroyed by freezing and thawing, the electrical resistance of thawed fish is also significantly reduced [315]. In view of the limitations of these initial technologies, which examined only optimum frequencies to make the measurements, other bioelectrical impedance spectroscopy methods that analyze the electrical response over an entire frequency region from 1 Hz to 1 MHz have been developed [347–350].

In addition to these technologies, other physical methods rely on measurement of dielectric properties in the microwave region as a function of frequency (100 MHz to 10 GHz) and multivariate analysis of the spectra obtained using principal component analysis [351]. Differentiation of fresh/frozen-thawed octopus (*Octopus vulgaris*) was successfully reported with the use of the Sequid RFQ-Scan® system (Sequid GmbH, Bremen, Germany), which is based on the principle of dielectric spectroscopy combined with time domain reflectometry (TDR) and applying an ultrawide band step signal to the material being tested [352]. The release of water and ions

during octopus thawing was suggested as an explanation of the differences found between fresh and frozen-thawed samples.

Taking into account that a nuclear magnetic resonance (NMR) signal is usually sensitive to water mobility and its interaction with other molecules, this technique has also been considered appropriate for determining freezing and thawing changes in fish flesh in a noninvasive and nondestructive way [353]. The effect of freezing and thawing on NMR signals (e.g., relaxation time and spin densities) was studied using magnetic resonance imaging (MRI) techniques in cod (*Gadus morhua*) and mackerel (*Scomber scombrus*) [354, 355], trout (*Salmo gairdneri*) [356, 357], salmon (*Salmo salar*) [358], and haddock (*Melanogrammus aeglefinus*) [355, 359]. Despite the acceptable results obtained and the potential of NMR/MRI analysis for fresh/frozen-thawed fish differentiation, the high investment costs, the still-significant size of the equipment, and the associated infrastructures needed for operation strongly limit its use at present as a standard analytical tool.

In addition to existing methods, volatile profiles measured by solid phase micro-extraction/gas chromatography/mass spectrometry analysis (SPME/GC/MS) were also used for authenticity purposes [360]. Analysis of fish samples of European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), and fillets of cod *(Gadus morhua*) and salmon (*Salmo salar*) using SPME/GC/MS showed four volatile compounds common to all species, dimethyl sulfide, 3-methylbutanal, ethyl acetate, and 2-methylbutanal, most likely derived from free amino acids, which are part of the soluble substance in the aqueous extract of the muscle tissue. Taking into account that the selected volatiles were shown in larger quantities after thawing but also increased with the duration of storage at −20 °C, they were considered as differentiation potential markers between fresh products and those that have been frozen.

New methods based on the electromagnetic absorption of organic compounds, such as near-infrared (NIR) spectroscopy, are gaining attention because of the major advantages they have over traditional chemical methods [315, 361–366]. As NIR is a physical and very fast method, it requires minimal or no sample preparation and its precision can be high [367]. Furthermore, and in contrast with traditional chemical analyses, no reagents are required and no waste is produced. The results from application of dry extract spectroscopy by infrared reflection (DESIR) on the meat juices of horse mackerel (*Trachurus japonicus*), bigeye tuna (*Thunnus obesus*), and amberjack (*Seriola purpurascens*) samples showed after differentiation by means of principal component analysis and multiple linear regressions that this technique could categorize fresh and frozen-thawed samples with 100% accuracy [367]. Likewise, a fiber-optic measurement surface probe using visible/NIR spectroscopy analysis was used on the skin of 108 fresh and frozen-thawed red sea bream samples (*Pagrus major*) in order to differentiate the origin of fresh or thawed samples [368]. After multivariate data analysis, the results showed that on account of the changes in light scatter, possibly arising from alterations in the physical structure of at least the surface layer of the fish, all the samples were correctly categorized according to their origin. The good results obtained, along with the fast data throughput and eco-friendly nature of the device, all supported the suggested application of this type of measurement in online or at-line processing control.

The effectiveness of near-infrared spectra used as a species-independent screening method for foodstuff classification in terms of fresh/frozen-thawed differentiation has also been validated in a database of more than 1200 samples of different fish species [361]. Analytical strategies based on partial least squares discriminant analysis of data from spectra of two NIR instruments exploring different spectral regions, respectively, from 680 to 1100 nm and from 1100 to 2500 nm, were developed using samples of species not included in the calibration data. The results showed the best validation classification accuracies with values between 91% and 88.4%. Besides being effective in fish differentiation, Vis/NIR spectroscopy combined with Savitzky-Golay 19-point smoothing of spectra and chemometrics methods (discriminant analysis and discriminant partial least squares) was also shown to correctly identify all 55 fresh shrimp and 50 frozen-thawed shrimp [369]. Given the advantages of NIR spectroscopy, this technology has been referred to as having high potential for performing efficiently in enforcing conformity with regulations [370]. Applying increasingly powerful and sophisticated chemometric tools to NIR databases improves calibration robustness and accuracy, whereas developing an extended range of algorithms in commercially available software packages is facilitating implementation.

Methods that distinguish fresh and frozen-thawed meats should not require reagents and should be fast, nondestructive, quantitative, and relatively inexpensive. Thus, as an emerging technique, hyperspectral imaging has also been suggested as being able to differentiate between fresh and frozen-thawed meat. Recent studies have demonstrated that hyperspectral imaging detects freshness in fish [363, 365, 371]. To assess the automatic freshness of cod fillets at an industrial speed of 400 $mm.s^{-1}$ or 1 $fillet.s^{-1}$, a hyperspectral imaging system with a handheld interactance probe was developed [363]. The results indicated that fresh cod (*Gadus morhua*) fillets could be fully separated from frozen-thawed fillets by using a small subset of wavelengths in the visible region (487, 557, 606, and 646 nm). In another application, fresh halibut (*Psetta maxima*) fillets were successfully separated from frozen-thawed fillets with a correct classification rate of 97%, based on both spectral and textural features that were extracted from hyperspectral images in the spectral region from 380 to 1030 nm [365].

Other new technologies such as Fourier transform infrared (FTIR) spectroscopy [372], and more recently Raman spectroscopy [373], are gaining wide attention because of their advantages, such as rapidity, on-site usability, and high accuracy. A patent has been filed for a rapid control method suitable for rapid differentiation of fresh and frozen/thawed meat or fish using FTIR spectroscopy and subsequent data processing based on hierarchical cluster analysis and artificial neuronal network analysis [374]. Raman spectroscopy and fatty acid profile analysis were combined to analyze 64 fish fat samples from 6 different species, namely, horse mackerel (*Trachurus trachurus*), European anchovy (*Engraulis encrasicolus*), red mullet (*Mullus surmuletus*), bluefish (*Pomatomus saltatrix*), Atlantic salmon (*Salmo salar*), and flying gurnard (*Trigla lucerna*) [373]. The Raman data collected were used as inputs for chemometric analysis and made it possible to develop a PCA model that successfully differentiated fresh and frozen-thawed fish samples.

Fresh and frozen-thawed fish can be differentiated by a wide range of methods, although until now the lack of standardization has deterred wider use in seafood authentication. In view of the need for food control, the authorities' methods should preferably be fast, nondestructive, and relatively inexpensive and not require reagents. Most of the present-day methods proposed need to be improved to match these requirements. For this, research needs to continue, not only in terms of the equipment but also on chemometrics in order to fully validate the best performing methodologies and standardization among laboratories.

7 Conclusion

This chapter has given an extensive overview of the most common methods used in seafood authenticity surveillance. Most are DNA-based methods, but some protein-based methods such as IEF or mass spectrometry have also been included because they are used as simple and cheap screening methods. For the emerging methods for seafood species identification, NGS is a method with great potential for species authentication in mixed products like surimi, cans of tuna, or wild shrimp. Nevertheless, at present, it is not yet possible to quantify the presence of seafood species with this method. Isothermal amplification methods, coupled with easy-to-handle detection devices such as dipsticks, are promising approaches for fast and on-site species detection and would be a valuable tool for official food surveillance, as well as companies' self-monitoring systems. In addition to DNA-based methods, promising methods in seafood species identification are those that use mass spectrometry – methods such as the very fast MALDI-TOF MS, for which some European laboratories (official and private) have already established fish species reference databases.

The methods reviewed for determining geographic origin and previously frozen seafood, and to distinguish wild from farmed seafood, are still under development. They are a very challenging field of research, in terms of their need to address all the traceability requirements and to tackle new questions such as how to distinguish double-freezing or super (or sub)-chilling. Nevertheless, reliable and standardized methods will be needed in the near future in order to fulfill the requirements of the new EU control regulation (Regulation (EU) 2017/625) which regulates the risk-based control of food authenticity. With regard to this, Germany is already in the process of establishing a national center for the authenticity and integrity of the agri-food chain. The development and standardization of new food authentication methods will be one of the priorities of this institution.

References

1. FAO (2016) The state of world fisheries and aquaculture 2016: contributing to food security and nutrition for all. Food and Agriculture Organization of the United Nations, Rome

2. European Commission (2016) Facts and figures on the Common Fisheries Policy. European Union. https://ec.europa.eu/fisheries/facts_figures_en. Accessed 24 Oct 2017
3. Pardo MÁ, Jiménez E, Pérez-Villarreal B (2016) Misdescription incidents in seafood sector. Food Control 62:277–283
4. Warner K, Mustain P, Lowell B, Geren S, Talmage S (2016) Deceptive dishes: seafood swaps found worldwide. https://www.google.com/search?q=Deceptive+dishes%3A+seafood+swaps&safe=strict. Accessed 23 Oct 2017
5. Agnew DJ, Pearce J, Pramod G, Peatman T, Watson R, Beddington JR, Pitcher TJ (2009) Estimating the worldwide extent of illegal fishing. PLoS One 4(2):e4570
6. Griffiths AM, Sotelo CG, Mendes R, Pérez-Martín RI, Schröder U, Shorten M, Silva HA, Verrez-Bagnis V, Mariani S (2014) Current methods for seafood authenticity testing in Europe: is there a need for harmonisation? Food Control 45:95–100
7. Kappel K, Schröder U (2016) Substitution of high-priced fish with low-priced species: adulteration of common sole in German restaurants. Food Control 59:478–486
8. Wang D, Hsieh YHP (2016) The use of imported pangasius fish in local restaurants. Food Control 65:136–142
9. Regulatory Fish encyclopedia: U. S. Food and Drug Administration (1993–2017) https://www.fda.gov/food/foodscienceresearch/rfe/default.htm
10. Kappel K, Schröder U (2015) Species identification of fishery products in Germany. J Verbrauch Lebensm 10:31–34
11. Rehbein H, Etienne M, Jerome M, Hattula T, Knudsen LB, Jessen F, Luten JB, Bouquet W, Mackie IM, Ritchie HA, Martin R, Mendes R (1995) Influence of variation in methodology on the reliability of the isoelectric focusing method of fish species identification. Food Chem 52:193–197
12. Altinelataman C et al (2009) Comparison of IEF patterns of sarcoplasmic proteins of fish from North Atlantic and Aegean Sea. Food Control 20:980–985
13. Piñeiro C, Barros-Velázquez J, Pérez-Martín R, Gallardo JM (2000) Specific enzyme detection following IEF as complimentary tool for the differentiation of related gadoid fish species. Food Chem 70:241–245
14. Abdullah A, Rehbein H (2015) Authentication of closely related scombrid, catfish and tilapia species by PCR-based analysis and isoelectric focusing of parvalbumin. Eur Food Res Technol 241:497–511
15. Schiefenhövel K, Rehbein H (2013) Differentiation of Sparidae species by DNA sequence analysis, PCR-SSCP and IEF of sarcoplasmic proteins. Food Chem 138:154–160
16. Schiefenhövel K, Rehbein H (2011) Identification of barramundi (*Lates calcarifer*) and tilapia (*Oreochromis* spp.) fillets by DNA- and protein-analytical methods. J Verbrauch Lebensm 6:203–214
17. Ataman C, Çelik U, Rehbein H (2006) Identification of some Aegean fish species by native isoelectric focusing. Eur Food Res Technol 222:99–104
18. Renon P, Bernardi C, Malandra R, Biondi PA (2005) Isoelectric focusing of sarcoplasmic proteins to distinguish swordfish, blue marlin and Mediterranean spearfish. Food Control 16:473–477
19. Chen TY, Shiau CY, Noguchi T, Wei CI, Hwang DF (2003) Identification of puffer fish species by native isoelectric focusing technique. Food Chem 83:475–479
20. Hsieh YHP, Chen FC, Nur M (1997) Rapid species identification of cooked red snapper using isoelectric focusing. J Food Sci 62:15–19
21. Rehbein H (1995) Differentiation of scombroid fish species (tunas, bonitos and mackerels) by isoelectric focusing, titration curve analysis and native polyacrylamide gel electrophoresis of sarcoplasmic proteins. Electrophoresis 16:820–822
22. Piñeiro C et al (1998) Two-dimensional electrophoretic study of the water-soluble protein fraction in white muscle of gadoid fish species. J Agric Food Chem 46:3991–3997
23. Berrini A, Tepedino V, Borromeo V, Secchi C (2006) Identification of freshwater fish commercially labelled "perch" by isoelectric focusing and two-dimensional electrophoresis. Food Chem 96:163–168

24. Piñeiro C, Barros-Velázquez J, Sotelo CG, Pérez-Martín RI, Gallardo JM (2000) Specific enzyme detection following IEF as complimentary tool for the differentiation of related gadoid fish species. Food Chem 70:241–245
25. Girija N, Rehbein H (1988) Comparison of parvalbumin patterns from different fish species by isoelectric focusing of muscle extracts. Comp Biochem Physiol B Comp Biochem 91:723–728
26. Arif SH, Jabeen M, Hasnain AU (2007) Biochemical characterization and thermostable capacity of parvalbumins: the major fish-food allergens. J Food Biochem 31:121–137
27. Sotelo CG, Piñeiro C, Gallardo JM, Pérez-Martin RI (1992) Identification of fish species in smoked fish products by electrophoresis and isoelectric focusing. Z Lebensm Unters Forsch 195:224–227
28. Etienne M, Jérôme M, Fleurence J, Rehbein H, Kundiger R, Yman IM, Ferm M, Craig A, Mackie I, Jessen F, Smelt A, Luten J (1999) A standardized method of identification of raw and heat-processed fish by urea isoelectric focusing: a collaborative study. Electrophoresis 20:1923–1933
29. Etienne M, Jérôme M, Fleurence J, Rehbein H, Kundiger R, Mendes R, Costa H, Martınez I (2001) Species identification of formed fishery products and high pressure-treated fish by electrophoresis: a collaborative study. Food Chem 72:105–112
30. Rehbein H, Kündiger I, Yman IM, Ferm M, Etienne M, Jerome M, Craig A, Mackie I, Jessen F, Martinez I, Mendes R, Smelt A, Luten J, Piñeiro C, Pérez-Martín R (1999) Species identification of cooked fish by urea isoelectric focusing and sodium dodecylsulfate polyacrylamide gel electrophoresis: a collaborative study. Food Chem 67:333–339
31. Etienne M, Jérôme M, Fleurence J, Rehbein H, Kündiger R, Mendes R, Costa H, Pérez-Martín R, Piñeiro-González C, Craig A, Mackie I, Yman IM, Ferm M, Martínez I, Jessen F, Smelt A, Luten J (2000) Identification of fish species after cooking by SDS−PAGE and urea IEF: a collaborative study. J Agric Food Chem 48:2653–2658
32. Mackie I, Craig A, Etienne M, Jérôme M, Fleurence J, Jessen F, Smelt A, Kruijt A, Yman IM, Ferm M, Martinez I, Pérez-Martín R, Piñeiro C, Rehbein H, Kündiger R (2000) Species identification of smoked and gravad fish products by sodium dodecylsulphate polyacrylamide gel electrophoresis, urea isoelectric focusing and native isoelectric focusing: a collaborative study. Food Chem 71:1–7
33. Asensio L, González I, García T, Martín R (2008) Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA). Food Control 19:1–8
34. Taylor WG, Jones JL (1992) An immunoassay for verifying the identity of canned sardines. Food Agric Immunol 4:169–175
35. Dominguez E, Perez MD, Puyol P, Calvo M (1997) Use of immunological techniques for detecting species substitution in raw and smoked fish. Z Lebensm Unters Forsch A 204:279–281
36. Céspedes A, García T, Carrera E, González I, Fernández A, Asensio L, Hernández PE, Martín R (1999) Indirect ELISA for the identification of sole (*Solea solea*), European plaice (*Pleuronectes platessa*), flounder (*Platichthys flesus*), and Greenland halibut (*Reinhardtius hippoglossoides*). J Food Prot 62:1178–1182
37. Asensio L, González I, Rodríguez MA, Mayoral B, López-Calleja I, Hernández PE, García T, Martín R (2003) Identification of grouper (*Epinephelus guaza*), wreck fish (*Polyprion americanus*), and Nile perch (*Lates niloticus*) fillets by polyclonal antibody-based enzyme-linked immunosorbent assay. J Agric Food Chem 51:1169–1172
38. Carrera E, Terni M, Montero A, García T, González I, Martín R (2014) ELISA-based detection of mislabeled albacore (*Thunnus alalunga*) fresh and frozen fish fillets. Food Agric Immunol 25:569–577
39. Chen YT, Hsieh YHP (2014) A sandwich ELISA for the detection of fish and fish products. Food Control 40:265–273
40. Verrez-Bagnis V, Escriche-Roberto I (1993) The performance of ELISA and dot-blot methods for the detection of crab flesh in heated and sterilized surimi-based products. J Sci Food Agric 63:445–449

41. Reed ZH, Park JW (2010) Quantification of Alaska pollock surimi in prepared crabstick by competitive ELISA using a myosin light chain 1 specific peptide. Food Chem 123:196–201
42. Ochiai Y, Watabe S (2003) Identification of fish species in dried fish products by immunostaining using anti-myosin light chain antiserum. Food Res Int 36:1029–1103
43. Huang T, Marshall MR, Kao K, Otwell WE, Wei C (1995) Development of monoclonal antibodies for red snapper (*Lutjanus campechanus*) identification using enzyme-linked immunosorbent assay. J Agric Food Chem 43:2301–2307
44. Asensio L, González I, Rodriguez MA, Mayoral B, López-Calleja I, Hernández PE, García T, Martin R (2003) Development of a specific monoclonal antibody for grouper (*Epinephelus guaza*) identification by an indirect enzyme-linked immunosorbent assay. J Food Prot 66:886–889
45. Asensio L, González I, Pavón MA, García T, Martín R (2008) An indirect ELISA and a PCR technique for the detection of grouper (*Epinephelus marginatus*) mislabeling. Food Addit Contam 25:677–683
46. Asensio L, González I, Rodríguez MA, Hernández PE, García T, Martín R (2003) Development of a monoclonal antibody for grouper (*Epinephelus marginatus*) and wreck fish (*Polyprion americanus*) authentication using an indirect ELISA. J Food Sci 68:1900–1903
47. McNulty ST, Klesius PH (2005) Development of an indirect enzyme-linked immunoabsorbent assay using a monoclonal antibody to identify *Ictalurus* sp. fillets. Aquac Res 36:1279–1284
48. Gajewski KG, Chen YT, Hsieh YH (2009) Production and characterization of monoclonal antibodies specific to pangasius catfish, basa, and tra. J Food Sci 74:C241–C247
49. Hsieh YH, Chen YT, Gajewski K (2009) Monoclonal antibody-based sandwich ELISA for reliable identification of imported Pangasius catfish. J Food Sci 74:C602–C607
50. Fernández A, García T, Asensio L, Rodríguez MA, González I, Lobo E (2002) Identification of the clam species *Ruditapes decussatus* (grooved carpet shell), *Venerupis romboides* (yellow carpet shell) and *Venerupis pullastra* (pullet carpet shell) by ELISA. Food Agric Immunol 14:65–71
51. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74:5463–5467
52. Garcia-Sancho M (2010) A new insight into Sanger's development of sequencing: from proteins to DNA, 1943–1977. J Hist Biol 43:265–323
53. Bartlett SE, Davidson WS (1991) Identification of *Thunnus* tuna species by the polymerase chain-reaction and direct sequence-analysis of their mitochondrial cytochrome-b genes. Can J Fish Aquat Sci 48:309–317
54. Bartlett SE, Davidson WS (1992) FINS (Forensically Informative Nucleotide Sequencing): a procedure for identifying the animal origin of biological specimens. BioTechniques 12:408–411
55. Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC (1989) Dynamics of mitocondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A 86:6196–6200
56. Quinteiro J, Sotelo CG, Rehbein H, Pryde SE, Medina I, Pérez-Martín RI, Rey-Méndez M, Mackie IM (1998) Use of mtDNA direct polymerase chain reaction (PCR) sequencing and PCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. J Agric Food Chem 46:1662–1669
57. Sanjuan A, Comesaña AS (2002) Molecular identification of nine commercial flatfish species by polymerase chain reaction- restriction fragment length polymorphism analysis of a segment if the cytochrome b region. J Food Protect 65:1016–1023
58. Pepe T, Trotta M, Di Marco I, Cennamo P, Anastasio A, Cortesi ML (2005) Mitochondrial cytochrome b DNA sequence variations: an approach to fish species identification in processed fish products. J Food Protect 68:421–425
59. Jérôme M, Martinsohn JT, Ortega D, Carreau P, Verrez-Bagnis V, Mouchel O (2008) Toward fish and seafood traceability: anchovy species determination in fish products by molecular markers and support through a public domain database. J Agric Food Chem 56:3460–3469

60. Blanco M, Perez-Martin RI, Sotelo CG (2008) Identification of shark species in seafood products by forensically informative nucleotide sequencing (FINS). J Agric Food Chem 56:9868–9874
61. Lago FC, Herrero B, Vieites JM, Espiñeira M (2011) Genetic identification of horse mackerel and related species in seafood products by means of forensically informative nucleotide sequencing methodology. J Agric Food Chem 59:2223–2228
62. Huang YR, Yin MC, Hsieh YL, Yeh YH, Yang YC, Chung YL, Hsieh C (2014) Authentication of consumer fraud in Taiwanese fish products by molecular trace evidence and forensically informative nucleotide sequencing. Food Res Int 55:294–302
63. Rasmussen RS, Morrissey MT (2009) Application of DNA-based methods to identify fish and seafood substitution on the commercial market. Compr Rev Food Sci Food Saf 8:118–154
64. Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc R Soc Lond B Biol Sci 270:313–321
65. Hebert PDN, Ratnasingham S, de Waard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc R Soc Lond B Biol Sci 270: S96–S99
66. Viñas J, Tudela S (2009) A validated methodology for genetic identification of tuna species (Genus *Thunnus*). PLoS One 4:e7606
67. Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes 7:544–548
68. Armani A, Guardone L, Castigliego L, D'Amico P, Messina A, Malandra R, Gianfaldoni D, Guidi A (2015) DNA and mini-DNA barcoding for the identification of Porgies species (family Sparidae) of commercial interest on the international market. Food Control 50:589–596
69. Shokralla S, Hellberg SE, Handy SM, King I, Hajibabaei M (2015) A DNA mini-barcoding system for authentication of processed fish products. Nature. Sci Rep 5:15894
70. Steinke D, Vences M, Salzburger W, Meyer A (2005) TaxI: a software tool for DNA barcoding using distance methods. Philos Trans R Soc B 360:1975–1980
71. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2013) GenBank. Nucleic Acids Res 41:D36–D42
72. Hellberg RS, Pollack S, Hanner RH (2016) Seafood species identification using DNA sequencing. In: Naaum AM, Hanner RH (eds) Seafood authenticity and traceability: a DNA perspective. Academic Press, London
73. Wong EK, Hanner RH (2008) DNA barcoding detects market substitution in North American seafood. Food Res Int 41:828–837
74. Zhang J, Hanner RH (2012) Molecular approach to the identification of fish in the South China sea. PlosOne 7:e30621
75. Lowenstein JH, Amato G, Kolokotronis SO (2009) The real maccoyii: identifying tuna sushi with DNA barcodes: contrasting characteristic attributes and genetic distances. PlosOne 4: e7866
76. Ferrito V, Bertolino V, Pappalardo AM (2016) White fish authentication by COIBar-RFLP: toward a common strategy for the rapid identification of species in convenience seafood. Food Control 70:130–137
77. Bustin SA (2005) Real-time PCR. In: Fuchs J, Podda M (eds) Encyclopedia of Diagnostic Genomics and Proteomics. CRC Press, New York
78. Kutyavin IV, Afonina IA, Mills A, Gorn VV, Lukhtanov EA, Belousov EA, Singer MJ, Walburger DK, Lokhov SG, Gall AA, Dempcy R, Reed MW, Meyer RB, Hedgpeth J (2000) 3′-Minor groove binder-DNA probes increase sequence specificity at PCR extension temperatures. Nucleic Acids Res 28:655–661
79. Ugozzoli LA, Latorra D, Pucket R, Arar K, Hamby K (2004) Real-time genotyping with oligonucleotide probes containing locked nucleic acids. Anal Biochem 324:143–152
80. Raymaekers M, Smets R, Maes B, Cartuyvels R (2009) Checklist for optimization and validation of real-time PCR assays. J Clinic Lab Anal 23:145–151

81. Broeders S, Huber I, Grohmann L, Berben G, Taverniers I, Mazzara M, Roosens N, Morisset D (2014) Guidelines for validation of qualitative real-time PCR methods. Trends Food Sci Technol 37:115–126
82. Taylor MI, Fox C, Rico I, Rico C (2002) Species specific probes for simultaneous identification of (*Gadus morhua* L.) haddock (*Melanogrammus aeglefinus* L.) and whiting (*Merlangius merlangus* L.) Mol Ecol Notes 2:599–601
83. Trotta M, Schonhuth S, Pepe T, Cortesi ML, Piyet A, Bautista JM (2005) Multiplex PCR method for use in real-time PCR for identification of fish fillets from grouper (*Epinephelus* and *Mycteroperca* species) and common substitute species. J Agric Food Chem 53:2039–2045
84. Espiñeira M, Vieites JM (2016) Genetic system for an integral traceability of European eel (*Anguilla anguilla*) in aquaculture and seafood products: authentication by fast real-time PCR. Eur Food Res Technol 242:25–31
85. Espiñeira M, Vieites JM (2016) Fast real time PCR for control of intra-species recycling in aquaculture feed, focused to the most relevant fish species farmed in Europe. Food Chem 204:352–357
86. Lopez I, Pardo MA (2005) Application of relative quantification TaqMan real-time Polymerase chain reaction technology for the identification and quantification of *Thunnus alalunga* and *Thunnus albacares*. J Agric Food Chem 53:4554–4560
87. Herrero B, Madriñan M, Vieites JM, Espiñeira M (2010) Authentication of Atlantic cod (*Gadus morhua*) using real-time PCR. J Agric Food Chem 58:4794–4799
88. Herrero B, Lago FC, Vieites JM, Espiñeira M (2011) Authentication of swordfish (*Xiphias gladius*) by RT-PCR and FINS methodologies. Eur Food Res Technol 233:195–202
89. Herrero B, Vieites JM, Espiñeira M (2011) Duplex real-time PCR for authentication of anglerfish species. Eur Food Res Technol 233:817–823
90. Rasmussen Hellberg RS, Morrissey MT, Hanner R (2010) A multiplex PCR method for the identification of commercially important salmon and trout species (*Oncorhynchus* and *Salmo*) in North America. J Food Sci 75:C595–C606
91. Chen S, Zhang J, Chen W, Zhang Y, Wang J, Xu D, Zhou Y (2012) Quick method for grouper species identification using real-time PCR. Food Control 27:108–112
92. Dalama J, Vieites JM, Espiñeira M (2015) Detection of the causal agents of Keriorrhea (*Lepidocybium flavobrunneum* and *Ruvettus pretiosus*) by means of real time PCR. Food Chem 174:326–329
93. Terio V, Di Pinto P, Decaro N, Parisi A, Desario C, Martella V, Buonavogli C, Tantillo MG (2010) Identification of tuna species in commercial cans by minor groove binder probe real-time polymerase chain reaction analysis of mitochondrial DNA sequences. Mol Cell Probes 24:352–356
94. Chuang PS, Chen M, Shiao JCH (2012) Identification of tuna species by real-time polymerase chain reaction technique. Food Chem 133:1055–1061
95. Bojolly D, Doyen P, Le Fur B, Christaki U, Verrez-Bagnis V, Grard T (2017) Development of a qPCR method for the identification of two related tuna species, bigeye tuna (*Thunnus obesus*) and yellowfin tuna (*Thunnus albacares*) in canned tuna. J Agric Food Chem 65:913–920
96. Giusti A, Castigliego L, Rubino R, Gianfaldoni D, Guidi A, Armani A (2015) A conventional multiplex PCR assay for the detection of toxic gemfish species (*Ruvettus pretiosus* and *Lepidocybium flavobrunneum*): a simple method to combat health frauds. J Agric Food Chem 64:960–968
97. Herrero B, Vieites JM, Espiñeira M (2011) Authentication of Atlantic salmon (*Salmo salar*) using real-time PCR. Food Chem 127:1268–1272
98. Herrero B, Lago FC, Vieites JM, Espiñeira M (2011) Real-time PCR method applied to seafood products for authentication of European sole (*Solea solea*) and differentiation of common substitute species. Food Addit Contam: Part A 29:12–18
99. Hird HJ, Hold GL, Chisholm J, Reece P, Russel VJ, Brown J, Goodie R, MacArthur R (2005) Development of a method for the quantification of haddock (*Melanogrammus aeglefinus*) in commercial products using real-time PCR. Eur Food Res Technol 220:633–637

100. Hird HJ, Chisholm J, Kaye J, Colyer A, Hold GL, Conyers CM, Núñez JI, MacArthur R (2012) Development of real-time PCR assay for the detection of Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*) and European plaice (*Pleuronectes platessa*) in complex food samples. Eur Food Technol 234:127–136
101. Vadopalas B, Bouma JV, Jackels CR, Friedmann CS (2006) Application of real-time PCR for simultaneous identification and quantification of larval abalone. J Exp Mar Biol Ecol 334:219–228
102. Dias PJ, Sollelis L, Cook EJ, Piertney SB, Davie IM, Snow M (2008) Development of a real-time PCR assay for detection of *Mytilus* species specific alleles: application to a sampling survey in Scotland. J Exp Mar Biol Ecol 367:253–258
103. Sánchez A, Quinteiro J, Rey-Méndez M, Perez-Martín RI, Sotelo CG (2014) Identification and quantification of two species of oyster larvae using real-time PCR. Aquat Living Resour 27:135–145
104. Espiñeira M, Vieites JM (2012) Rapid method for controlling the correct labelling of products containing common octopus (*Octopus vulgaris*) and main substitute species (*Eledone cirrhosa* and *Dosidicus gigas*) by fast real-time PCR. Food Chem 135:2439–2444
105. Herrero B, Lago FC, Vieites JM, Espiñeira M (2012) Rapid method for controlling the correct labelling of products containing European squid (*Loligo vulgaris*) by fast real-time PCR. Eur Food Res Technol 234:77–85
106. Ye J, Feng J, Liu S, Zhang Y, Jiang X, Dai Z (2016) Identification of four squid species by quantitative real-time polymerase chain reaction. Mol Cell Probes 30:22–29
107. Swoboda I (2011) Fish allergy: new strategies for improvement of diagnosis and treatment. Allergologie 34:388–397
108. Sun M, Liang C, Gao H, Li C, Deng M (2009) Detection of parvalbumin, a common fish allergen gene in food, by real-time polymerase chain reaction. J AOAC Int 92:234–240
109. Herrero B, Vieites JM, Espiñeira M (2014) Development of an in-house fast real-time PCR method for detection of fish allergen in foods and comparison with a commercial kit. Food Chem 151:415–420
110. Tetzlaff C, Mäde D (2016) Development of a real-time PCR system for the detection of the potential allergen fish in food. Eur Food Res Technol 243:849–857
111. Herrero B, Vieites JM, Espiñeira M (2012) Fast real-time PCR for the detection of crustacean allergen in foods. J Agric Food Chem 60:1893–1897
112. Eischeid AC, Kim B, Kasko SM (2012) Two quantitative real-time PCR assays for the detection of penaeid shrimp and Blue crab, crustacean shellfish allergen. J Agric Food Chem 61:5669–5674
113. Zagon J, Schmidt J, Schmidt AS, Broll H, Lampen A, Seidler T, Braeunin A (2017) A novel screening approach based on six real-time PCR systems for the detection of crustacean species in food. Food Control 79:27–34
114. Martin I, García T, Rojas M, Pegels N, Pavón M, Hernández PE, González I, Martin R (2010) Real-time polymerase chain reaction detection of fishmeal in feedstuffs. J AOAC Int 93:1768–1777
115. Pegels N, González I, López-Calleja I, García T, Martin R (2013) Detection of fish-derived ingredients in animal feeds by a Taqman real-time PCR assay. Food Anal Met 6:1040–1048
116. Benedetto A, Abete MC, Squadrone S (2011) Towards a quantitative application of real-time PCR technique for fish DNA detection in feedstuffs. Food Chem 126:1436–1442
117. Kochzius M, Nölte M, Weber H, Silkenbeumer N, Hjörleifsdottir S, Hreggvidsson GO, Marteinsson V, Kappel K, Planes S, Tinti F, Magoulas A, Vazquez EG, Turan C, Hervet C, Falgueras DC, Antoniou A, Landi M, Blohm D (2008) DNA microarrays for identifying fishes. Mar Biotechnol 10:207–217
118. Kochzius M, Seidel C, Antoniou A, Botla SK, Campo D, Cariani A, Vazquez EG, Hauschild J, Hervet C, Hjörleifsdottir S, Hreggvidsson G, Kappel K, Landi M, Magoulas A, Marteinsson V, Nölte M, Planes S, Tinti F, Turan C, Venugopal MN, Weber H, Blohm D (2010) Identifying fishes through DNA barcodes and microarrays. PLoS One 5:e12620

119. Chitipothu S, Cariani A, Bertasi F, Stagioni M, Kochzius M, Blohm D, Tinti T, Landi M (2014) Invertebrate DNA chip: opportunities and challenges in the development and application of microarrays for marine biodiversity studies. In: Rogers JV (ed) Microarrays: principles, applications and technologies, Genetics – research and issues. Nova Science Publishers, New York
120. Teletchea F, Bernillon J, Duffraisse M, Laudet V, Hanni C (2008) Molecular identification of vertebrate species by oligonucleotide microarray in food and forensic samples. J Appl Ecol 45:967–975
121. Yoon HK, Kim GE, Jeong D, Jung JW, Chung IH, Kang S, Kim CG, Hwang SY, Lee YH (2008) Development of salmon identification DNA chip based on mitochondrial COIII-ND3-ND4L variations. Biochip J 2:287–295
122. Yoon HK, Jeong D, Chung IH, Jung JW, Oh MJ, Kim S, Lee YH, Kim CG, Hwang SY (2009) Rapid species identification of elasmobranch fish (skates and rays) using oligonucleotide microarray. Biochip J 3:87–96
123. Kim JH, Park JY, Jung JW, Kim MJ, Lee WS, An CM, Kang JH, Hwang SY (2011) Species identification of filefishes (Monacanthidae) using DNA microarray in Korean marketplace. Biochip J 5:229–235
124. Park JY, Kim JH, Kim EM, Kang JH, Kang HS, An CM, Lee WS, Hwang SY (2013) Development of a DNA chip to identify the place of origin of hairtail species. Biochip J 7:136–142
125. Park JY, Cho H, Kang JH, Kim EM, An CM, Kim JH, Lee WS, Hwang SY (2014) Development of DNA microarray for species identification of eels (Anguilliformes and Myxiniformes) in Korean fisheries markets. Biochip J 8:310–316
126. Park JY, Kim JH, An YR, Kim MJ, Lee WS, An CM, Jung JW, Kang JH, Moon HB, Hwang SY (2010) A DNA microarray for species identification of cetacean animals in Korean water. Biochip J 4:197–203
127. Handy SM, Chizhikov V, Yakes BJ, Paul SZ, Deeds JR, Mossoba MM (2014) Microarray chip development using infrared imaging for the identification of catfish species. Appl Spectrosc 68:1365–1373
128. Chisholm J, Conyers CM, Hird H (2008) Species identification in food products using the bioMerieux FoodExpert-ID (R) system. Eur Food Res Technol 228:39–45
129. Applewhite L, Rasmussen R, Morrissey M (2012) Species identification of seafood. In Granata LA, Flick GJ, Martin RE (eds.), The Seafood Industry, Wiley-Blackwell Oxford
130. Iwobi AN, Huber I, Hauner G, Miller A, Busch U (2011) Biochip technology for the detection of animal species in meat products. Food Anal Method 4:389–398
131. http://www.fei-bonn.de/gefoerderte-projekte/projektdatenbank/aif-18667-n.projekt
132. Reed GH, Wittwer CT (2004) Sensitivity and specificity of single-nucleotide polymorphism scanning by high-resolution melting analysis. Clin Chem 50:1748e1754
133. Druml B, Cichna-Markl M (2014) High resolution melting (HRM) analysis of DNA: its role and potential in food analysis. Food Chem 158:245–254
134. Madesis P, Ganopoulos I, Anagnostis A, Tsaftaris A (2012) The application of Bar- HRM (Barcode DNA-High Resolution Melting) analysis for authenticity testing and quantitative detection of bean crops (Leguminosae) without prior DNA purification. Food Control 25:576–582
135. Sakaridis I, Ganopoulos I, Argiriou A, Tsaftaris A (2013) High resolution melting analysis for quantitative detection of bovine milk in pure water buffalo mozzarella and other buffalo dairy products. Int Dairy J 28:32–35
136. Akiyama H, Nakamura F, Yamada C, Nakamura K, Nakajima O, Kawakami H, Harikai N, Furui S, Kitta K, Teshima R (2009) A screening method for the detection of the 35S promoter and the nopaline synthase terminator in genetically modified organisms in a real-time multiplex polymerase chain reaction using high-resolution melting-curve analysis. Biol Pharm Bull 32:1824–1829

137. Klomtong P, Phasuk Y, Duangjinda M (2016) Animal species identification through high resolution melting real time PCR (HRM) of the mitocondrial 16SrRNA gene. Ann Anim Sci 16:415–424
138. Fernandes TJR, Costa J, Oliveira MBPP, Mafra I (2017) DNA barcoding coupled to HRM analysis as a new and simple tool for the authentication of Gadidae fish species. Food Chem 230:49–57
139. Tomas C, Ferreira IMLPLVO, Faria MA (2017) Codfish authentication by a fast short amplicon high resolution melting analysis (SA-HRMA) method. Food Control 71:255–263
140. Jilberto F, Araneda C, Larrain MA (2017) High resolution melting analysis for identification of commercially-important Mytilus species. Food Chem 229:716–720
141. Yang S, Li C, Wu Q, Zhu C, Xu X, Zhou G (2014) High-resolution melting analysis: a promising molecular method for meat traceability. Eur Food Res Technol 239:473–480
142. Vogelstein B, Kinzler KW (1999) Digital PCR. Proc Natl Acad Sci U S A 96:9236–9241
143. Pinheiro LB, Coleman VA, Hindson CM, Herrmann J, Hindson BJ, Bhat S, Emslie KR (2012) Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification. Anal Chem 84:1003–1011
144. Sanders R, Hugget JF, Bushell CA, Cowen S, Scott DJ, Foy CA (2011) Evaluation of digital PCR for absolute DNA quantification. Anal Chem 83:6474–6484
145. Cai Y, He Y, Lu R, Chen H, Wang Q, Pan L (2017) Detection and quantification of beef and pork materials in meat products by duplex droplet digital PCR. PlosOne 12:e0181949
146. Shehata HR, Li J, Chen S, Redda H, Cheng S, Tabujara N, Li H, Warriner K, Hanner R (2017) Droplet digital polymerase chain reaction (ddPCR) assays integrated with an internal control for quantification of bovine, porcine, chicken and turkey species in food and feed. PLoS One 12:e0182872
147. Valentini A, Taberlet P, Miaud C, Civade R, Herder J, Thomsen PF, Bellemain E, Besnard A, Coissac E, Boyer F, Gaboriaud C, Jean P, Poulet N, Roset N, Copp GH, Geniez P, Pont D, Argillier C, Baudoin JM, Peroux T, Crivelli AJ, Olivier A, Acqueberge M, Le Brun M, Møller PR, Willerslev E, Dejean T (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol Ecol 25:929–942
148. Lacoursiere-Roussel A, Cote G, Leclerc V, Bernatchez L (2016) Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. J Appl Ecol 53:1148–1157
149. Bertolini F, Ghionda MC, D'Alessandro E, Geraci C, Chiofalo V, Fontanesi L (2015) A next generation semiconductor based sequencing approach for the identification of meat species in DNA mixtures. PLoS One 10:e0121701
150. Tillmar AO, Dell'Amico B, Welander J, Holmlund G (2013) A universal method for species identification of mammals utilizing next generation sequencing for the analysis of DNA mixtures. PLoS One 8:e83761
151. Muñoz-Colmenero M, Martínez JL, Roca A, Garcia-Vazquez E (2017) NGS tools for traceability in candies as high processed food products: Ion Torrent PGM versus conventional PCR-cloning. Food Chem 214:631–636
152. Cheng X, Su X, Chen X, Zhao H, Bo C, Xu J, Bai H, Ning K (2014) Biological ingredient analysis of traditional Chinese medicine preparation based on high-throughput sequencing: the story for Liuwei Dihuang wan. Sci Rep 4:5147
153. Coghlan ML, Haile J, Houston J, Murra DC, White NE, Moolhuijzen P, Bellgard ML, Bunce M (2012) Deep sequencing of plant and animal DNA contained within traditional Chinese medicines reveals legality issues and health safety concerns. PLoS Genet 8:e1002657
154. Ivanova NV, Kuzmina ML, Braukmann TWA, Borisenko AV, Zakharov EV (2016) Authentication of herbal supplements using next generation sequencing. PLoS One 11:e0156426
155. Park JY, Lee SY, An CM, Kang JH, Kim JH, Chai JC, Chen J, Kang JS, Ahn JJ, Lee YS, Hwang SY (2012) Comparative study between next generation sequencing technique and identification of microarray for species identification within blended food products. Biochip J 6:354–361

156. De Battisti C, Marciano S, Magnabosco C, Busato S, Arcangeli G, Cattoli G (2014) Pyrosequencing as a tool for rapid fish species identification and commercial fraud detection. J Agric Food Chem 62:198–205
157. Abbadi M, Marciano S, Tosi F, De Battisti C, Panzarin V, Arcangeli G, Cattoli G (2017) Species identification of bivalve molluscs by pyrosequencing. J Sci Food Agric 97:512–519
158. Kappel K, Haase I, Käppel C, Sotelo CG, Schröder U (2017) Species identification in mixed tuna samples with next-generation sequencing targeting two short cytochrome b gene fragments. Food Chem 234:212–219
159. Carvalho DC, Palhares RM, Drummond MG, Gadanho M (2017) Food metagenomics: next generation sequencing identifies species mixtures and mislabeling within highly processed cod products. Food Control 80:183–186
160. Pompanon F, Deagle BE, Symondson WOC, Brown DS, Jarman SN, Taberlet P (2012) Who is eating what: diet assessment using next generation sequencing. Mol Ecol 21:1931–1950
161. Ripp F, Krombholz CF, Liu Y, Weber M, Schäfer A, Schmidt B, Köppel R, Hankeln T (2014) All-food-Seq (AFS): a quantifiable screen for species in biological samples by deep DNA sequencing. BMC Genomics 15:639
162. Liu YC, Ripp F, Koeppel R, Schmidt H, Hellmann SL, Weber M, Krombholz CF, Schmidt B, Hankeln T (2017) AFS: identification and quantification of species composition by metagenomic sequencing. Bioinformatics 33:1396–1398
163. Lan F, Haliburton JR, Yuan A, Abate AR (2016) Droplet barcoding for massively parallel single-molecule deep sequencing. Nat Commun 7:11784
164. Tewhey R, Warner JB, Nakano M, Libby B, Medkova M, David PH, Kotsopoulos SK, Samuels ML, Hutchison JB, Larson JW, Topol EJ, Weiner MP, Harismendy O, Olson J, Link DR, Frazer KA (2009) Microdroplet-based PCR enrichment for large-scale targeted sequencing. Nat Biotechnol 27:1025–U1094
165. Clark LF (2015) The current status of DNA barcoding technology for species identification in fish value chains. Food Policy 54:85–94
166. Compton J (1991) Nucleic acid sequence-based amplification. Nature 350:91–92
167. Kievits T, Van Gemen B, Van Strijp D, Schukkink R, Dircks M, Adriaanse H, Malek L, Sooknanan R, Lens P (1991) NASBA isothermal enzymatic in vitro nucleic acid amplification optimized for the diagnosis of HIV-1 infection. J Virol Methods 35:273–286
168. Ulrich RM, John DE, Barton GW, Hendrick GS, Fries DP, Paul JH (2013) Ensuring seafood identity: grouper identification by real-time nucleic acid sequence-based amplification (RT-NASBA). Food Control 31:337–344
169. Ulrich RM, John DE, Barton GW, Hendrick GS, Fries DP, Paul JH (2015) A handheld sensor assay for the identification of grouper as a safeguard against seafood mislabeling fraud. Food Control 53:81–90
170. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28:e63–e63
171. Saull J, Duggan C, Hobbs G, Edwards T (2016) The detection of Atlantic cod (*Gadus morhua*) using loop mediated isothermal amplification in conjunction with a simplified DNA extraction process. Food Control 59:306–313
172. Ye J, Feng J, Dai Z, Meng L, Zhang Y, Jiang X (2017) Application of loop-mediated isothermal amplification (LAMP) for rapid detection of jumbo flying squid *Dosidicus gigas* (D'Orbigny, 1835). Food Anal Method 10:1452–1459
173. Piepenburg O, Williams CH, Stemple DL, Armes NA (2006) DNA detection using recombination proteins. PLoS Biol 4:e204
174. Vincent M, Xu Y, Kong H (2004) Helicase-dependent isothermal DNA amplification. EMBO Rep 5:795–800
175. Coutlée F, Bobo L, Mayur K, Yolken RH, Viscidi RP (1989) Immunodetection of DNA with biotinylated RNA probes: a study of reactivity of a monoclonal antibody to DNA-RNA hybrids. Anal Biochem 181:96–105

176. Sue MJ, Yeap SK, Omar AR, Tan SW (2014) Application of PCR-ELISA in molecular diagnosis. Biomed Res Int 2014:653014
177. Sotelo CG, Pérez-Martin RI (2003) Species identification in processed seafoods. In: Lees M (ed) Food authenticity and traceability. Woodhead Publishing, London
178. Holzhauser T, Stephan O, Vieths S (2002) Detection of potentially allergenic hazelnut (*Corylus avellana*) residues in food: a comparative study with DNA PCR-ELISA and protein sandwich-ELISA. J Agric Food Chem 50:5808–5815
179. Milne SA, Gallacher S, Cash P, Lees DN, Henshilwood K, Porter AJR (2007) A sensitive and reliable reverse transcriptase PCR-enzyme-linked immunosorbent assay for the detection of human pathogenic viruses in bivalve molluscs. J Food Protect 70:1475–1482
180. Musiani M, Gallinella G, Venturoli S, Zerbini M (2007) Competitive PCR– ELISA protocols for the quantitative and the standardized detection of viral genomes. Nat Protoc 2:2511–2519
181. Asensio L, González I, Rodríguez MA, Hernández PE, García T, Martín R (2004) PCR-ELISA for the semiquantitative detection of Nile perch (*Lates niloticus*) in sterilized fish muscle mixtures. J Agric Food Chem 52:4419–4422
182. Santaclara FJ, Velasco A, Pérez-Martín RI, Quinteiro J, Rey-Méndez M, Pardo MA, Jimenez E, Sotelo CG (2015) Development of a multiplex PCR-ELISA method for the genetic authentication of *Thunnus* species and *Katsuwonus pelamis* in food products. Food Chem 180:9–16
183. Taboada L, Sánchez A, Velasco A, Santaclara FJ, Pérez-Martín RI, Sotelo CG (2014) Identification of Atlantic cod (*Gadus morhua*), ling (*Molva molva*), and Alaska pollock (*Gadus chalcogrammus*) by PCR-ELISA using duplex PCR. J Agric Food Chem 62:5699–5706
184. Trantakis IA, Spaniolas S, Kalaitzis P, Ioannou PC, Tucker GA, Christopoulos TK (2012) Dipstick test for DNA-based food authentication: application to coffee authenticity assessment. J Agric Food Chem 60:713–717
185. Taboada L, Sánchez A, Pérez-Martín RI, Sotelo CG (2017) A new method for the rapid detection of Atlantic cod (*Gadus morhua*), Pacific cod (*Gadus macrocephalus*), Alaska pollock (*Gadus chalcogrammus*) and ling (*Molva molva*) using a lateral flow dipstick assay. Food Chem 233:182–189
186. Arunrut N, Prombun P, Saksmerprome V, Flegel TW, Kiatpathomchai W (2011) Rapid and sensitive detection of infectious hypodermal and hematopoietic necrosis virus by loop-mediated isothermal amplification combined with a lateral flow dipstick. J Virol Methods 171:21–25
187. Surasilp T, Longyant S, Rukpratanporn S, Sridulyakul P, Sithigorngul P, Chaivisuthangkura P (2011) Rapid and sensitive detection of *Vibrio vulnificus* by loop-mediated isothermal amplification combined with lateral flow dipstick targeted to rpoS gene. Mol Cell Probes 25:158–163
188. Huang X, Zhai C, You Q, Chen H (2014) Potential of cross-priming amplification and DNA-based lateral-flow strip biosensor for rapid on-site GMO screening. Anal Bioanal Chem 406:4243–4249
189. Pineiro C, Barros-Velázquez J, Vázquez J, Figueras A, Gallardo JM (2003) Proteomics as a tool for the investigation of seafood and other marine products. J Proteome Res 2:127–135
190. Marcos B, Liu J, Rai DK, Di Luca A, Mullen AM (2011) Assessment in the quality and safety of food of animal origin. In: Eckersall PD, Whitfield PD (eds) Methods in animal proteomics, 1st edn. Wiley, Chichester
191. Carrera M, Cañas B, Gallardo JM (2013) Proteomics for the assessment of quality and safety of fishery products. Food Res Int 54:972–979
192. Tedesco S, Mullen W, Cristobal S (2014) High-throughput proteomics: a new tool for quality and safety in fishery products. Curr Protein Pept Sci 15:118–133
193. Mazzeo MF, Siciliano RA (2016) Proteomics for the authentication of fish species. J Proteome 147:119–124

194. Ortea I, O'Connor G, Maquet A (2016) Review on proteomics for food authentication. J Proteome 147:212–225
195. Jagadeesh DS, Kannegundla U, Reddy RK (2017) Application of proteomic tools in food quality and safety. Adv Anim Vet Sci 5:213–225
196. Ortea I, Böhme K, Calo-Mata P, Barros-Velázquez J (2017) Molecular techniques–genomics and proteomics. In: Georgiou C (ed) Food authentication: management analysis and regulation, 1st edn. Wiley, Chichester
197. Pandey A, Mann M (2000) Proteomics to study genes and genomes. Nature 405:837–846
198. McLafferty FW, Breuker K, Jin M, Han X, Infusini G, Jiang H, Kong X, Begley TP (2007) Top-down MS, a powerful complement to the high capabilities of proteolysis proteomics. FEBS J 274(24):6256–6268
199. López JL, Marina A, Álvarez G, Vázquez J (2002) Application of proteomics for fast identification of species-specific peptides from marine species. Proteomics 2:1658–1665
200. Ortea I, Cañas B, Calo-Mata P, Barros-Velázquez J, Gallardo JM (2009) Arginine kinase peptide mass fingerprinting as a proteomic approach for species identification and taxonomic analysis of commercially relevant shrimp species. J Agric Food Chem 57:5665–5672
201. Ortea I, Canas B, Gallardo JM (2009) Mass spectrometry characterization of species-specific peptides from arginine kinase for the identification of commercially relevant shrimp species. J Proteome Res 8:5356–5362
202. Pascoal A, Ortea I, Gallardo JM, Cañas B, Barros-Velázquez J, Calo-Mata P (2012) Species identification of the northern shrimp (*Pandalus borealis*) by polymerase chain reaction–restriction fragment length polymorphism and proteomic analysis. Anal Biochem 421:56–67
203. Ortea I, Cañas B, Gallardo JM (2011) Selected tandem mass spectrometry ion monitoring for the fast identification of seafood species. J Chrom A 1218:4445–4451
204. Salla V, Murray KK (2013) Matrix-assisted laser desorption ionization mass spectrometry for identification of shrimp. Anal Chim Acta 794:55–59
205. Carrera M, Cañas B, Piñeiro C, Vázquez J, Gallardo JM (2006) Identification of commercial hake and grenadier species by proteomic analysis of the parvalbumin fraction. Proteomics 6:5278–5287
206. Carrera M, Canas B, López-Ferrer D, Pineiro C, Vázquez J, Gallardo JM (2011) Fast monitoring of species-specific peptide biomarkers using high-intensity-focused-ultrasound-assisted tryptic digestion and selected MS/MS ion monitoring. Anal Chem 83:5688–5695
207. Carrera M, Cañas B, Piñeiro C, Vázquez J, Gallardo JM (2007) De novo mass spectrometry sequencing and characterization of species-specific peptides from nucleoside diphosphate kinase B for the classification of commercial fish species belonging to the family Merlucciidae. J Proteome Res 6:3070–3080
208. Barik SK, Banerjee S, Bhattacharjee S, Gupta SKD, Mohanty S, Mohanty BP (2013) Proteomic analysis of sarcoplasmic peptides of two related fish species for food authentication. App Biochem 171:1011–1021
209. Mazzeo MF, Giulio BD, Guerriero G, Ciarcia G, Malorni A, Russo GL, Siciliano RA (2008) Fish authentication by MALDI-TOF mass spectrometry. J Agric Food Chem 56:11071–11076
210. Del Prete E, d'Esposito D, Mazzeo MF, Siciliano RA, Facchiano A (2016) Comparative analysis of MALDI-TOF mass spectrometric data in proteomics: a case study. In: Angelini C, Rancoita P, Rovetta S (eds) Computational intelligence methods for bioinformatics and biostatistics, CIBB 2015. Springer, Cham
211. Wulff T, Nielsen ME, Deelder AM, Jessen F, Palmblad M (2013) Authentication of fish products by large-scale comparison of tandem mass spectra. J Proteome Res 12:5253–5259
212. Wulff T, Jessen F, Palmblad M, Nielsen ME (2013) Tandem mass spectrometry for species recognition and phenotyping in fish. In: Rodregues P, Eckersall D, de Almeida A (eds) Farm animal proteomics 2013. Wageningen Academic Publishers, Wageningen

213. Siciliano RA, d'Esposito D, Mazzeo MF (2015) Food authentication by MALDIMS: MALDI-TOF MS analysis of fish species. In: Cramer R (ed) Advances in MALDI and laser-induced soft ionization mass spectrometry. Springer, Cham
214. Nessen MA, van der Zwaan DJ, Grevers S, Dalebout H, Staats M, Kok E, Palmblad M (2016) Authentication of closely related fish and derived fish products using tandem mass spectrometry and spectral library matching. J Agric Food Chem 64:3669–3677
215. Ulrich S, Beindorf PM, Biermaier B, Schwaiger K, Gareis M, Gottschalk C (2017) A novel approach for the determination of freshness and identity of trouts by MALDI-TOF mass spectrometry. Food Control 80:281–289
216. Stahl A, Schröder U (2017) Development of a MALDI–TOF MS-based protein fingerprint database of common food fish allowing fast and reliable identification of fraud and substitution. J Agric Food Chem 65:7519–7527
217. Spielmann G, Huber I, Maggipinto M, Haszprunar G, Busch U, Pavlovic M (2017) Comparison of five preparatory protocols for fish species identification using MALDI-TOF MS. Eur Food Res Technol (in press). https://doi.org/10.1007/s00217-017-2983-2
218. Yamashita M, Namikoshi A, Iguchi J, Takashima Y, Hossain MA, Yabu T, Yamashita Y (2008) Molecular identification of species and the geographic origin of seafood. In: Tsukamoto K, Kawamura T, Takeuchi T, Beard TD, Kaiser MJ Jr (eds) Fisheries for Global Welfare and Environment, 5th World Fisheries Congress. TerraPub, Tokyo
219. Leal MC, Pimentel P, Ricardo F, Rosa R, Calado R (2015) Seafood traceability: current needs, available tools, and biotechnological challenges for origin certification. Trends in Biotechnol 33:331–336
220. Ensing D, Crozier WW, Boylan P, O'Maoiléidigh N, McGinnity P (2013) An analysis of genetic stock identification on a small geographical scale using microsatellite markers, and its application in the management of a mixed-stock fishery for Atlantic salmon (*Salmo salar*) in Ireland. J Fish Biol 82:2080–2094
221. El Sheikha AF, Montet D (2016) How to Determine the geographical origin of seafood? Crit Rev Food Sci Nutr 56:306–317
222. Arechavala-Lopez P, Sanchez-Jerez P, Bayle-Sempere J, Fernandez-Jover D, Martinez-Rubio L, Lopez-Jimenez JA, Martinez-Lopez FJ (2010) Direct interaction between wild fish aggregations at fish farms and fisheries activity at fishing grounds: a case study with *Boops boops*. Aquaculture Res 42:996–1010
223. Tanner DK, Brazner JC, Bardy VJ (2000) Factors influencing carbon, nitrogen, and phosphorus content of fish from a Lake Superior coastal wetland. Can J Fish Aquatic Sci 57:1243–1251
224. Stowasser G, Pond DW, Collins MA (2012) Fatty acid trophic markers elucidate resource partitioning within the demersal fish community of South Georgia and Shag Rocks (Southern Ocean). Mar Biol 159:2299–2310
225. Hoelzel AR (1992) Molecular genetic analysis of populations: a practical approach. IRL Press, Cambridge
226. Teletchea F (2009) Molecular identification methods of fish species: reassessment and possible applications. Rev Fish Biol Fisheries 19:265–293
227. Boudinar AS, Chaoui L, Quignard JP, Aurelle D, Kara MH (2016) Otolith shape analysis and mitochondrial DNA markers distinguish three sand smelt species in the *Atherina boyeri* species complex in western Mediterranean. Estuar Coast Shelf Sci 182:202–210
228. Standish JD, Sheehy M, Warner RR (2008) Use of otolith natal elemental signatures as natural tags to evaluate connectivity among open-coast fish populations. Mar Ecol Prog Ser 356:259–268
229. Ricardo F, Genio L, Leal MC, Albuquerque R, Queiroga H, Rosa R, Calado R (2015) Trace element fingerprinting of cockle (*Cerastoderma edule*) shells can reveal harvesting location in adjacent areas. Sci Rep 5:11932
230. Tanner SE, Reis-Santos P, Vasconcelos RP, França S, Thorrold SR, Cabral H (2012) Otolith geochemistry discriminates among estuarine nursery areas of *Solea solea* and *S. senegalensis* over time. Mar Ecol Progr Ser 452:193–203

231. Tanner SE, Reis-Santos P, Cabral HN (2016) Otolith chemistry in stock delineation: a brief overview, current challenges and future prospects. Fish Res 173:206–213
232. Wells BK, Thorrold SR, Jones CM (2000) Geographic variation in trace element composition of juvenile weakfish scales. Trans Am Fish Soc 129:889–900
233. Danezis GP, Tsagkaris AS, Brusic V, Georgiou CA (2016) Food authentication: state of the art and prospects. Curr Opin Food Sci 10:22–31
234. Leatherhead Food Research (2015) Literature review on isotope ratios in seafood. Factsheet FS81:7–15
235. Camin F, Boner M, Bontempo L, Fauhl-Hassek C, Kelly SD, Riedl J, Rossmann A (2016) Stable isotope techniques for verifying the declared geographical origin of food in legal cases: review. Trends Food Sci Technol 61:176–187
236. Krivachy N, Rossmann A, Schmidt HL (2015) Potentials and caveats with oxygen and sulfur stable isotope analyses in authenticity and origin checks of food and food commodities. Food Control 48:143–150
237. Li L, Boyd CE, Sun Z (2016) Authentication of fishery and aquaculture products by multi-element and stable isotope analysis: review. Food Chem 194:1238–1244
238. Ashford J, Jones CM (2007) Oxygen and carbon stable isotopes in otoliths record spatial isolation of Patagonian toothfish (*Dissostichus eleginoides*). Geochim Cosmochim Acta 71:87–94
239. Whitledge G (2008) Assessment of otolith chemistry as an indicator of fish movement or transfer between the Illinois river system and lake Michigan. Reports, Paper 6
240. Matta ME, Orland IJ, Ushikubo T, Helser TE, Black BA, Valley JW (2013) Otolith oxygen isotopes measured by high-precision secondary ion mass spectrometry reflect life history of a yellowfin sole (*Limanda aspera*). Rapid Commun Mass Spectrom 27:691–699
241. Thomas CJ, Cahoon LB (1993) Stable isotope analyses differentiate between different trophic pathways supporting rocky-reef fishes. Mar Ecol Prog Ser 95:19–24
242. Olsen SA, Hansen PK, Givskud H, Ervik A, Samuelsen OB (2015) Changes in fatty acid composition and stable isotope signature of Atlantic cod (*Gadus morhua*) in response to laboratory dietary shifts. Aquaculture 435:277–285
243. Dixon HJ, Dempson JB, Power M (2015) Assessing the use of different marine growth zones of adult Atlantic salmon scales for studying marine trophic ecology with stable isotope analysis. Fish Res 164:112–119
244. Curtis JM, Stunza GW, Overath RD, Vegac RR (2014) Otolith chemistry can discriminate signatures of hatchery-reared and wild spotted seatrout. Fish Res 153:31–40
245. Moreno-Rojas JM, Tulli F, Messina M, Tibaldi E, Guillou C (2008) Stable isotope ratio analysis as a tool to discriminate between rainbow trout (*O. mykiss*) fed diets based on plant or fish-meal proteins. Rapid Commun Mass Spectrom 22:3706–3710
246. Morrison DJ, Preston T, Bron JE, Hemderson RJ, Cooper K, Strachan F, Bell JG (2007) Authenticating production origin of gilthead sea bream (*Sparus aurata*) by chemical and isotopic fingerprinting. Lipids 42:537–545
247. Wolff BA, Johnson BM, Breton AR, Martinez PJ, Winkelman DL (2012) Origins of invasive piscivores determined from the strontium isotope ratio (87Sr/86Sr) of otoliths. Can J Fish Aquatic Sci 69:724–739
248. Carrera M, Gallardo JM (2017) Determination of the geographical origin of all commercial hake species by stable isotope ratio (SIR) analysis. J Agric Food Chem 65(5):1070–1077
249. Li L, Boyd C, Sun Z (2016) AuAuthentication of fishery and aquaculture products by multi-element and stable isotope analysis. Food Chem 194:1238–1244
250. Kim H, Kumar KS, Hwang SY, Kang BC, Moon HB, Shin KNH (2015) Utility of stable isotope and cytochrome oxidase I gene sequencing analyses in inferring origin and authentication of hairtail fish and shrimp. J Agric Food Chem 63:5548–5556
251. Chaguri MP, Maulvault AL, Costa S, Gonçalves A, Nunes ML, Carvahlo ML, Sant'Ana LS, Bandarra N, Marques A (2017) Chemometrics tools to distinguish wild and farmed meagre (*Argyrosomus regius*). J Food Process Pres 41:e13312

252. Ménard F, Lorrain A, Potier M, Marsac F (2007) Isotopic evidence of distinct feeding ecologies and movement patterns in two migratory predators (yellowfin tuna and swordfish) of the western Indian Ocean. Mar Biol 153(2):141–152
253. Carter JF, Tinggi T, Yang X, Fry B (2015) Stable isotope and trace metal compositions of Australian prawns as a guide to authenticity and wholesomeness. Food Chem 170:241–248
254. Ellegren H (2004) Microsatellites: simple sequences with complex evolution. Nature Reviews 5:435–445
255. Chistiakov DA, Hellemans B, Volckaert FAM (2006) Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. Aquaculture 255:1–29
256. Lane H, Symonds JE, Ritchie PA (2016) The phylogeography and population genetics of *Polyprion oxygeneios* based on mitochondrial DNA sequences and microsatellite DNA markers. Res 174:19–29
257. Sanchez G, Tomano S, Yamashiro C, Fujita R, Wakabayashi T, Sakai M, Umino S (2016) Population genetics of the jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae) in the northern Humboldt current system based on mitochondrial and microsatellite DNA markers. Fish Res 175:1–9
258. Cabranes C, Fernandez-Rueda P, Martínez JL (2008) Genetic structure of *Octopus vulgaris* around the Iberian Peninsula and Canary Islands as indicated by microsatellite DNA variation. J Marine Sci 65:12–16
259. De Luca D, Catanese G, Procaccini G, Fiorito G (2016) *Octopus vulgaris* (Cuvier, 1797) in the Mediterranean sea: genetic diversity and population structure. PLoS One 11:e0149496
260. Andre C, Larsson LC, Laikre L, Bekkevold D, Brigham J, Carvalho GR, Dahlgren TG, Hutchinson WF, Mariani S, Mudde K, Ruzzante DE, Ryman N (2011) Detecting population structure in a high gene-flow species, Atlantic herring (*Clupea harengus*): direct, simultaneous evaluation of neutral vs putatively selected loci. Heredity 106:270–280
261. Wang L, Liu S, Zhuang Z, Guo L, Meng Z, Lin H (2013) Population genetic studies revealed local adaptation in a high gene-flow marine fish, the small yellow croaker (*Larimichthys polyactis*). PLoS One 8:e83493
262. Zhigileva ON, Baranova OG, Pozhidaev VV, Brol IS, Moiseenko TI (2013) Comparative analysis of using isozyme and Issr-Pcr markers for population differentiation of Cyprinid Fish. Turk J Fish Aquat Sc 13:159–168
263. Le Nguyen DD, Ngoc HH, Dijoux D, Loiseau G, Montet D (2008) Determination of fish origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: an application on *Pangasius* fish from Viet Nam. Food Control 19:454–460
264. Montet D, Le Nguyen DD, El Sheikha AF, Condur A, Métayer I, Loiseau G (2008) Application of PCR-DGGE in determining food origin: cases studies of fish and fruits. Asp Appl Biol 87:11–22
265. Cuéllar-Pinzón J, Presa P, Hawkins SJ, Pita A (2016) Genetic markers in marine fisheries: types, tasks and trends. Fish Res 173:194–205
266. Davey JW, Blaxter ML (2010) RADSeq: next-generation population genetics. Brief Funct Genomics 9:416–423
267. Martinsohn JT, Ogden R, FishPopTrace Consortium (2009) Developing SNP-based population genetic assignment methods to investigate illegal fishing. Forensic Sci Int Genet Suppl Ser 2:294–296
268. Milano I, Babbucci M, Panitz F, Ogden R, Nielsen RO, Taylor MI, Heylar SJ, Carvalho GR, Espiñeira M, Atanassova M, Tinti F, Maes GE, Patarnello T, FishPopTrace Consortium, Bargelloni L (2011) Novel tools for conservation genomics: comparing two high-throughput approaches for SNP discovery in the transcriptome of the European hake. PLoS One 6(11): e28008
269. Nielsen EE, Cariani A, Mac Aoidh E, Maes GE, Milano I, Ogden R, Taylor M, Hemmer-Hansen J, Babbucci M, Bargelloni L, Bekkevold D, Diopere E, Grenfell L, Helyar S, Limborg MT, Martinsohn JT, McEwing R, Panitz F, Patarnello T, Tinti F, Van Houdt JKJ,

Volckaert FAM, Waples RS, Albin JEJ, Vieites JMB, Barmintsev V, Bautista JM, Bendixen C, Bergé JP, Blohm D, Cardazzo B, Diez A, Espiñeira M, Geffen AJ, Gonzalez E, González-Lavín N, Guarniero I, Jerôme M, Kochzius M, Krey G, Mouchel O, Negrisolo E, Piccinetti C, Puyet A, Rastorguev S, Smith JP, Trentini M, Verrez-Bagnis V, Volkov A, Zanzi A, Carvalho GR (2012) Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. Nat Commun 3:851

270. Arechavala-Lopez P, Fernandez-Jover D, Black KD, Ladoukakis E, Bayle-Sempere JT, Sanchez-Jerez P, Dempster T (2013) Differentiating the wild or farmed origin of Mediterranean fish: a review of tools for sea bream and sea bass. Rev Aquacult 5:137–157
271. Bylemans J, Maes GE, Diopere E, Cariani A, Senn H, Taylor MI, Helyar S, Bargelloni L, Bonaldo A, Carvalho G, Guarniero I, Komen H, Martinsohn JT, Nielsen EE, Tinti F, Volckaert FAM, Ogden R (2016) Evaluating genetic traceability methods for captive-bred marine fish and their applications in fisheries management and wildlife forensics. Aquacult Environ Interact 8:131–145
272. Burch R (2015) Literature review: isotope ratios in seafood. Leatherhead Food Research. FS81_7_15 Isotope ratios in seafood. http://www.seafish.org/media/Publications/FS81_7_15_Isotope_ratios_in_seafood.pdf. Accessed June 2017
273. Kim H, Kumar KS, Shin KY (2015) Applicability of stable C and N isotope analysis in inferring the geographical origin and authentication of commercial fish (mackerel, yellow croaker and pollock). Food Chem 172:523–527
274. Chaguri MP, Maulvault AL, Nunes ML, Santiago DA, Denadai JC, Fogaca FH, Sant'Ana LS, Ducatti C, Bandarra N, Carvalho ML, Marques A (2015) Different tools to trace geographic origin and seasonality of croaker (*Micropogonias furnieri*). LWT – Food Sci Technol 61:194–200
275. Kelly S, Heaton K, Hoogewerff J (2005) Tracing the geographical origin of food: the application of multi-element and multi-isotope analysis. Trends Food Sci Tech 16(12):555–567
276. Bell JG, Preston T, Henderson RJ, Strachan F, Bron JE, Cooper K, Douglas JM (2007) Discrimination of wild and cultured European sea bass (*Dicentrarchus labrax*) using chemical and isotopic analyses. J Agric Food Chem 55:5934–5941
277. Fasolato L, Novelli E, Salmaso L, Corain L, Camin F, Perini M, Antonetti P, Balzan S (2010) Application of nonparametric multivariate analyses to the authentication of wild and farmed European sea bass (*Dicentrarchus labrax*). Results of a survey on fish sampled in the retail trade. J Agric Food Chem 58:10979–10988
278. Moreno-Rojas JM, Serra F, Giani I, Moretti VM, Reniero F, Guillou C (2007) The use of stable isotope ratio analyses to discriminate wild and farmed gilthead sea bream (*Sparus aurata*). Rapid Commun Mass Sp 21:207–211
279. Serrano R, Blanes MA, Orero L (2007) Stable isotope determination in wild and farmed gilthead sea bream *(Sparus aurata*) tissues from the western Mediterranean. Chemosphere 69:1075–1080
280. Molkentin J, Lehmann I, Ostermeyer U, Rehbein H (2015) Traceability of organic fish – authenticating the production origin of salmonids by chemical and isotopic analysis. Food Control 53:55–66
281. Gamboa-Delgado J, Molina-Poveda C, Godinez-Siordia DE, Villareal-Cavazos D, Ricque-Marie D, Cruz-Suarez LE (2014) Application of stable isotope analysis to differentiate shrimp extracted by industrial fishing or produced through aquaculture practices. Can J Fish Aquat Sci 71:1520–1528
282. Molkentin J, Meisel H, Lehmann I, Rehbein H (2007) Identification of organically farmed Atlantic salmon by analysis of stable isotopes and fatty acids. Eur Food Res Technol 224:535–543
283. Thomas F, Jamin E, Wietzerbin K, Guérin R, Lees M, Morvan E, Billault I, Derrien S, Moreno Rojas JM, Serra F, Guillou C, Aursand M, McEvoy L, Prael A, Robins RJ (2008) Determination of origin of Atlantic salmon (*Salmo salar*): the use of mulitprobe and multielement

isotopic analysis in combination with fatty acid composition to assess wild or farmed origin. J Agric Food Chem 56:989–997
284. Mnari A, Boundel I, Chraief I, Hammami M, Romdhane MS, El Cafsi M, Chaouch A (2007) Fatty acids in muscle and liver of Tunisian wild and farmed gilthead sea bream, *Sparus aurata*. Food Chem 100:1393–1397
285. Periago MJ, Ayala MD, López-Albors O, Abdel I, Martínez C, Garcia-Alcázar A, Ros G, Gil F (2005) Muscle cellularity and flesh quality of wild and farmed sea bass, *Dicentrarchus labrax* L. Aquaculture 249:175–188
286. Šimat V, Bogdanović T, Krželj M, Soldo A, Maršić-Lučić J (2012) Differences in chemical, physical and sensory properties during shelf life assessment of wild and farmed gilthead sea bream (*Sparus aurata*, L.) J Applied Ichthyol 28:95–101
287. Attouchi M, Sadok S (2012) The effects of essential oils addition on the quality of wild and farmed sea bream (Sparus Aurata) stored in ice. Food Bioprocess Tech 5:1803–1816
288. Berge GM, Witten PE, Baeverfjord G, Vegusdal A, Wadsworth S, Ruyter B (2009) Diets with different n-6/n-3 fatty acid ratio in diets for juvenile Atlantic salmon, effects on growth, body composition, bone development and eicosanoid production. Aquaculture 296:299–308. https://doi.org/10.1016/j.aquaculture.2009.08.029
289. Strobel C, Jahreis G, Kuhnt K (2012) Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products. Lipids Health Dis 11:144
290. Yildiz M, Sener E, Timur M (2008) Effects of differences in diet and seasonal changes on the fatty acid composition in fillets from farmed and wild sea bream (*Sparus aurata* L.) and sea bass (*Dicentrarchus labrax* L.) Int J Food Sci Tech 43:853–858
291. Dubois V, Breton S, Linder M, Fanni J, Parmentier M (2003) Fatty acid profiles of vegetable oils with regard to their nutritional potential. European J Lipid Sci Tech 109:710–732
292. Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci 11:107–184
293. Ferreira M, Caetano M, Antunes P, Costa J, Gil O, Bandarra N, Pousão-Ferreira P, Vale C, Reis-Henrique MA (2010) Assessment of contaminants and biomarkers of exposure in wild and farmed sea bass. Ecotox Environ Safe 73:579–588
294. Sharma P, Kumar V, Sinha AK, Ranjan J, Kithsiri HM, Venkateshwarlu G (2010) Comparative fatty acid profiles of wild and farmed tropical freshwater fish rohu (*Labeo rohita*). Fish Physiol Biochem 36:411–417
295. Lenas DS, Triantafillou DJ, Chatziantoniou S, Nathanailides C (2011) Fatty acid profile of wild and farmed gilthead sea bream (*Sparus aurata*). J Verbrauch Lebensm 6:435–440
296. Ottavian M, Facco P, Fasolato L, Novelli E, Mirisola M, Perini M, Barolo M (2012) Use of near-infrared spectroscopy for fast fraud detection in seafood: application to the authentication of wild European sea bass (*Dicentrarchus labrax*). J Agr Food Sci 60:639–648
297. Fuentes A, Fernández-Segovia I, Serra JA, Barat JM (2010) Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. Food Chem 119:1514–1518
298. Mnari A, Bouhlel I, Chouba L, Hammami M, El Cafsi M, Chaouch A (2010) Total lipid content, fatty acid and mineral compositions of muscles and liver in wild and farmed sea bass (*Dicentrarchus labrax*). Afr J Food Sc 4:522–530
299. Turchini GM, Torstensen BE, Ng WG (2009) Fish oil replacement in finfish nutrition. Rev Aquacult 1:10–57
300. Asdari R, Aliyu-Paiko M, Hashim R, Ramachandran S (2011) Effects of different dietary lipid sources in the diet for *Pangasius hypophthalmus* (Sauvage, 1878) juvenile on growth performance, nutrient utilization, body indices and muscle and liver fatty acid composition. Aquac Nutr 17:44–53
301. Petenuci ME, Rocha INA, de Sousa SC, Schneider VV, Alves da Costa LA, Visentainer JV (2016) Seasonal variations in lipid content, fatty acid composition and nutritional profiles of five freshwater fish from the Amazon basin. J Am Oil Chem Soc 93:1373

302. Khitouni IK, Mihoubi NB, Bouain A, Rebah FB (2014) Seasonal variation of the chemical composition, fatty acid profiles and mineral elements of *Diplodus annularis* (Linnaeus, 1758) caught in the Tunisian coastal water. J Food Nutr Res 2:306–311
303. Grigorakis K, Alexis MN, Taylor KDA, Hole M (2002) Comparison of wild and cultured gilthead sea bream; composition, appearance and seasonal alterations. Int J Food Sci Tech 37:477–484
304. Busetto ML, Moretti VM, Moreno-Rojas JM, Caprino F, Giani I, Malandra R, Bellagamba F, Guillou CJ (2008) Authentication of farmed and wild turbot (*Psetta maxima*) by fatty acid and isotopic analyses combined with chemometrics. Agric Food Chem 56:2742–2750
305. Fernandez-Jover D, Martinez-Rubio L, Sanchez-Jerez P, Bayle-Sempere JT, Lopez-Jimenez JA, Martínez-Lopez FJ, Pål-Arne B, Uglem I, Dempster T (2011) Waste feed from coastal fish farms: a trophic subsidy with compositional side-effects for wild gadoids. Estuar Coast Shelf S 91:568–559
306. Fernandez-Jover D, Lopez-Jimenez JA, Sanchez-Jerez P, Bayle-Sempere J, Gimenez-Casalduero F, Martínez-Lopez FJ, Dempster T (2007) Changes in body condition and fatty acid composition of wild Mediterranean horse mackerel (*Trachurus mediterraneus*, Steindachner, 1868) associated with sea cage fish farms. Mar Environ Res 63:1–18
307. Aursand M, Standal IB, Praël A, McEvoy L, Irvine J, Axelson DE (2009) ^{13}C NMR pattern recognition techniques for the classification of Atlantic salmon (*Salmo salar* L.) according to their wild, farmed, and geographical origin. J Agric Food Chem 57(9):3444–3451
308. Megdal PA, Craft NA, Handelman GJ (2009) A simplified method to distinguish farmed (*Salmo salar*) from wild salmon: fatty acid ratios versus astaxanthin chiral isomers. Lipids 44(6):569–576
309. Mannina L, Sobolev AP, Capitani D, Iaffaldano N, Rosato MP, Ragni P, Reale A, Sorrentino E, D'Amico I, Coppola R (2008) NMR metabolic profiling of organic and aqueous sea bass extracts: implications in the discrimination of wild and cultured sea bass. Talanta 77:433–444
310. Del Coco L, Papadia P, De Pascali SA, Bressani G, Storelli C, Zonno V, Fanizzi FP (2009) Comparison among different gilthead sea bream (*Sparus aurata*) farming systems: activity of intestinal and hepatic enzymes and ^{13}C-NMR analysis of lipids. Forum Nutr 1:291–301
311. Costa R, Albergamo A, Piparo M, Zaccone G, Capillo G, Manganaro A, Dugo P, Mondello L (2017) Multidimensional gas chromatographic techniques applied to the analysis of lipids from wild-caught and farmed marine species. Eur J Lipid Sci Technol 119:1600043
312. Martinez I, Stendhal I, Aursand M, Yamashita Y, Yamashita M (2009) Analytical methods to differentiate farmed from wild seafood. In: Nollet LML, Toldra F (eds) Handbook of seafood and seafood products analysis, 1st edn. CRC Press/Taylor and Francis Group, Boca Raton
313. Gram L, Huss HH (1996) Microbiological spoilage of fish and fish products. Int J Food Microbiol 33:121–137
314. Matsumoto J (1979) Denaturation of fish muscle proteins during frozen storage. In: Fennema O (ed) Proteins at low temperatures, Advances in chemistry series. American Chemical Society, Washington, DC
315. Uddin M (2009) Differentiation of fresh and frozen-thawed fish. In: Nollet LML, Toldra F (eds) Handbook of seafood and seafood products analysis. CRC Press/Taylor and Francis Group, Boca Raton
316. Pavlov A, Garcia de Fernando GD, Diaz O, Fernandez M, Lopez D, Ordonez JA, Hoz L (1994) Effect of freezing on the b-hydroxyl-CoA-dehydrogenase (HADH) activity of fish meat. Z Lebensm Unters For 198:465–468
317. Fernandez M, Mano S, Garcia de Fernando GD, Ordonez JÁ, Hoz L (1999) Use of b-hydroxyacyl-CoA-dehydrogenase (HADH) activity to differentiate frozen from unfrozen fish and shellfish. Eur Food Res Technol 209:205–208
318. Salfi V, Fucetola F, Pannunzio G (1985) A micromethod for the differentiation of fresh from frozen fish muscle. J Sci Food Agric 36:811
319. Nambudiri DD, Gopakumar K (1990) Effect of freezing and thawing on press juices enzyme activity in the muscle of farmed fish and shellfish. IIF–IIR, commission C2. Aberdeen 3:229–233

320. Karvinen VP, Bamford DH, Granroth B (1982) Changes in muscle subcellular fraction of Baltic herring (Clupea Harengus Membras). J Sci Food Agric 33:763–772
321. Barbagli C, Crescenzy GS (1981) Influence of freezing and thawing on the release of cytochrome oxidase from chicken's liver and from beef and trout muscle. J Food Sci 46:491–496
322. Chhatbar SK, Velankar NK (1977) A biochemical test for the distinction of fresh fish from frozen and thawed fish. Fish Technnol 14:131–133
323. Rehbein H (1979) Development of an enzymatic method to differentiate fresh and sea frozen and thawed fish fillets. Z Lebensm Unters Fors 169:263–265
324. Frigerio R, Ardemagni A, Cantoni C (1980) Variazoni quantitative della succino-deidrogenasi durante la lavorazione di molluschi. Arch Vet Ital 31:162–166
325. Salfi V, Fucetola F, Verticelli V, Arata P (1986) Optimized procedures of biochemical analysis for the differentiation between fresh and frozen thawed fish products. Test of mitochondrial malate dehydrogenase. Ind Alim 25:634
326. Gould E (1971) An objective test for determining whether fresh fish have been frozen and thawed. In: Kreuzer R (ed) Fish inspection quality control. Fishing News (Books) Ltd, London
327. Kitamikado M, Yuan CS, Ueno R (1990) An enzymatic method designed to differentiate between fresh and frozen–thawed fish. J Food Sci 55:74–76
328. Yuan CS, Yoshioka K, Ueno R (1988) Differentiation of frozen–thawed fish from unfrozen fish by determination of neutral b-N-acetylglucosaminidase activity in the blood. Bull Jpn Soc Sci Fish 54:2143–2148
329. Rehbein H (1992) Physical and biochemical methods for the differentiation between fresh and frozen–thawed fish or fillets. Ital J Food Sci 2:75–86
330. Nilsson K, Ekstrand B (1993) The effect of storage on ice and various freezing treatments on enzyme leakage in muscle tissue of rainbow trout. Z Lebensm Unters For 197:3–7
331. Rehbein H, Cakli S (2000) The lysosomal enzyme activities of fresh, cooled, frozen and smoked salmon fish (*Onchorhyncus keta* and *Salmo salar*). Turk J Vet Anim Sci 24:103–108
332. Erickson MC (2012) Chemical measurements. In: Sun D-W (ed) Handbook of frozen food processing and packaging. CRC Press/Taylor & Francis Group, Boca Raton, pp 563–586
333. Okazaki E, Yamashita Y, Uddin M (2006) Classification of fresh and frozen-thawed fish–a review. Refrigeration 81:175–181
334. Love RM (1956) Post-mortem changes in the lenses of fish eyes. II. Effects of freezing, and their usefulness in determining the past history of the fish. J Sci Food Agric 7:220–226
335. Duflos G, Le Fur B, Mulak V, Becel P, Malle P (2002) Comparison of methods of differentiating between fresh and frozen–thawed fish or fillets. J Sci Food Agric 82:1341–1345
336. Yoshioka K, Kitamikado M (1983) Differentiation of freeze–thawed fish from fresh fish by the examination of medulla of crystalline lens. Bull Jpn Soc Sci Fish 49:151–154
337. Yoshioka K (1983) Differentiation of freeze-thawed fish from fresh fish by the determination of hematocrit value. Bull Jpn Soc Sci Fish 49:149–151
338. Yoshioka K, Kitamikado M (1988) Differentiation of freeze-thawed fish fillet from fresh fish fillet by the examination of erythrocyte. Nippon Suisan Gakk 54:1221–1225
339. Karoui R, Thomas E, Dufour E (2006) Utilisation of a rapid technique based on front-face fluorescence spectroscopy for differentiating between fresh and frozen–thawed fish fillets. Food Res Int 39(3):349–355
340. Karoui R, Hassoun A, Ethuin P (2017) Front face fluorescence spectroscopy enables rapid differentiation of fresh and frozen-thawed sea bass (*Dicentrarchus labrax*) fillets. J Food Eng 202:89–98
341. Yawen G, Haiqing T, Changrong O, Yamin L, Caiye W, Jinxuan C (2016) Differentiation between fresh and frozen-thawed large yellow croaker based on front-face fluorescence spectroscopy technique. Trans Chin Soc Agric Eng 32:279–285
342. Kim JB, Murata M, Sagakuchi M (1987) A method for the differentiation of frozen–thawed from unfrozen fish fillets by a combination of torrymeter readings and K values. Nippon Suisan Gakk 53:159–164

343. Oehlenschläger J (2003) Measurement of freshness of fish based on electrical properties. In: Luten JB, Oehlenschläger J, Olafsdottir G (eds) Quality of fish from catch to consumer: labelling, monitoring and traceability. Wageningen Academic Publishers, Wageningen
344. Kent M, Oehlenschläger J (2009) Measuring electrical properties. In: Rehbein H, Oehlenschläger J (eds) Fishery products: quality, safety and authenticity. Wiley-Blackwell, Oxford, UK
345. Sakaguchi M, Murata M, Kim JB (1989) The effects of repeated freeze-thaw cycle on torrymeter readings of carp fillets. Nippon Suisan Gakk 55:1665–1669
346. Zhang L, Shen H, Luo Y (2010) Study on the electric conduction properties of fresh and frozen–thawed grass carp (*Ctenopharyngodon idellus*) and tilapia (*Oreochromis niloticus*). Int J Food Sci Tech 45:2560–2564
347. Vidaček S, Medića H, Botka-Petrakb K, Nežakc J, Petrak T (2008) Bioelectrical impedance analysis of frozen sea bass (*Dicentrarchus labrax*). J Food Eng 88:263–271
348. Fernández-Segovia I, Fuentes A, Aliño M, Masot R, Alcañiz M, Barat JM (2012) Detection of frozen–thawed salmon (*Salmo salar*) by a rapid low-cost method. J Food Eng 113:210–216
349. Charpentier J, Goutefongea R, Salé P, Thomasset A (1972) La discrimination des viandes fraiches et congelées par mesures d'impédance à deux fréquences. Ann Biol, Biochim Biophys 12:173–178
350. Fuentes A, Masot R, Fernández-Segovia I, Ruiz-Rico M, Alcañiz M, Barat JM (2013) Differentiation between fresh and frozen–thawed sea bream (*Sparus aurata*) using impedance spectroscopy techniques. Innov Food Sci Emerg 19:210–217
351. Kent M, Oehlenschlager J, Mierke-Klemeyer S, Knöchel R, Daschner F, Schimmer O (2004) Estimation of the quality of frozen cod using a new instrumental method. Eur Food Res Tech 219:540–544
352. Mendes R, Schimmer O, Vieira H, Pereira J, Teixeira B (2017) Control of abusive water addition to *Octopus vulgaris* with non-destructive methods. J Sci Food Agric (in press). 98:369–376
353. Aursand M, Veliyulin E, Standal IB, Falch E, Aursand IG, Erikson U (2009) Nuclear magnetic resonance. In: Rehbein H, Oehlenschläger J (eds) Fishery products: quality, safety and authenticity. Wiley-Blackwell, Oxford, UK
354. Nott KP, Evans SD, Hall LD (1999) The effect of freeze-thawing on the magnetic resonance imaging parameters of cod and mackerel. LWT - Food Sci Technol 32(5):261–268
355. Howell N, Shavila Y, Grootveld M, Williams S (1996) High resolution NMR and MRI studies on fresh and frozen cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). J Sci Food Agric 72:49–56
356. Nott KP, Evans SD, Hall LD (1999) Quantitative magnetic resonance imaging of fresh and frozen–thawed trout. Magn Reson Imag 17:445–455
357. Foucat L, Taylor RG, Labas R, Renou JP (2001) Characterization of frozen fish by NMR imaging and histology. Am Lab 33:38–43
358. Aursand IG, Veliyulin E, Böcker U, Ofstad R, Rustad T, Erikson U (2009) Water and salt distribution in Atlantic salmon (*Salmo salar*) studied by low-field ^{1}H NMR, ^{1}H and ^{23}Na MRI and light microscopy: effects of raw material quality and brine salting. Agric Food Chem 57(1):46–54
359. Veliyulin E, Borge A, Singstad T, Gribbestad I, Erikson U (2006) Post-mortem studies of fish using magnetic resonance imaging. In: Webb GA (ed) Modern magnetic resonance. Springer, The Netherlands
360. Leduc F, Krzewinski F, Le Fur B, N'Guessan A, Malle P, Kol O, Duflos G (2012) Differentiation of fresh and frozen/thawed fish, European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), cod (*Gadus morhua*) and salmon (*Salmo salar*), using volatile compounds by SPME/GC/MS. J Sci Food Agric 92:2560–2568
361. Ottavian M, Fasolato L, Facco P, Barolo M (2013) Foodstuff authentication from spectral data: toward a species-independent discrimination between fresh and frozen–thawed fish samples. J Food Eng 119:765–775

362. Ottavian M, Fasolato L, Serva L, Facco P, Barolo M (2014) Data fusion for food authentication: fresh/frozen–thawed discrimination in west African goatfish (*Pesudupeneus prayensis*) fillets. Food Bioprocess Technol 7:1025–1036
363. Sivertsen AH, Kimiya T, Heia K (2011) Automatic freshness assessment of cod (*Gadus morhua*) fillets by VIS/NIR spectroscopy. J Food Eng 103:317–323
364. Fasolato L, Balzan S, Riovanto R, Berzaghi P, Mirisola M, Ferlito JC, Serva L, Benozzo F, Passera R, Tepedino V, Novelli E (2012) Comparison of visible and near-infrared reflectance spectroscopy to authenticate fresh and frozen–thawed swordfish (*Xiphias gladius* L). J Aquat Food Prod T 21:493–507
365. Zhu F, Zhang D, He Y, Liu F, Sun DW (2012) Application of visible and near infrared hyperspectral imaging to differentiate between fresh and frozen–thawed fish fillets. Food Bioprocess Tech 6:2931–2937
366. Kimiya T, Sivertsen AH, Heia K (2013) VIS/NIR spectroscopy for non-destructive freshness assessment of Atlantic salmon (*Salmo salar* L) fillets. J Food Eng 116:758–764
367. Uddin M, Okazaki E (2004) Classification of fresh and frozen-thawed fish by near-infrared spectroscopy. J Food Sci 69:665–668
368. Uddin M, Okazaki E, Turza S, Yumiko Y, Tanaka M, Fukuda Y (2005) Non-destructive visible/NIR spectroscopy for differentiation of fresh and frozen-thawed fish. J Food Sci 70: c506–c510
369. Zhang A, Cheng F (2013) Identification of fresh shrimp and frozen-thawed shrimp by Vis/NIR spectroscopy. 2nd International Conference on Nutrition and Food Sciences IPCBEE, vol 53. IACSIT Press, Singapore
370. Weeranantanaphan J, Downey G, Allen P, Sun DW (2011) A review of near infrared spectroscopy in muscle food analysis: 2005–2010. J Near Infrared Spec 19:61–104
371. Sone I, Olsen RL, Sivertsen AH, Eilertsen G, Heia K (2012) Classification of fresh Atlantic salmon (*Salmo salar* L.) fillets stored under different atmospheres by hyperspectral imaging. J Food Eng 109:482–489
372. Chaijan M, Benjakul S, Visessanguan W, Faustman C (2006) Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. Food Chem 99(1):83–91
373. Velioğlua HM, Temizb HT, Boyacib IH (2015) Differentiation of fresh and frozen-thawed fish samples using Raman spectroscopy coupled with chemometric analysis. Food Chem 172:283–290
374. Grunert T, Stephan R, Ehling-Schulz M, Johler S (2017) Rapid differentiation of fresh and thawed meat or fish by FTIR spectroscopy. Technology opportunity, ref. no. UZ-17/359. http://www.switt.ch/adminall2/userfiles/technologien/608_top_uz17359_johler.pdf accessed June 2017

Some Statistical Considerations Regarding the Occurrence and Analysis of Bioactive Materials in Foods

71

Basil Jarvis

Contents

Abstract

Bioactive materials (BMs) include a diversity of reactive chemicals that occur in foods and feeds. Some are natural constituents of specific foods, while others may be developed as a consequence of processing or microbial growth, as environmental contaminants, or as additives and adulterants. Many BMs have potential health-giving benefits, but those that are potentially harmful to the consumer are

B. Jarvis (✉)
Daubies Farm, Ross-on-Wye, UK

Department of Food and Nutritional Sciences, School of Chemistry, Food and Pharmacy, The University, Reading, UK
e-mail: basil.jarvis@btconnect.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_77

of equal, or possibly, greater importance. Examples of BMs that occur in foods are discussed briefly and some examples of the statistical methods used in planning and/or analysis of experimental work are described. Of critical importance is the manner in which the distribution of specific compounds within a food material may impact the outputs of statistical procedures.

Keywords

Adulterants · Analytical methods · ANOVA · Bioactive materials · Chemometrics · Clinical trials · Cluster analysis · Latin Square designs · Microbial toxins · Principle components analysis · Measurement uncertainty · Residues · Sampling uncertainty · Statistical distributions · Statistical methods · Statistical planning · Validation · Verification

List of Abbreviations

ANOVA	Analysis of variance
BMs	Bioactive materials
KW	Kruskal-Wallis
NBD	Negative binomial distribution
ND	Normal distribution
PCA	Principle components analysis
SAP	Sampling and analytical plan

1 Introduction

Bioactive materials (BMs) have been defined as "... extra-nutritional constituents that typically occur in small quantities in foods." Many such materials are being intensively studied to evaluate their effects on health [1] since BMs that occur in both plant and animal products have pharmacological effects on living organisms. The range of beneficial BMs include fatty acids, flavonoids, caffeine, carnitine, choline, polysaccharides, polyphenols, and anthocyanins. In-depth study of such compounds has shown that many are able to promote good health by acting as antitumor, immunological, anti-inflammatory, hypoglycemic, or antiviral agents. One consequence is that BMs may play a vital role in the development of new drugs. However, it should not be overlooked that while a compound may be beneficial at low concentration, it may also be harmful at higher concentrations. Paracelsus (1493–1541) noted, "Everything is poison; it is just the concentration that will decide if something is nontoxic."

But this is only one aspect of the occurrence and role of BMs in relation to health and well-being [2]. Although many raw foods, especially plant materials, contain a diversity of BMs that have a potential beneficial effect on health [3], many foods are not eaten raw – most are cooked or are processed commercially using thermal and other process conditions that affect the composition of the food. Such processes may reduce, or increase, the nature and the availability of BMs. Many processed foods also contain food additives that have bioactive effects – although such materials may

not be harmful (to some a matter of debate), deliberate adulteration of foods can result in the addition of harmful substances. The growth of microorganisms in the field or during storage of raw and processed food materials can also result in the formation of harmful materials, including bacterial toxins, such as staphylococcal enterotoxin and botulinum toxin. But possibly the most important microbial products are mycotoxins, such as aflatoxin and ochratoxin, which contaminate a wide range of food commodities. Finally, it is important not to overlook the potential impact of food contaminants such as agrochemical residues at levels in excess of those permitted by legislation in most countries. Examples of some beneficial and harmful BMs are shown in Tables 1 and 2.

Table 1 Some beneficial bioactive materials that occur in foods

Chemical group	Examples	Source
Glycosides	Glucosinolates Anthraquinone glycosides	*Brassica* spp.
		Rhubarb
Flavonoids and proanthocyanidins	Phytoestrogens	Fruits, nuts, beans, cereals
Terpenoids	Diterpenoids	Coffee beans
Lignans	Phytoestrogens	Oil seeds
Alkaloids	Methylxanthines	Coffee and cocoa beans
Proteins and peptides	Lectins[a]	Most foods including beans and cereals

[a]See also Table 2

Table 2 Some harmful bioactive materials that occur in foods

Group	Examples	Foods
Plant toxins	Ricin and other lectins	Castor beans
	Furocoumarins	Citrus peel and oils
	Muscarine; psilocybin	Wild mushrooms (toadstools)
Microbial toxins	*Staphylococcus* enterotoxin	Cheese, cured meats
	Botulinum toxin	Cured meats and sausage
	Mycotoxins, e.g., aflatoxins, ochratoxins	Cereals, nuts, milk
Agrochemicals	Pesticides, e.g., chlorpyrifos, captan	Plant foods
	Herbicides, e.g., glyphosate	
Food adulterants	Melamine, urea, formalin, arsenic	Dried and fresh milk
	Diethylene glycol	Wine
	"Denatured" rapeseed oil containing derivatives of OOPAP[a]	Olive oil
	Metanilic acid	Spices, e.g., turmeric
	Methylmercury	Grain

[a]OOPAP = 1,2 dioleoyl-3-(*N*-phenylamino)-1,2-propanediol

2 Distribution of Bioactive Materials in Food Materials

2.1 Natural Occurrence

Levels of naturally occurring BMs in raw foods vary considerably, depending on the species, the cultivar, and the environmental conditions under which they are grown. Even for crops grown under "ideal" conditions, the range of concentrations can vary tenfold or more between different cultivars. For instance, the glucosinolate content of broccoli genotypes grown under controlled conditions was reported to vary, on average, from about 0.2 to 1.8 μmol/g dry weight [4]. The concentration of glucoraphanin in the commercial broccoli variety "Marathon" is about five times higher than that in the variety "Everest" [4]; it is not possible for the commercial user or the consumer to differentiate between varieties in order to select for enhanced levels of glucoraphanin.

The presence of specific nutrients in the soil influences the pattern and levels of glucosinolates in broccoli [5]. In field experiments, fertilization with increased sulfur levels generally gave a higher glucosinolates concentration in *Brassica* species [6]. However, the levels of the alkyl glucosinolates, glucoraphanin and glucoiberin, were reduced by 70% in broccoli supplied with enhanced nitrogen levels in comparison with plants receiving no added nitrogen [7]. Nitrogen fertilization also lowers the anthocyanin content in Merlot grapes [8]. Anthocyanin levels in red cabbage are reported to be reduced by high nitrogen levels, but potassium and phosphorous have little effect [9]. The highest lycopene concentrations, and the best color, in tomatoes were reached under low nitrogen levels, but the yield was increased by higher nitrogen levels; however, the lycopene concentration increased with increasing phosphorous and potassium [10]. In carrots, the carotene content seems to increase with increasing nitrogen supply [11].

Similarly, the concentrations of BMs in many plants are affected by the levels of solar radiation, temperature, and moisture content. Generally, levels of BMs are increased significantly when grown in conditions where the soil moisture content is low, while levels were increased by exposure to sunlight and low mean temperatures [12].

The importance of BMs in relation to the quality of foods and feeds has created interest in their natural biosynthetic pathways and in the possibility of manipulating the levels to produce new and improved varieties. Breeding projects to develop F1 hybrids and genetic manipulation procedures have been used to improve agronomic and quality traits and to select for specific characteristics such as increased total phenols content and total antioxidant capacity [13].

A good example of deliberate breeding for high levels of health-promoting glucosinolates is the selection of broccoli cultivars for higher levels of glucoiberin and glucoraphanin, the precursors of the isothiocyanates iberin and sulforaphane, respectively [14]. This was achieved by crossing a standard broccoli cultivar with *B. villosa*, a wild relative of *B. oleracea* from Sicily, which accumulates high levels of glucoiberin in the flower buds. The F1 hybrids had high levels of both glucosinolates. By using a series of backcrosses, two regions of the *B. villosa* genome for high glucosinolate content were introduced into a commercial broccoli. These high glucosinolate broccoli cultivars have subsequently been used in human

intervention trials in the UK and shown to deliver about four times the amount of sulforaphane to the systemic circulation than standard cultivars [15]. It is important to note that the isothiocyanates derived from these glucosinolates contribute little to flavor so that it is possible to enhance their levels. However, increasing the levels of certain other glucosinolates may result in more pungent isothiocyanates, such as 2-propenyl or 3-butenyl, which are commercially undesirable.

2.2 Contaminants in Foods

This diverse category of BCs includes both agrochemical residues and microbial toxins. Generally, the levels of pesticide and herbicide residues in plants are below the limits set by law in most countries. However, the most recent (2016) results of pesticide residue monitoring in the UK shows that residues were detected in about 48% of all commodities tested and levels exceeded the legislative limits in some 3.2% of all samples tested, mainly in imported foods [16]. Similar levels of incidence are found in most developed countries although recent US studies suggest a much higher incidence of residues. But in the third world, residue levels are often much higher, and the adverse consequences for health of consumers, especially children, are significant [17].

However, the situation is different for microbial toxins, both those produced within an agrochemical commodity and, potentially, as a consequence of microbial growth in a manufactured food product. Growth of fungi within a primary product may occur in the field or during postharvest storage of crops; fungi and their metabolites are perpetuated along the food chain as a consequence of blending of primary raw materials prior to, or as part of, a manufacturing process. Possibly the best-known example of such contaminants is the occurrence of the potentially carcinogenic aflatoxins in nuts, cereals, and other food crops. Growth of the producer organisms, *Aspergillus flavus* and *A. parasiticus* strains, occurs within individual nuts. While widespread infection by the fungi may occasionally affect entire batches of a crop, it is widely recognized that often there is only a small number of infected nuts within a consignment that is otherwise largely unaffected. In certain instances, aflatoxins may occur at levels of 1000 μg g-1 in peanut kernels and up to 5000 μg g-1 in cottonseed [18, 19]. Good Manufacturing Practices use color, UV fluorescence, or other forms of automated detection to screen out damaged and highly contaminated nuts, [20] but in rural communities in Africa, South America, and elsewhere, such screening is not possible. As a consequence, a population that consumes the nuts as a primary food source is exposed to high levels of dietary aflatoxin and/or other mycotoxins [21–23]. The presence of aflatoxin B_1 in the feed of dairy animals results in the excretion of aflatoxin M_1 in the milk.

2.3 Distribution of BMs in Food Materials

The distribution of naturally occurring BMs in food plants generally conforms reasonably to a normal distribution (ND), as do those BMs developed as a

consequence of food processing operations, such as heating, for instance, the development of furans in pasteurized fruit juices and acrylamide in high temperature-cooked products. Similarly, BMs added as food supplements, food ingredients, or food additives will generally be well mixed during the food preparation stages so that the distribution in the food conforms reasonably to the expectation of a ND. Generally, the distribution of agrochemical residues in crops also conforms reasonably to the ND.

However, the variability in the levels of contamination by mycotoxins is considerable, even within a "lot," and the distribution in primary food materials does not conform to a ND. The most usual conformation is to a negative binomial (Gamma-Poisson) distribution (NBD) [24–27]. By contrast, aflatoxin M_1 in "lots" of milk and in products such as cheese and yogurt is normally distributed [28] although there may be significant differences between "lots" depending on the level of contamination of the animal feed.

Toxins derived following growth of bacteria, such as staphylococcal enterotoxin and botulinum toxin, will normally occur in the vicinity of microcolonies of the producer organisms but will diffuse into the product over time. The initial bacterial contamination will usually be distributed randomly, although sometimes high-level microbial contamination occurs in localized areas, such that distribution of the cells conforms to a Beta- or Gamma-Poisson NBD [29]. If the initially contaminated product is subsequently processed by blending, then the organisms, and their toxins, will more usually be distributed more randomly in accordance with the expectation of a Poisson distribution [27].

3 Statistical Aspects of Sampling for Analysis

3.1 Sampling Protocols for Surveillance and Monitoring

The underlying principles of sampling are "dependent on purpose," but the primary objective is to obtain a series of samples that is "representative of the batch of material under investigation." Regulators may seek more samples than a producer may wish to have tested so that the question "how many samples do I need to take?" has often been answered with the general advice "more than you can afford to test." By contrast, the analyst generally sees a sample purely as "a quantity of material selected for analysis from a larger quantity of material" – but this misses the point: the selected sample(s) may not be truly "representative" of the whole [30].

In many countries, regulations concerned with control of contaminants often set a level of compliance as a limit value (e.g., a maximum or average value depending on the nature of the test). Demonstrating compliance against a limit requires a sampling and analytical plan (SAP) that often specifies the need for "representative" samples and analysis by an accredited laboratory. The SAP rarely requires measurement uncertainty to be reported nor does it require that the samples were truly representative of the "lot" which provides evidence regarding

the variability of the analyte concentration in the material. Merely replicating the sampling and analysis may provide some evidence that sampling is representative but ignores the possibility of a common sampling bias affecting a series of tests. It is relevant, therefore, that ISO 3534-4 [31] states that "the notion of a representative sample is fraught with controversy with some practitioners rejecting the term altogether." Even changing the concept to one of "appropriate" sampling [30] may not solve this dilemma especially when investigating the levels of a specific constituent, such as a contaminant in a food material. Part of the dilemma lies in the difference of understanding between the analyst and the statistician. To the analyst, the term "representative sample" usually refers to the material that arrives for laboratory analysis, whereas the survey statistician considers the sample as "one for which the observed values have the same distribution as that in the population sampled."

Wherein lies the problems of sampling? A generally homogeneous material, such as a nonviscous liquid, can be mixed relatively easily; but, even then, there will be variation between results on replicate samples that reflect both the distribution of the analyte within the bulk material, inaccuracies in the preparation of the sample for analysis (e.g., compounding of bulk materials and extraction of the analyte), the imprecision of the method, and the way that it is used by the analyst. Sampling of solid materials (e.g., plant materials) requires homogenization of the target material in order to draw a uniform sample for analysis. But which target material is to be used? If a BM (or other analyte) is present only in the flower bud, should the "buds" be separated before analysis, or should the buds and stalks be analyzed together? If the latter, how does the analyst ensure that a similar admixture of plant materials is always analyzed?

Bulk granular materials are even more variable! A "lot" of, e.g., corn or peanuts will contain different quantities of the analyte throughout the bulk such that very large samples of the target material require effective grinding and mixing to give a relatively homogeneous primary target sample that can be further ground, mixed, and subjected to extraction. While such procedures can be carried out for chemical analysis, they can rarely be done for microbiological analysis [27].

3.2 Sampling Plans and Analytical Methods

For monitoring and control purposes, a SAP is generally based on a specification relating to the number of individual samples to be examined for a given bulk of material using defined sample preparation and analytical methods. The results of the analyses are then compared against criteria that define an acceptable upper or lower limit for the analyte.

By contrast, surveillance studies aim to determine the frequency and levels of occurrence of the analyte. If the analyte is known to be a constituent of the material under investigation, then only quantitative tests will usually be undertaken, but if one is concerned with ascertaining whether a contaminant might be present in a product, both qualitative and quantitative methods may be required. For instance, surveys of

the incidence of aflatoxin in a commodity or processed food are usually predicated on the use of semiquantitative methods that provide both a measure of frequency and an indication of the level of contamination in a proportion of samples. The choice of method should therefore be determined by the precision and accuracy required for the result. For analytes which are known to be highly variable, the number and size of primary samples for analysis should always be much greater than for a more homogeneous target.

Figure 1, using data reported by Whitaker et al [24], shows the distribution of the mean levels of detection of aflatoxins in a total of 290 samples of shelled peanuts, being 10 sub-samples taken from each of 29 lots. Whilst some 57% of the samples contained less than 20 μg kg-1, including no detectable aflatoxin, 43% contained aflatoxins at levels up to almost 300 μg kg-1. The range of mean aflatoxin levels within individual lots ranged from 0 to 14 μg kg-1 through to 0 to 285 μg kg-1 in another lot. The 'box and whisker' plot (Fig. 2) shows a selection of the results for individual lots. Lot 16 is particularly interesting because although nine of the ten samples contained aflatoxin at < 20 μg kg-1 one sample contained an average level of 285 μg kg-1. Although not unusual, such an observation illustrates the diversity of contamination levels that may result from only a small number of contaminated peanuts within a lot. Other surveys for mycotoxin contamination of food and feed materials have shown similar distribution patterns, reflecting the nature of microbial activity.

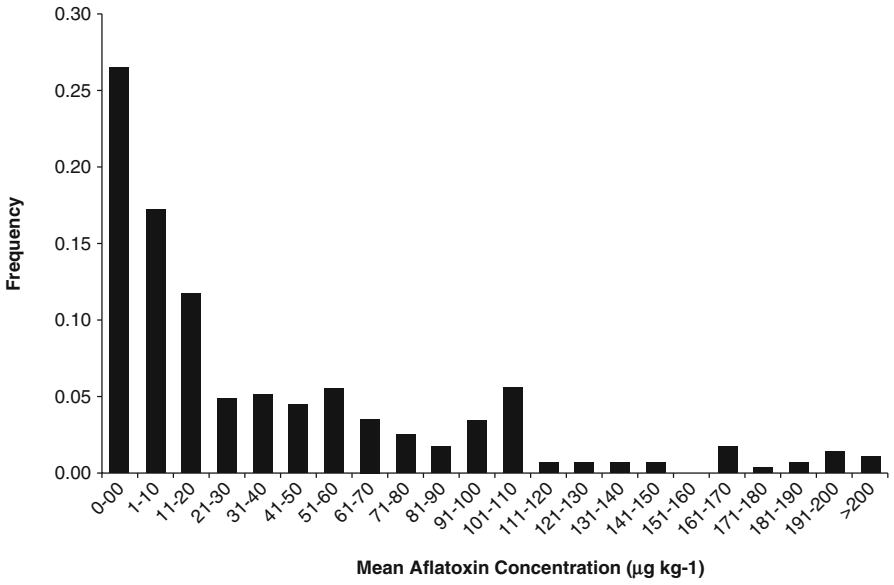


Fig. 1 Frequency distribution plot of the mean aflatoxin levels in each of 10 replicate subsamples taken from primary samples of 29 lots of shelled peanuts. The data, reported by Whitaker et al [24], were used originally to demonstrate that aflatoxin contamination follows a NBD

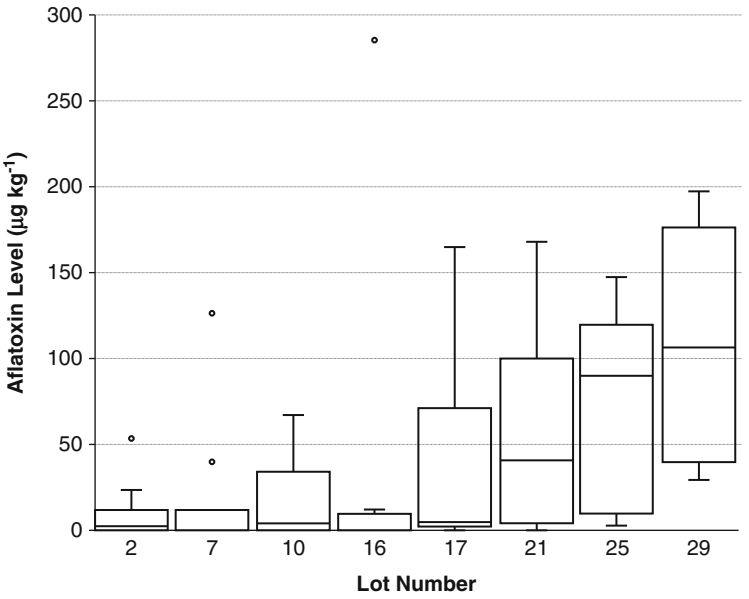


Fig. 2 'Box and Whisker' plots to illustrate the diversity of aflatoxin contamination levels on 8 of the 29 lots of peanuts reported by Whitaker et al [24]. Whilst a general low level of contamination was found in lots 2, 7 and 16 individual test samples from these lots contained higher levels in one or more samples. By contrast a more widespread contamination occurred in lots 17, 21, 25 and 29

The choice of method should be determined by the precision and accuracy required for the result. For analytes which are known to be highly variable, the number and size of primary samples for analysis should always be much greater than for a more homogeneous target.

3.3 Analytical Uncertainty

For any SAP, there will always be a level of uncertainty in the results. In this context, uncertainty takes into account both the precision of the method and the accuracy of the result [32]. Three kinds of uncertainty occur: sampling uncertainty, sample preparation uncertainty, and measurement uncertainty.

Sampling uncertainty is a measure of the variability of the analyte in a "lot" of the target material and, even with relatively homogeneous materials, is usually much greater than measurement uncertainty for both chemical [32, 33] and microbiological procedures [34].

Sample preparation uncertainty relates to the variability associated with the production of individual test samples taken from a primary target sample. Techniques such as grinding and mixing of a relatively large quantity of primary sample may be needed to reduce the bulk of material to provide a more

homogeneous matrix that will be sampled again in order to provide a "test sample" for analysis.

Analytical (measurement) uncertainty is an estimate of the variability of the results of an analysis that is related to the analytical method and its use in an individual laboratory and/or in a number of collaborating laboratories [35, 36]. Usually the statistical error (variability) of sample preparation is included as part of analytical uncertainty, but some workers may treat it separately.

The overall estimate of uncertainty is determined from the square root of the combined variances of the individual stages (Eq. 1):

$$U = \sqrt{s_{\text{sam}}^2 + s_{\text{prep}}^2 + s_{\text{an}}^2} \tag{1}$$

where $U =$ the estimate of overall uncertainty measured as a standard deviation; s_{sam}^2 = the estimate of sampling variance, i.e., the "between sample" variance; s_{prep}^2 = "within" sample (preparation) variance; and s_{an}^2 = the analytical variance. Statistical analysis of variance (ANOVA) is usually applied to derive the individual variances, but reliable results for the variances require that estimates conform to a ND. If this is not so, then the original data need to be "normalized" before an analysis of variance is undertaken. If, for instance, the distribution of the analyte in the product is believed to conform to a negative binomial distribution (as in the case of many mycotoxins), it is necessary to transform the original data before determining the estimates of variance. In the case of the negative binomial, the usual transformation is $\sinh^{-1}\sqrt{\frac{x+0.375}{k-2(0.375)}}$, where x is the analytical result and k is the negative binomial exponent. In the case of the distribution of aflatoxins in peanuts, Whittaker et al. [24] determined the value to be $k = (2.0866 + 2.3898\,\text{m}) \times 10^{-6}$ (in terms of the μg kg-1 contamination levels), where m was the mean concentration of aflatoxin.

The overall uncertainty (U) is multiplied by a "coverage factor" (K) to give an expanded standard deviation that is an approximate estimate of the confidence limits of the analysis. The coverage factor used is normally a value of 2, so that the approximate 95% expanded uncertainty is given (Eq. 2) by

$$U_{\text{exp}} = K\text{U} = 2U \tag{2}$$

By way of example, let us assume that analysis of ten samples of a product for a natural constituent gives a mean concentration of 6.8 μgkg^{-1} product and a test for "normality" is not rejected. ANOVA gives a between sample variance of 0.80 μgkg^{-1}, a within sample variance of 0.15 μgkg^{-1}, and an analytical variance (reproducibility) of 0.22 μgkg^{-1}. Then the uncertainty estimate is given by $U = \sqrt{0.80 + 0.15 + 0.2} = \sqrt{1.05} = 1.0724$. Expanded uncertainty is $2U = 2 \times 1.0724 \approx 2.145$ μgkg^{-1}, so the overall test result is 6.8 ± 2.145 μgkg^{-1} and the bounds of the lower and upper 95% confidence limits are 4.655–8.945 μgkg^{-1}, respectively.

4 Sampling for Special Purposes

4.1 Planning and Analysis of Trials

Trials can take many forms depending on the overall objectives. The requirements for growth trials of new cultivars and trials of cultivation conditions (soil type, moisture, solar effects, temperature, etc.) are all well described in scientific literature, yet in so many cases, one finds that proper randomly controlled trials are not undertaken. There are three key elements: replication of each test factor (e.g., soil) for each test subject (e.g., a cultivar), replication of each test subject between each test factor, and a consistent approach to analysis for the defined analyte between subjects and test factors [37].

The original examples using the Latin Square approach to controlled replication of experiments, were described almost 250 years ago by Leonhard Euler (1707–1783). More recently, Williams [38], Laywine and Mullen [39], and Fisher [40] have provided working tools to enable the researcher to apply the concept of Latin Squares in the design of experiments. The primary objective of Latin Squares is to ensure randomization and replication under controlled conditions, as illustrated in Fig. 3 for a series of 25 "plots" of land used to trial four new cultivars and to provide a comparison to a "standard" cultivar.

As shown in the figure, each cultivar is replicated five times to make allowance for variations in the soil. Of course, replication of plants within a "plot" permits estimation

a

A	B	C	D	E
B	C	D	E	A
C	D	E	A	B
D	E	A	B	C
E	A	B	C	D

b

A	E	C	D	B
E	C	A	B	D
D	B	E	C	A
B	A	D	E	C
C	D	B	A	E

Fig. 3 Two forms (**a**, **b**) of Latin Squares. Note that each letter (from A to E) occurs once in each row and each column. Form (**a**) is a cyclic orthogonal Latin Square, where letters in succeeding rows move to the left one row at a time; by contrast, form (**b**) shows a random orthogonal allocation. The control (or reference) trial and the four test trials (cultivars or treatments) would each be allocated randomly to a letter location. For a field trial, unless testing for effects of soil type, the underlying assumption is that the composition and type of soil are essentially uniform across all test plots

of variation between plants of each cultivar so that the "within plot" and "between plots" variance can be determined. The Latin Square approach is not restricted to just a few cultivars or treatments; Latin Square designs allowing 20 or more sets can be produced, but the level of statistical analysis is increased proportionately to the increased number of parameters. For larger numbers of parameters, an alternative approach is the use of a Quasi-Latin Square where twice the number of factors can be tested but on half the number of replicate occasions but with slightly reduced precision [41].

4.2 Example

Suppose that in a simple experimental trial, we wish to assess and compare the levels of a metabolite X in four new plant cultivars against an existing commercial cultivar. We plant three replicates of each cultivar in each of five trial plots (25 plots in total). Throughout the trial, we monitor and record the condition of each plant (in terms of size, conformation, appearance, etc.), and at harvest time we again record condition and take the materials for analysis. Table 3 illustrates the sort of results that might be found using a categorical scoring system of 0 = no growth, 3 = good growth, and 5 = luxuriant growth. From the scores, it is clear that plants of cultivar C grew poorly and that cultivar D grew more strongly than did the control plants (A); cultivars B and E grew at a rate comparable to A. Table 3 also demonstrates that overall growth in row 1 was slightly less than in the other four rows. The question now arises as to whether the differences are of statistical significance. The null hypothesis is that all cultivars grow equally well; the alternative hypothesis is that they do not.

Since the data are categorical (i.e., scored by reference to preset criteria), it is necessary to use a nonparametric analysis of variance, such as the Kruskal-Wallis (KW), test to assess whether the results differ significantly. The total score from each plot in Table 3 is ranked across all plots (Table 4), where if two or more results have the same value, the average rank is used. Then for each set of data, the ranked values are summated to give a value (*Tc*), which is squared and divided by the number of replicates to determine a value, *H*, using Eq. (3):

Table 3 Categorical results for cultivar growth over an 8-week period. The control (reference) cultivar was allocated to A and the other four cultivars to B, C, D, or E. The results are based on growth of three replicates in each plot scored a five-point scale with 0 representing no growth, 3 good growth, and 5 excessive growth

Cultivar	Growth in row					Total
	1	2	3	4	5	
A	3,3,4 = 10	4,4,3 = 11	3,3,3 = 9	3,2,3 = 8	3,3,3 = 9	47
B	3,2,3 = 8	3,3,3 = 9	4,3,3 = 10	3,3,2 = 8	4,3,3 = 10	45
C	0,1,1 = 2	1,1,1 = 3	1,2,1 = 4	1,2,2 = 5	1,1,1 = 3	17
D	5,4,4 = 13	5,5,3 = 13	5,5,4 = 14	5,5,5 = 15	4,5,5 = 14	69
E	3,3,3 = 9	3,4,4 = 11	3,4,3 = 10	3,3,4 = 10	3,3,4 = 10	50
Total	42	47	47	46	46	228

$$H = \left[\frac{12}{N(N+1)} \times \sum \frac{\mathrm{Tc}^2}{n_c}\right] - 3 \times (N+1) \quad (3)$$

where H is the KW value, N is the total number of tests in all groups, n_c is the number of tests in a specific group (in this case it equals 5), Tc^2 is the square of the sum of the ranked values for each group (c), and $\sum$ means "sum of" for all groups. Critical value tables for the H can be found in Meyer and Seaman [42], but the value of χ^2, available in statistical tables, provides an approximation for H. For the ranked data shown in Table 4, $H = 19.298$ with four degrees of freedom (five test series – 1). From tables of χ^2 for 4 df, the value of H is greater than the critical value (18.467) for $p = 0.01$, so the differences in the results are highly significant. However, a word of caution: if many values are of equal rank, the result of this nonparametric form of ANOVA may be misleading – theory suggests that ideally there should be few tied ranks.

For this example, it was not really necessary to carry out a statistical analysis because, just by inspection, we could rate cultivar C as poor while cultivar D obviously grew much better than the control (A) or the two other cultivars (B and E). But for other data, it could be important to have the statistical confirmation.

Suppose also that the mature plants have been analyzed for a hypothetical constituent X and, since these are actual values, it is appropriate to compare them using a parametric ANOVA. As before we have three replicate plants from five cultivars each grown in five different plots of land. We can assess the mean and variance for the level of X in all three plants from each plot, but, for the sake of simplicity, I will assume that we analyze only the mean level of X in the plants in any one plot. Preliminary tests for ND [43] showed that for cultivar C both the mean value and the variance of the results were low compared to the other cultivars (Table 5). The results for cultivar C were therefore removed from the dataset, and a test for homoscedasticity (equality of variances) was performed on the four remaining cultivars using Levene's test [43] which did not reject the hypothesis that the variances of the remaining four populations were equal ($F = 0.16$ with 3 and 16 df and $p = 0.9203$). ANOVA was then carried out on the data for the four cultivars (Table 6), and the results demonstrate a highly significant difference ($p < 0.001$) that requires rejection of the null hypothesis that the mean values of all the results are equal. A post hoc test, using pair-wise

Table 4 Ranking of cultivar growth in a Latin Square trial of four new cultivars (B to E) against a control cultivar (A). Note that if two, or more, results are the same, then each is given the average rank

	Ranked values for each plot					
Cultivar	1	2	3	4	5	Tc sum
A	15.5	19	10.5	7	10.5	63.0
B	7	10.5	15.5	7	15.5	55.5
C	1	2.5	4	5	2.5	15.0
D	21.5	21.5	23.5	25	23.5	115.0
E	10.5	20	15.5	15.5	15.5	76.5
Sum	55.5	73.5	69	59.5	67.5	325

Table 5 Hypothetical results for a compound (X) determined on cultivars grown in a 5 × 5 Latin Square trial

Cultivar	Mean level of X (μg/g) in plot					Mean	Variance
	A	B	C	D	E		
A	37.6	37.1	35.9	35.6	36.1	36.46	0.723
B	31.6	33.5	33.6	33.2	33.4	33.06	0.688
C	2.6	3.6	2.1	2.8	2.3	2.68	0.337
D	40.2	39.4	41.6	40.2	39.8	40.24	0.688
E	36.5	38.1	37.2	36.3	38.1	37.24	0.728

Table 6 Results of ANOVA of the data from Table 5 after removal of data for Cultivar C

Source	SS	DF	MS	F	*p*-value
Cultivar	130.60	3	43.53	61.60	<0.0001
Error	11.31	16	0.71		
Total	141.91	19			

comparisons of the four sets of data using Tukey's "least significant difference" test [42] showed that the level of compound X in cultivar E did not differ from that in the reference cultivar (A), that B was significantly lower, and that D was significantly higher than the reference value (both at $p < 0.01$).

By comparison with the results for the reference cultivar, we can conclude that cultivar B grew at a similar rate but produced a lower level of compound (X); that cultivar C did not grow well or produce much X; that cultivar E was not different in terms of growth or production of X; but that cultivar D was more prolific in its growth and also produced significantly higher levels of X. It is important to recognize that differences in growth and metabolic output between cultivars need not only to be observed but also to be demonstrated as being of statistical significance.

5 Chemometric Methods

Surveillance work for trace compounds in foods, and for investigation of food adulteration, often produces a database with results from many tests; some of which may be purely categorical, while others are analytical data [43]. Categorical (observational) data may be of three types: nominal (e.g., three or more types of foods, etc.), dichotomous (e.g., authentic or adulterated), and ordinal (data ordered by criteria, e.g., three levels of sensorial evaluation such as 1 = unacceptable, 2 = marginally acceptable, or 3 = acceptable). On the other hand, quantitative variables include continuous scales (temperature, pressure, time, concentration, mass, etc.), intervals, and ratios. In recent times, many investigations use multivariate statistical procedures such as principle components analysis (PCA), cluster analysis, and other more complex procedures, to associate the level of BMs with in vivo or in vitro functional properties.

PCA is a statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called "principal components." The number of distinct principal components is equal to the smaller of the number of original variables minus one. The transformation is defined in such a way that the first principal component has the largest possible variance (i.e., accounts for as much of the variability in the data as possible); each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to the preceding components. The resulting vectors form an uncorrelated orthogonal base set. PCA is sensitive to the relative scaling of the original variables and is mostly used as a tool in exploratory data analysis and for making predictive models. It is also used in canonical correlation analysis to define coordinate systems that optimally describe the cross-covariance between two datasets so that the PCA defines a new orthogonal coordinate system that optimally describes variance in a single dataset.

"Cluster analysis" is a procedure for grouping a set of objects in such a way that objects in the same group (a cluster) are more similar to each other than to those in other groups (clusters). Its main task is for exploratory data mining.

A recent paper [43] describes and reviews the use of these, and many other forms of, chemometric analysis that have been used in food-related studies. For instance, chemometrics based on appropriate analytical techniques have been used to confirm adulteration of wines, honey, essential oils, and many other high-value food products. Aside from food adulterations, chemometrics have been used to analyze data on soil toxicity, influences of climate change on the nutritional value of foods, and changes in functional properties consequent on processing.

6 Clinical Trials

Validation of beneficial or harmful effects in experimental animals requires careful planning and ethical approval. Is the target species appropriate and how will the effects be recognized? Even if beneficial effects are observed in, e.g., rats, mice, or hamsters, will the effects also be seen in humans? Clinical trials on humans require both careful practical and statistical planning and ethical review before the trials commence. It is not possible to summarize all the planning and statistical issues that must be reviewed, but guidance is available in publications such as the US FDA regulations [44] and the European Commission Guidelines [45].

7 Validation and Verification of Methods

Whenever possible analytical methods that have been collaboratively tested under AOAC [46], ISO [47], or other recognized national schemes should be used for detection and estimation of levels of occurrence. The primary objective of validation and verification of methods is to ensure that the results are obtained using the most reproducible procedures, rather than by an ad hoc procedure developed in a specific

laboratory. Unfortunately, in many cases workers tend to use their own methods of analysis. Of course, new developments in analytical technology offer opportunities to improve sensitivity, precision, and accuracy of methods – but, such developments should always be validated against reference methods that have been internationally approved as standard methods. Observation of a new potentially beneficial or toxic constituent requires validation using approved methods.

A similar stricture applies also to the choice and use of statistical methods of analysis. An experimental or surveillance program needs to be reviewed and approved by a statistician before the work is undertaken to ensure that the work plan is appropriate. All too often one finds that results given in papers submitted for publication have been obtained using nonstandard methods or that the statistical methods used to substantiate the findings are not appropriate. It is then difficult to assess whether a reported finding is genuine or merely a consequence of the analytical or statistical procedure used by the authors of the paper. Even relatively minor changes to methodology can affect the validity of the results!

8 Conclusions

The occurrence of BMs in raw and processed foods may offer benefits or risks to the health of consumers, be they human or animal. Surveys for presence and levels of BMs need to be undertaken using statistically sound experimental sampling plans with adequate levels of control and to use appropriate analytical and statistical methods. Key among these is the need to develop an appropriate statistical approach before practical work is undertaken.

Acknowledgments I am grateful to Dr Alan Hedges of the University of Bristol for his inciteful and helpful comments on the draft of this chapter.

References

1. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113(Suppl 9B):71S–88S
2. Bernhoft A (ed) (2010) Bioactive compounds in plants – benefits and risks for man and animals. The Norwegian Academy of Science and Letters, Novus Forlag, Oslo
3. Srivastava R, Kulshreshtha DK (1989) Bioactive polysaccharides from plants. Phytochemistry 28:2877–2883
4. Farnham MW, Wilson PE, Stephenson KK, Fahey JW (2004) Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli. Plant Breed 123:60–65
5. Aires A, Rosa E, Carvalho R (2006) Effect of nitrogen and sulphur fertilization on glucosinolates in the leaves and roots of broccoli sprouts (*Brassica oleracea* var. *italica*). J Sci Food Agric 86:1512–1516

6. Rangkadilok N, Nicolas ME, Bennett RN, Eagling DR (2004) The effect of sulfur fertilizer on glucoraphanin levels in broccoli (*B. olera*cea L. var. *italica*) at different growth stages. J Agric Food Chem 52:2632–2639
7. Verkerk R, Schreiner M, Krumbein A, Ciska E, Holst B, Rowland I, De Schrijver R, Hansen M, Gerhäuser C, Mithen R, Dekker M (2009) Glucosinolates in brassica vegetables: the influence of the food supply chain on intake, bioavailability and human health. Mol Nutr Food Res 53:S219–S265
8. Giraudon J, Soyer JP, Molot C, Milin S, Gaudillère J, Hilbert G (2003) Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot J Grapewine Res 42:69–76
9. Piccaglia R, Marotti M, Baldoni G (2002) Factors influencing anthocyanin content in red cabbage (*Brassica oleracea* var. *capitata* L. f. *rubra* (L.) Thell.) J Sci Food Agric 82:1504–1509
10. Dumas Y, Dadomo M, Di Lucca G, Grolier P (2003) Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J Sci Food Agric 83:369–382
11. Cserni I, Prohászka K (1998) The effect of N supply on the nitrate, sugar and carotene content of carrots. Acta Hortic 220:303–308
12. Schreiner M (2005) Vegetable crop management strategies to increase the quantity of phytochemicals. Eur J Nutr 44:85–94
13. Diamanti J, Capocasa F, Tulipani S, Battino M, Mezzetti B (2008) Breeding strawberry (*Fragaria* x *Ananassa duch*) to increase fruit nutritional quality. (Abstract) Workshop on Bioactive compounds in berry fruits: genetic control, breeding, cultivar, analytical aspects and human health, 3–5 Dec 2008, Zürich
14. Mithen R, Faulkner K, Magrath R, Rose P, Williamson G, Marquez J (2003) Development of isothiocyanate-enriched broccoli, and its enhanced ability to induce phase 2 detoxification enzymes in mammalian cells. Theor Appl Genet 106:727–734
15. Gasper AV, Al-Janobi A, Smith JA, Bacon JR, Fortun P, Atherton C, Taylor MA, Hawkey CJ, Barrett DA, Mithen RF (2005) Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. Am J Clin Nutr 82:1283–1291
16. Health & Safety Executive (2017) Pesticide residues in foods. Annual report for 2016. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/655035/expert-committee-pesticide-residues-food-annual-report-2016.pdf. Accessed 23 Nov 2017
17. Galt RE (2014) Food systems in an unequal world: pesticides, vegetables and agrarian capitalism in Costa Rica. University of Arizona Press, Tucson
18. Cucullu AF, Lee LS, Mayne RY, Goldblatt LA (1986) Determination of aflatoxin in individual peanuts and peanut sections. J Am Oil Chem Soc 43:89–92
19. Cucullu AF, Lee LS, Pons WA (1977) Relationship of physical appearance of individual mold damaged cottonseed to aflatoxin content. J Am Oil Chem Soc 54:235A–2237
20. Pelletier MJ, Reizner JR (1992) Comparison of fluorescence sorting and colour sorting for the removal of aflatoxin from large groups of peanuts. Peanut Sci 19:15–20
21. Darwish WS, Ikenaka Y, Nakayama SMM, Isizuka Y (2014) An overview on mycotoxin contamination of foods in Africa. J Vet Med Sci 76:789–797
22. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr 80:1106–1122
23. Galvano F, Ritieni A, Piva G, Pietri A (2005) Mycotoxins in the human food chain. In: Diaz DE (ed) The mycotoxin blue book. Nottingham University Press, Nottingham, pp 187–224
24. Whitaker TB, Dickens JW, Monroe RJ, Wiser EH (1972) Comparison of the observed distribution of aflatoxin in shelled peanuts to the negative binomial distribution. J Am Oil Chem Soc 49:590–593
25. Whitaker TB, Dickens JW, Monroe RJ (1974) Variability of aflatoxin test results. J Am Oil Chem Soc 51:214–218
26. FAO (1993) Sampling plans for aflatoxin analysis in peanuts and corn. FAO food and nutrition paper 55. FAO, Rome

27. Jarvis B (2016) Statistical aspects of the microbiological examination of foods, 3rd edn. Academic, Oxford
28. Iha MH, Barbosa CB, Okada IA, Trucksess MW (2013) Aflatoxin M1 in milk and distribution and stability of aflatoxin M_1 during production and storage of yoghurt and cheese. Food Control 29:1–6
29. Jongenberger I, den Besten HM, Zwietering MH (2015) Statistical aspects of food sampling. Annu Rev Food Sci Technol 6:479–503
30. Ramsey MH, Barnes B (2016) Representative sampling? Views from a regulator and a measurement scientist. Anal Methods 8:4783–4784
31. ISO (2014) Statistics – vocabulary and symbols. Part 4: survey sampling. ISO 3534:2014. International Standards Organization, Geneva
32. Ramsay MH, Lyn JH, Wood R (2001) Optimised uncertainty at minimum overall cost to achieve fitness-for-purpose in food analysis. Analyst 126:1777–1783
33. Ramsay MH, Ellison SLR (eds) (2007) Eurachem/EUROLAB/CITAC/Nordtest/AMC guide: measurement uncertainty arising from sampling: a guide to methods and approaches. ISBN 978 0 948926 26 6
34. Jarvis B, Hedges AJ, Corry JEL (2012) The contribution of sampling uncertainty to total measurement uncertainty in the enumeration of microorganisms in foods. Food Microbiol 30:367–371
35. ISO 5725 (1994–1996) Accuracy (Trueness and precision) of measurement methods and results. Parts 1–6. International Standards Organization, Geneva
36. ISO-CD 19036:2017E. Microbiology of the food chain – estimation of measurement uncertainty for quantitative determinations. Final committee draft. International Standards Organization, Geneva
37. Williams EJ (1949) Experimental designs which are balanced for the estimation of residual effects of treatments. Aust J Sci Res 2:149–164
38. Laywine CF, Mullen CM (1998) Discrete mathematics using Latin squares. Wiley Interscience, New York
39. Fisher RA (1958) Statistical methods for research workers, 13th edn. Oliver & Boyd, Edinburgh
40. Meyer JP, Seaman MA (2014) A comparison of the exact Kruskal-Wallis distribution to asymptotic approximations for all sample sizes up to 105. J Exp Educ 81:139–156
41. Brien CJ, Bailey RA, Tran TT, Boland J (2012) Quasi-Latin designs. Electron J Stat 6:1900–1925
42. Granato D, Calado VMA, Jarvis B (2014) Observations on the use of statistical methods in food science and technology. Food Res Int 55:137–149
43. Granato D, Putnik P, Kovačević DB, Santos JS, Calado VMA, Cruz AJ, Jarvis B, Rodionova OY, Pomerantsev A (2018) Trends in chemometrics: food authentication, microbiology, and effects of processing. Compr Rev Food Sci F. https://doi.org/10.1111/1541-4337.12341
44. US FDA (2016) Clinical trials and human subject protection. FDA Regulations relating to Good Clinical Practice and Clinical Trials. https://www.fda.gov/ScienceResearch/SpecialTopics/RunningClinicalTrials/ucm155713.htm#FDARegulations. Accessed 02 Dec 2017
45. EC (2016) Clinical trials guidelines. https://ec.europa.eu/health/documents/eudralex/vol-10_en. Accessed 02 Dec 2107
46. AOAC International (2016) Official methods of analysis of AOAC international, 20th edn. AOAC International, Rockville. http://www.aoac.org/AOAC_Prod_Imis/AOAC/AB/AOAC_Member/AOACACF/AOACOCF/AOACA14.aspx?hkey=adb377f8-2682-4109-b058-fdfd147ca013. Accessed 29 Nov 2017
47. International Organization for Standardization, ISO Central Secretariat, Vernier, Geneva, Switzerland. https://www.iso.org/standards.html

LC-MS/MS Determination of Pesticide Residues in Fruits and Vegetables

72

Anna Stachniuk

Contents

Abstract

A proper diet is commonly regarded as one of the most important factors determining one's health. A key role in such a diet is played by unprocessed food of plant origin, mainly fruits and vegetables, as they contain many important dietary bioactive compounds such as polyphenols, carotenoids, fiber, antioxidants, several important vitamins, and minerals. Despite the nutritional benefit, they may also contain substances that adversely affect human health. Pesticides constitute a special group of contaminants as even small amounts of these substances can result in acute poisoning, lead to cancer, and have an adverse impact on the endocrine, immune, and nervous system. A considerable number of pesticides have a harmful effect already in low concentrations, within the range of μg kg^{-1} and below μg kg^{-1}; hence, there is a great need for identifying and determining them by means of highly selective and sensitive methods. In the analysis of pesticide residue, similarly to the analysis of other food contaminants,

A. Stachniuk (✉)
Laboratory of Separation and Spectroscopic Method Applications, Center for Interdisciplinary Research, The John Paul II Catholic University of Lublin, Lublin, Poland
e-mail: lianna@kul.pl

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_82

there is a clear tendency to prepare multiresidue methods that enable monitoring a large number of compounds in a great number and variety of samples. Most multiresidue methods reported for fruits and vegetables in the last decade are based mostly on the use of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which is the technique of choice for the majority of pesticides and their metabolites nowadays.

Keywords
Pesticides · Fruits and Vegetables · Liquid chromatography–mass spectrometry

Abbreviations

APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
ASE	Accelerated solvent extraction
C18	Octadecylated silica bounded stationary phase
C8	Octasilyl silica bounded stationary phase
CHEMAC	Conservative homogenizing extraction and multifunction adsorption cleanup
CID	Collision-induced dissociation
CNTs	Carbon nanotubes
dSPE	Dispersive solid phase extraction
ESI	Electrospray ionization
EU	European union
GC	Gas chromatography
GCB	Graphitized carbon black
GC-MS	Gas chromatography coupled with mass spectrometer
GC-MS/MS	Gas chromatography coupled with tandem mass spectrometer
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
LC-MS	Liquid chromatography coupled with mass spectrometer
LC-MS/MS	Liquid chromatography coupled with tandem mass spectrometer
LLE	Liquid–liquid extraction
MAE	Microwave-assisted extraction
MRL	Maximum residue level
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MSPD	Matrix solid phase dispersion
MWCNTs	Multiwalled carbon nanotubes
OPPs	Organophospohorus pesticides
PSA	Primary–secondary amine
Q	Quadrupole
QQQ	Triple quadrupoles
Q-TOF	Quadrupole-time of flight
Q-Trap	Quadrupole-linear ion trap

QuEChERS	Quick, easy, cheap, effective, rugged, safe
SCAN	Scan monitoring
SFE	Supercritical-fluid extraction
SIM	Selected ion monitoring
SPE	Solid phase extraction
SPME	Solid phase microextraction
SRM	Selected reaction monitoring
STEMIT	Single-tube extraction with multisorbent impurity trapping
SWCNTs	Single-walled carbon nanotubes
TIC	Total ion current
TOF	Time of flight
UHPLC	Ultrahigh-performance liquid chromatography
WHO	World health organization

1 Introduction

Recent years have seen a clear trend encouraging people to consume unprocessed food. An active lifestyle and a balanced diet have a decisive impact on protecting human health. Fresh fruits and vegetables hold a prominent place in this diet as they are a rich source of fiber and several vitamins and minerals important for health. According to the World Health Organization (WHO), consumption of fruits and vegetables in Europe constituted over 30% of consumer diet [1]. In unprocessed food, including raw fruits and vegetables, a considerable number of healthy bioactive compounds can be accompanied by contaminants occurring in atmospheric air, soil, and water. Unfortunately, almost all of the agricultural commodities currently cultivated and distributed are exposed to contaminants. Three main groups of contaminants are distinguished based on the impact of environmental factors: physical, chemical, and biological [2]. Chemical contamination, mainly caused by the increasing use of agrochemicals in the agricultural and food industry, constitutes a special group of contaminants. Unlike dangerous biological contamination of food immediately resulting in poisoning, chemical contaminants usually emerge with a delay because they can accumulate in human tissue over a long time and cause symptoms of disease only after reaching a critical level. The presence of chemical contaminants in food is one of the basic criteria for assessing the safety and quality of foodstuffs [2]. Plant protection products, i.e., pesticides, constitute the most varied source of food contaminants. Owing to their long-term persistence and long-term effects in living organisms, they are ranked among the most toxic chemical substances in the environment. Their presence in food poses a serious threat to the health and life of consumers. One of the protection measures against pesticide residues in food is to determine the maximum residue levels permitted in a food product, and to carry out an obligatory and systematic analytical testing of food for pesticide residue. For each pesticide used in food production, legislators specify the definition of residue, which enables the assessment of the health hazard. In Europe, maximum residue levels (MRL) of pesticides permitted in food and feed, including fruits and vegetables,

intended for human consumption were established by Regulation (EC) No 396/2005 of the European Parliament and Council on pesticide residues [3].

Recently we have seen growing public concern and scientific investigations related to the presence and control of pesticide residues in plant products to assess the potential health hazards more thoroughly. Before food products can enter a particular market, requirements for MRL must be met for a variety of pesticides [4]. Therefore, there is a need for reliable and sensitive analytical methods that are able to quantify a large number of compounds at the low limits set out in legislation [3]. With over a thousand compounds to monitor [5] in various matrices, multiresidue methods using gas and liquid chromatography coupled with tandem mass spectrometry have become the Gold Standard methodology for both quantification and semiquantitative screening of food contaminants, such as pesticides [6, 7]. Nowadays, both liquid and gas chromatography are complementary techniques covering the full range of pesticides [6, 8–12]. However, gas chromatography has the drawback of being unsuitable for a number of pesticides because of their instability and polarity [13]. Compounds which are thermally labile, not volatile, and have not been derivatized, can be separated by liquid chromatography [13]. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) can analyze a much wider range of contaminants than gas chromatography coupled with tandem mass spectrometry (GC-MS, GC-MS/MS). A general discussion of the application of GC-MS/MS and LC-MS/MS for the analysis of different chemical classes of pesticides can be found in the book chapter by Raina [14]. Liquid chromatography coupled with mass spectrometry provides a large amount of information about complex mixtures and the structure of the analyte without the need to derivatize the analyte. It also enables the screening, confirmation, and quantification of hundreds of components with one analysis. Its sample purity requirements are not stringent, and it enables a simultaneous analysis of contaminations with considerable variations of polarity [13]. Indeed, to achieve a wide scope and better sensitivity for a wide range of new generation pesticides and their metabolites, which are more polar compounds [6], it is not surprising that LC-MS/MS has become the method of choice for routine pesticides multi-residue analysis in vegetables and fruits.

This chapter presents general information about pesticides and modern methods of their identification by means of LC-MS/MS. A review and assessment was carried out of the existing techniques and methods of sample preparation, separation of multicomponent mixtures, as well as kinds of detection used in the analysis of pesticides in fruits and vegetables by means of LC-MS/MS. Special attention is paid to the QuEChERS technique and the triple quadrupole analyzer (QQQ) most frequently used in MS detection. Furthermore, pesticide residues most frequently occurring in fruits and vegetables are presented.

2 Pesticides: General Information

Pesticides have been widely used around the world since the middle of the twentieth century [8]. About 1000 active ingredients have been employed and are currently formulated in thousands of different commercial products [5]. They are part of a

large group of compounds with extremely diverse physicochemical properties and large differences with regard to polarity, volatility, and persistence [15]. They are widely used in the control or prevention plant crop diseases. Their extensive applications go beyond agriculture. Pesticides are used to reduce the number of plant and animal parasites, combat plant diseases, regulate the growth and development of plants, remove weeds, and reduce losses in food during storage and transport. Furthermore, they help increase personal hygiene by destroying domestic insects (fleas, lice, and cockroaches) and insects bearing diseases hazardous to humans such as malaria, the plague, or spotted fever [16]. Their introduction contributed significantly to increased productivity in agriculture through the stabilization of agricultural crop volume and quality. Alongside unquestionable benefits, the common use of pesticides also has an adverse impact on the natural environment, including human and animal's health and life, mainly because of the varying persistence, toxicity, bioaccumulation capacity, and mobility of pesticides [17–21]. Pesticides are classified as chemicals with a high level of toxicological hazard. They have a carcinogenic, teratogenic, and embryotoxic effects [20]. Several studies have found that human exposure to pesticides underlies many disorders such as Alzheimer's disease [22], infant mortality [23], carcinogenicity [24], neurotoxicity [25], reproductive toxicity [26], and metabolic toxicity [27]. Pesticides have an effect on both harmful and useful organisms. Their benefits and hazards are summarized in Fig. 1. The influence of pesticides on the natural environment and food quality was described extensively in studies by Aktar [28] and Fenik [15].

The great diversity of compound structure and broad spectrum of effects and applications makes a clear classification of pesticides difficult. The following criteria are used most frequently: purpose of application, chemical structure, toxicity, environmental stability, and pathways along which they penetrate target organisms [15, 16]. The classification by purpose of application is presented in Table 1. In terms of

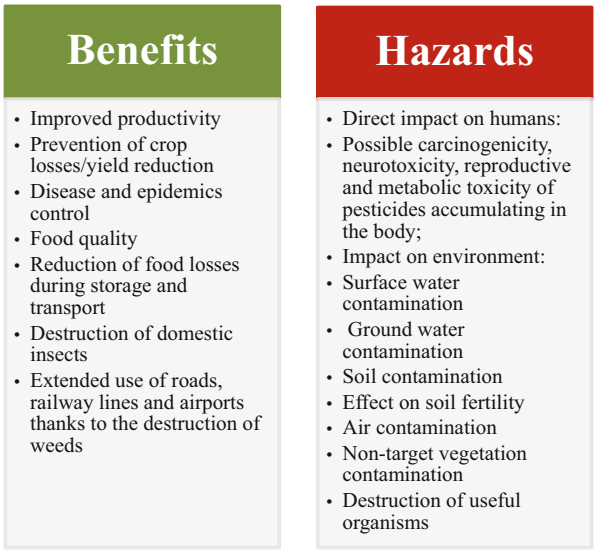


Fig. 1 The effects of using pesticides: their benefits and hazards

Table 1 Classification of pesticides based on their application [16]

Type	Purpose of application	Type	Purpose of application
Zoocides	Used to exterminate harmful vertebrates	Fungicides	Control or kill fungi
Insecticides	Control or kill insects	Herbicides	Kill or inhibit the growth of unwanted plants
Rodenticides	Control mice and other rodents		
Molluscicides	Control slugs, snails and other mollusks	Plant growth regulatory	Alter the growth of plants
Disinfectants	Control germs and microorganisms		
Bactericides	Control or kill bacteria	Defoliants	Cause plants to drop their leaves
Algicides	Control or kill algae	Desiccants	Are used to dry up living plant tissues
Virucides	Control or kill viruses	Fumigants	Produce gas or vapor intended to destroy pests
Acaricides	Control or kill mites	Repellents	Repel unwanted pests
Nematocides	Kill roundworms	Pheromones	Used to attract insects or disrupt their mating behavior
Ovicides	Control eggs of insects and mites	Synergists	Make certain pesticides more effective

chemical structure, pesticides are divided into organic and inorganic. Inorganic pesticides are used very rarely nowadays. Arsenic insecticides, fluoride insecticides, inorganic fungicides, and inorganic herbicides are examples of typical inorganic pesticides [15]. Organic pesticides are an extremely numerous group of chemical compounds, among which the following pesticides occur most often in fruits and vegetables: organophosphorus pesticides (OPPs), organochlorine pesticides, carbamate pesticides, synthetic pyrethroids, triazoles, triazines, imidazoles, strobilurin, and neonicotinoid pesticides [15, 29–31]. The most widespread food contaminants include organophosphorus pesticides (OPPs) (e.g., dichlorvos, methamidofos, malathion, methyl parathion, chlorpyrifos, diazinon, phosalone, and monocrotophos). They include all organic compounds containing phosphorus [15]. They are used mainly as insecticides but also as herbicides, fungicides, and acaricides. Their presence has been confirmed in a wide variety of fruits and vegetables such as oranges [31], apples and pears [30], bananas [30], peaches [32], grapes [10], olives [9], cauliflowers [11], cabbages [33], celeries and peppers [34], eggplants [35], potatoes and tomatoes [11].

The overview above mentions only the most frequently encountered groups of chemical compounds contaminating food, and encompasses just a fragment of the list of all pesticides permitted for use. In the literature on the subject, one can find many extensive monographic studies containing the basic definitions, bases for classification, ranges of toxicity, and persistence in the environment [16–20, 28, 36]. It should be remembered that, along with the progress of science and technology, each year encounters more and more active compounds belonging to various chemical groups permitted for use as pesticides.

3 Pesticide Legislation

In the European Union (EU), there are a number of pieces of legislation which regulate the presence of pesticide residues in food, including fruits and vegetables. The most important ones include Regulation EC/396/2005 that came into force on 1 September 2008 and defines a fully harmonized set of rules for pesticide residues for all EU countries [3]. The European Commission sets the maximum permitted levels of pesticide residues to ensure that they do not pose an unacceptable health risk for consumers. This regulation simplifies the previously existing legislation by setting European Maximum Residue Levels (MRLs). Until 5 January 2018, 120 amendments were made to the regulation (the first one on 22 February 2006 and the last one of 4 January 2018) [3]. The list of the MRLs for all the various commodities is easily accessible in the EU Pesticides database [37]. Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results [38] is another extremely important piece of legislation that must be observed by laboratories where food is analyzed by means of LC-MS/MS. This document includes definitions and descriptions of how to assess trueness, recovery, repeatability, ruggedness, as well as detailed requirements for MS detection and identification of targeted substances. This piece of legislation is critical in the context of the required number of identification points corresponding to the accurate number of precursor ion–product ion transitions, guaranteeing the reliability of the analytical method based on MS detection. Nowadays, many laboratories all over the world develop and validate their own methods for pesticide residue analysis because, depending on the analytical technique chosen, different approaches for sample treatment can be considered. Therefore, it is extremely important for the newly developed methods to be reliable. The European Commission's Directorate for Health and Food Safety (DG SANTE) provides a guidance document [39] on analytical quality control (SANCO) to laboratories for the validation of methods for pesticide residue analysis in food and feed. Laboratories performing pesticide residue analyses should meet the performance requirements detailed in the SANCO Guidance [39]. An overview of the identification criteria for LC-MS/MS in various application areas are summarized by Mol et al. [40].

The regulatory frameworks require each chemist analyst dealing with the analysis of pesticides in food to have an excellent knowledge of the current law and to apply it while carrying out LC-MS/MS analyses in the laboratory, particularly when new detection methods are being developed.

4 Approach to Pesticide Identification by Means of LC-MS/MS

In pesticide residue analysis, similarly to the analysis of other food contaminants, there is a clear tendency to prepare multiresidue methods that enable the determination of hundreds of analytes in single analysis [5, 13, 41, 42]. This approach has become a necessity because of the use of diverse active substances in agricultural

pesticides, which can lead to the cooccurrence of a wide range of pesticides with different chemical properties in food. A growing need for such analyses is also a natural consequence of introducing low maximum pesticide residue levels in the European Union and their obligatory control. The increased awareness of consumers who demand products that are safe and free from chemical contaminants is also significant.

Multiresidue methods follow the basic analytical steps including sample preparation (homogenization, extraction, and cleanup) and determination (separation and detection).

4.1 Sample Preparation

Sample preparation is the cornerstone of any effective multiresidue method. The correct extraction and preparation of the sample are of fundamental importance because they determine the quality and reliability of the determination result. The choice of the sample preparation procedure is determined by the kind of foodstuff and the chemical structure of the determined contaminants. Many different extraction procedures are used in laboratories [41, 43–45]. So far, to purify and extract organic compounds from fruits and vegetable samples, various preparation techniques have been employed, such as liquid–liquid extraction (LLE) [46], microwave-assisted extraction (MAE) [47], accelerated solvent extraction (ASE) [48], matrix solid-phase dispersion (MSPD) [49], solid-phase extraction (SPE) [50], solid-phase microextraction (SPME) [51], supercritical fluid extraction (SFE) [52], and membrane extraction techniques [53]. Advantages and disadvantages of these techniques have been discussed extensively in many papers [43–45]. The majority of the aforementioned methods are rather time-consuming, labor-intensive, complicated, and expensive, and they consume a large volume of solvent. Therefore, they fail to meet the standards of a multiresidue application [30]. Nowadays, there is a marked trend to have simple, fast, easy-to-perform procedures [54]. One example of such a procedure is the so-called Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method of pesticide analysis. This technique involves liquid–liquid partitioning using acetonitrile and purifying the extracts using dispersive solid-phase extractions. Since its development and publication [55], the QuEChERS method has been gaining significant popularity. According to Web of Science (January 2018), over 1200 papers on using the QuEChERS method for pesticide analysis in food have been already published. Now, it is the method of choice for food analysis because it combines several steps and extends the range of pesticides recovered in comparison with older, more difficult, and laborious extraction techniques (Fig. 2).

The QuEChERS procedure was developed in the years 2000–2002, and for the first time it was presented at the 4th European Pesticide Residue Workshop in Rome in 2002 by Anastassiades et al. [56]; it was published a year later [55]. It was used as a method to prepare samples for the determination of pesticide residues in fruits and

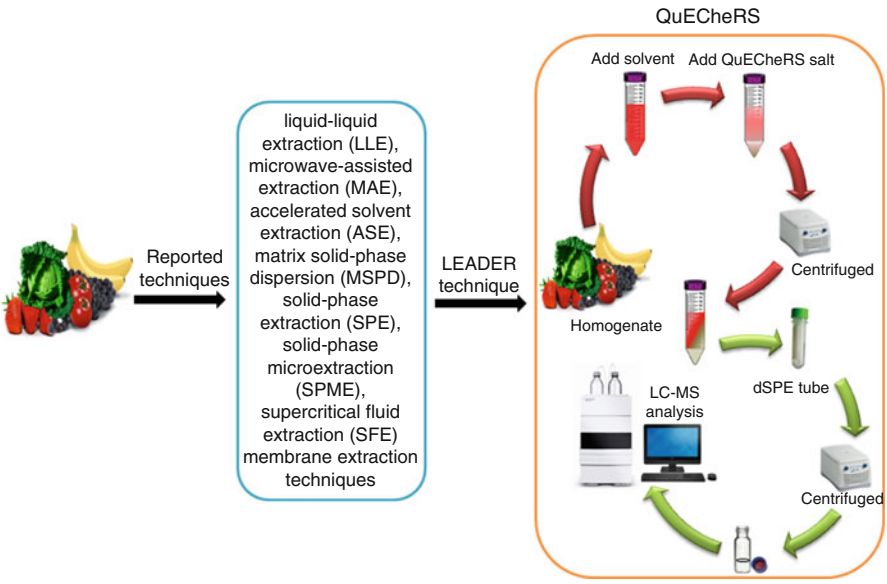


Fig. 2 Extraction techniques used to isolate pesticides from food by means of the LC-MS/MS method

vegetables by means of GC-MS [55]. Its first application in conjunction with LC-MS methods was recorded in 2005 as a continuation of research conducted by Anastassiades in 2003 [57]. The results obtained then from the validation method for over 200 different pesticides in a dozen or so fruits and vegetable samples with a varied matrix composition attracted an enormous interest in the procedure and led to its very fast growth. The basic concept of QuEChERS [55], which was developed to determine pesticide residues in fruits and vegetable samples with a high water content, is based on extraction by centrifugation of the food matrix with acetonitrile. Water is separated from acetonitrile through the addition of adequately mixed salts (anhydrous magnesium sulfate and sodium chloride). The extract is then cleaned up using dispersive solid-phase extractions (d-SPE) with anhydrous magnesium sulfate and primary-secondary amine (PSA), which efficiently removes many polar interfering substances present in the matrix.

The QuEChERS procedure [55] consists of several steps [54]. The first step is to weigh 10 g of the homogenized sample into a 50 mL polypropylene tube, then add 10 ml of acetonitrile and shake the sample vigorously for 1 min. After that, an internal standard (ISTD) is added and the tube is shaken intensively for another 1 min by hand. Next, an addition of salt mixture (4 g anhydrous magnesium sulfate and 1 g sodium chloride) is followed by intense agitation. The whole sample is again shaken vigorously for 1 min and centrifuged. Afterwards, 1 mL of organic supernatant (upper acetonitrile layer) is transferred into dispersive solid phase extraction (d-SPE) tubes containing 150 mg of anhydrous magnesium and 50 mg of PSA. Then the sample is shaken vigorously for 1 min and centrifuged. The obtained supernatant

is transferred to an autosampler vial for LC-MS/MS analysis. The schematic flow chart for the main steps is presented in Fig. 2.

Depending on the objective of the analysis, the type of analysed compounds, kind and composition of the matrix, the original procedure [55] is subjected to various modifications. To apply the QuEChERS method to a wide range of pesticides, including pH-sensitive pesticides, the original procedure was modified with buffers. Lehotay et al. modified the method to use a relatively strong acetate as a buffer and the method became the AOAC Official Method 2007.01 [58]. Annastassiades et al. chose citrate as the buffer and this version was named by the European Committee for Standardization (CEN) as Standard Method EN 15662 [59]. Other frequent modifications include: changes in the composition and/or proportion of the salts used in extraction [60], changes in the composition and/or proportion of the sorbents used [10, 12, 61], using various proportions of buffers: citrate [12, 32, 62, 63] or acetate [12, 32, 63] and (in order to maintain the optimum pH of the sample within the 5–5.5 range) using appropriate acids [29, 60] or bases [29]. One should also note other variants of the method that are based on omitting the cleanup step completely [40], using the sorbent deposit in the form of a column [64], replacing acetonitrile with ethyl acetate as the organic solvent [65], or introducing an additional step where the analytes are concentrated in the cleaned up extract by evaporating and/or replacing the solvent [61, 66, 67]. Lee et al. proposed an interesting modification of the method [68]. To improve the recovery of some problematic highly polar pesticides in pepper (*Capsicum*), they introduced the dry ice-partitioning QuEChERS method. This approach uses dry ice to separate a sample extract into the acetonitrile layer and aqueous layer without the need for salting out and centrifugation [68]. The protocol developed involves two extraction methods. The first detects the acetonitrile layer for general pesticides, and the second combines the acetonitrile and the aqueous layer for propamocarb, pymetrozine, and flonicamid metabolites [68].

The selection of the right sorbent in sample cleanup is a key stage of the QuEChERS procedure. In dispersive solid-phase extractions (d-SPE) used in QuEChERS, the traditional sorbent deposit was replaced with loose sorbent, added directly to the sample solution. The method involves the d-SPE cleanup procedure whereby the extract is shaken with a small amount of sorbents and $MgSO_4$ [45]. Among the different kinds of d-SPE sorbents, primary secondary amine (PSA) is the most commonly used in the QuEChERS method; it is used to remove polar organic acid, fatty acids, polar pigments, and some sugar [69]. In some modified QuEChERS methods, graphitized carbon black (GCB) and octadecyl silica (C_{18}) are applied to remove sterols, pigments, and nonpolar interfering substances [69]. GCB is useful for the purification of carotinoids, chlorophyll, and sterols with the disadvantage of losses of planar analytes [54]. C_{18} provides good results in the purification of samples with significant fat and wax content but recoveries of the more lipophilic pesticides may suffer [54]. One of the new sorbents recently used in the QuEChERS method are carbon nanotubes (CNTs). Based on the principle of carbon atom layers in the wall of nanotubes, the carbon nanotubes are classified as multiwalled carbon nanotubes (MWCNTs) and single-walled carbon nanotubes (SWCNTs) [69–72].

MWCNTs exhibit a good affinity for polycyclic compounds and could be used as effective d-SPE sorbents to adsorb the interfering substances in fruits and vegetable matrices owing to their unique structure and huge surface area [69]. They have been used for the cleanup of complex matrices such as grape, mango juice, wheat, spinach, carrot, apple, citrus, peanut, leek, and leaf lettuce [10, 50, 69, 71]. The application of different d-SPE sorbents for the QuEChERS cleanup step of various matrix types is extensively described by Rajczak and Tuzimski [54].

The QuEChERS extraction technique makes it possible to reduce the number of the necessary steps of analysis, reagents, and laboratory glassware. It also simplifies the step of analyte extraction and extract cleanup. In comparison with other extraction techniques, it is characterized by very good results in removing matrix ingredients and high recovery of the target compounds [45, 54, 69]. The QuEChERS approach is also in accordance with the principles of green chemistry due to low solvent consumption and absence of chlorinated solvents and very small generation of waste [54]. Since the introduction of the QuEChERS method, the literature on the subject has presented numerous examples of its use to determine pesticides in a whole range of food products [12, 73] as well as studies reviewing the method [44, 54, 73] and comparing it with other extraction techniques [12, 45].

4.2 Sample Determination

The accurate determination of pesticides in complex matrices such as fruits and vegetables is a challenge due to the complexity of the matrix and low mass fraction levels to be dealt with. The choice of appropriate separation and detection techniques is a step of fundamental importance.

Most multiresidue methods (MRMs) reported for fruits and vegetables are based on the use of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which is a powerful technique that provides excellent selectivity and sensitivity, allowing a reliable identification and quantification of a wide range of pesticides and their metabolites [42]. The most important advantages of coupling LC with MS/MS include: a clear identification of sample components, very high sensitivity of the analytical system, possibility of identifying all or most of the sample components, high repeatability of measurements, possibility of quantitative analysis (generating real chromatographic peaks by the LC system), possibility of distinguishing substances with identical molecular mass but different retention times (isomers), and possibility of obtaining additional information about each sample component by means of fragmentation [42, 74, 75]. Using multiresidue methods in chromatographic determinations, the problem of the coelution of sample components occurs. Coupling liquid chromatography with mass spectrometry allows the identification of the studied compound based on the mass spectrum, enabling the quantification of substances even if they are not fully chromatographically separated [74]. This technique provides information regarding the characteristic ion of each analyte as well as two or more transitions of these ions, which is useful for

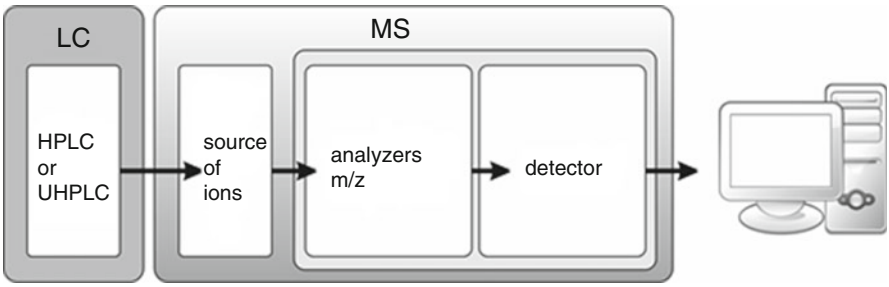


Fig. 3 Block diagram of the LC-MS/MS system

quantifying and confirming analytes at concentrations consistent with the maximum residue levels (MRLs) established [76].

In a typical LC-MS/MS system, the studied sample is first separated in the LC system and its consecutive fractions flowing out of the chromatographic column are subjected to ionization and introduced into the spectrometer. The typical LC-MS/MS system is shown in Fig. 3.

Liquid chromatography is one of the most widespread methods of separating complex samples. It is used for the analysis polar and/or nonvolatile and thermally labile pesticides for which GC conditions are not suitable. Most of the procedures used to determine pesticides in fruits and vegetables employ the HPLC technique in a reversed phase (RP) system, using gradient elution with a linear increase of the percentage of the organic solvent [69, 77]. The common stationary phases are based mainly on the nonpolar octadecylsilane phase (C_{18}) [69, 78, 79]; the medium-polar octylsilane (C_8) phase is used much more rarely [80]. The mobile phase consists of a mixture of acetonitrile, methanol, and water in various volume ratios [40, 60, 78]. In order to improve the ionization capacity, the use of different additives to the mobile phases has been described such as organic acid (formic [69, 78] and acetic [34]), ammonium salts (ammonium formate [60, 77, 78] or ammonium acetate [33, 67]) or a combination of an organic acid with its ammonium salt [77, 79]. One of the crucial technological revolutions in LC was the implementation of ultrahigh pressure liquid chromatography (UHPLC). The particle size of the solid phase was reduced from 5 μm to sub-2 μm resulting in an enhanced resolution in a shorter runtime [81]. The use of short columns with the particle diameter not larger than 2 μm was first proposed by Waters in 2004 [82]. The UHPLC system is currently the optimum solution for the separation of multicomponent mixtures.

Tandem mass spectrometry (MS/MS) is a key analytical technique enabling the identification of pesticides in different kinds of fruits and vegetables. The basic principle of MS/MS is the selection of the precursor ion, fragmentation of this ion, usually by collision-induced dissociation (CID), and measurement of the m/z ratio of the product ion formed [75]. Ionization in LC-MS/MS is mainly based on atmospheric pressure ionization (API), which enables the introduction of considerable

volumes of solutions flowing out of the chromatographic column into the mass spectrometer without the risk of losing a high vacuum [83]. The analyzers and detector of the mass spectrometer are kept in a high vacuum to avoid accidental collisions with air molecules [13]. The most frequently used ionization methods in pesticide determination include electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) [7, 13]. However, APCI appears to have been overshadowed by the extreme popularity of ESI [7]. A discussion about the application of both of these ionization methods in the LC-MS/MS analysis of pesticides can be found in our previous paper [13]. The analyzer is the most important part determining the parameters of a spectrometer. Tandem mass spectrometry (MS/MS) is a coupled system of two analyzers of the same type or different types, characterized by a high separation efficiency [13]. The current trend in food control is the use of tandem MS such as a triple quadrupole (QQQ) as well as high-resolution mass spectrometers (HRMS) such as quadrupole-time of flight (Q-TOF) and quadrupole-linear ion trap (Q-Trap). Among all of the above, the triple quadrupole (QQQ) is the most common MS analyzer in LC-based methods for multiresidue analysis in food nowadays. This is because of their excellent quantification and identification properties for a group of target compounds [7, 41]. As has been described previously by Stachniuk and Fornal [13], the conventional QQQ instrument is a serial connection of quadrupole analyzers, the middle of which performs the role of the collision cell. The first quadrupole only allows the so-called precursor ions (ions of a specific m/z value) to pass through. The precursor ions selected in the first analyzer are fragmented in the collision cell, filled with neutral gas and constituting the second quadrupole. The fragmentation spectrum, i.e., the m/z values of product ions, is recorded in the detector. When the third quadrupole allows only the selected product ions, the spectrometer works in the multiple reaction monitoring mode (MRM). When the third quadrupole allows all product irons, it works in the scan mode (full mass spectrum collection). Working in the scan mode, a tandem system of the QQQ type operates like a classic quadrupole analyzer. When measuring the full spectrum, the first two quadrupoles function as beam collimators, and the entire analysis is performed in the third quadrupole [13]. Thus, a mass spectrometer of the QQQ type can work with different settings for ion analysis parameters. First, MS can work in the positive or negative ion detection mode. Second, several ways of ion detection are possible. The first one is based on the monitoring of the total ion current (TIC) from the preset m/z range. The second one is based on monitoring only selected ions (SIM) characteristic of the compounds searched for. The third one, multiple reaction monitoring (MRM) (also called selected reaction monitoring (SRM)), enables the confirmation of the presence of fragmented ions formed from a specific precursor ion [13]. QQQ analyzers are used mainly for quantification and confirmation purposes. Typically, two or three MRM transitions are selected for a targeted analysis of pesticide residues: one for quantification and an additional one for confirmation purposes [62].

The use of the MRM mode in QQQ instruments for the determination of pesticide residue in food seems to have certain limitations: they require acquisition parameter

optimization for each compound analyzed, the number of the analyzed compounds is limited, only compounds from the target list can be detected, and retrospective data analysis is impossible [78]. Therefore, there has been a move to an alternative approach using HRMS capable of providing full spectrum data. Operating in a full scan mode, HRMS offer a significantly higher analysis selectivity and can detect an unlimited number of compounds. HRMS analyzers used for pesticide residue analysis in fruits and vegetables are the Q-TOF [84] and Q-Trap [78]. However, only few papers have described the use of HRMS for quantitative purpose [6, 11, 66, 78, 84]. The triple quadrupole remains the most common instrument in quantitative target analysis. Several reviews on the subject help to interpret the trends within the field [7, 13, 41, 42, 85]. The current LC-MS/MS strategies for multiresidue methods are summarized in Table 2.

One of the most limiting factors in LC-MS/MS pesticide residue analysis is the occurrence of the matrix effect. The ionization of target analytes can be affected when competition between the analyte and coeluting components for the available charge occurs. Exo- and endogenic substances present in the matrix can interfere with the analyte in the ion source [13]. This effect may cause the suppression or enhancement of ionization, leading to quantification errors. In various review articles, different approaches have been discussed to overcome this analytical problem [13, 41, 102, 103]. As reported by Ucles et al. [103], several approaches are used to eliminate or reduce matrix effects; they include optimizing sample preparation to remove interfering compounds from the samples [104], changing LC conditions to avoid the coelution of analytes and interfering compounds [105], changing MS conditions to reduce the occurrence of the matrix effect in the ion source [106], dilution of the sample [106] or using chemical treatment measures [107] or calibration techniques such as matrix-matched calibrations [29], and the standard addition method [13].

To reduce the matrix effect and increase the accuracy of determination during the sample preparation step in multiresidue analysis of pesticides in a wide range of food matrices, matrix-matched calibrations with the addition of a single internal standard are mainly used.

5 The Most Frequently Detected Pesticides in Fruits and Vegetables

Based on the review of the literature (Table 2), it was determined how frequently pesticide residues, determined by means of LC-MS/MS, occur in fruits and vegetables. The obtained results are presented in Fig. 4.

The most frequently detected pesticide residues in the fruit group included: carbendazim, azoxystrobin, chlorpyrifos, imazalil, imidacloprid, acephate, acetamiprid, cyprodinil, dimethoate, cypermethrin, metalaxyl, methomyl, and thiabendazole. As Fig. 4 indicates, carbendazim is one of the main detectable residues in fruit, and occurred in the concentration range of 0.0026–3.4 mg kg^{-1} as reported in eight different papers. The residues of carbendazim detected in

Table 2 Current LC-MS/MS strategies for the analysis of pesticide residues in fruits and vegetables (2017–2010)

No.	Matrix	No. of analytes	Extraction methods	Detector MS	Ionization methods	MS mode	Refs.
1	Mango	68	modified QuEChERS	Q-TOF	ESI	scan, EIC	[84]
2.	Goji berry	8	QuEChERS	QQQ	ESI	MRM	[86]
3.	Fruits and vegetables	60	QuEChERS	QQQ	ESI	MRM	[29]
4.	Cabbage	7	QuEChERS	QQQ	ESI	SRM	[33]
5.	Spring onion	5	QuEChERS	QQQ	ESI	MRM	[87]
6.	Tomato	109	QuEChERS	QQQ	ESI	MRM	[60]
7.	Oranges	115	QuEChERS	QQQ	ESI	MRM	[31]
8.	Peach, grapes, bananas, apples, pears, strawberries	17	QuEChERS	QQQ	ESI	MRM	[30]
9.	Tropical fruits	30	QuEChERS	QQQ	ESI	MRM	[77]
10.	Grape and mango juices	41	QuEChERS	Q-trap	APPI	MRM	[10]
11.	Tomato	57	QuEChERS	QQQ	ESI	MRM	[88]
12.	Leek, leaf lettuce,	70	Modified QuEChERS	QQQ	ESI	SRM	[69]
13.	Soya	10	QuEChERS	QQQ	ESI	MRM	[61]
14.	Apple, cucumber	120	QuEChERS	QQQ	ESI	SRM	[62]
15.	Fruits and vegetables	60	Modified QuEChERS	TOF	ESI	Scan, EIC	[11]
16.	Fruits and vegetables	100	LLE	Q-TOF	ESI	Scan, EIC	[6]
17.	Fruits and vegetables	186	QuEChERS	QQQ	ESI	MRM	[89]
18.	Apple, guava, kaki, peach	46	Modified QuEChERS	QQQ	ESI	MRM	[65]
19.	Fruits and vegetables	199	QuEChERS	Q-TOF	ESI	Scan, EIC	[66]
20.	Bananas	128	QuEChERS	QQQ	ESI	MRM	[90]
21.	Olives	90	QuEChERS	QQQ	ESI	MRM	[9]
22.	Fruits and vegetables	6	LLE	QQQ	ESI, APCI	SRM	[91]
23.	Fruits and vegetables	100	QuEChERS	QQQ	ESI	SRM	[34]
24.	Vegetables	44	QuEChERS	QQQ	ESI	MRM	[92]
25.	Fruits	29	LLE	Q-TOF	ESI	MRM	[93]
26.	Fruits and vegetables	17	QuEChERS	QQQ	ESI, APCI	MRM	[94]
27.	Vegetables	18	QuEChERS	Q-trap	ESI	MRM	[35]

(*continued*)

Table 2 (continued)

No.	Matrix	No. of analytes	Extraction methods	Detector MS	Ionization methods	MS mode	Refs.
28.	Cucumber, tomato, apple, lettuce, grape	5	QuEChERS	QQQ	ESI	MRM	[81]
29.	Fruits and vegetables	150	QuEChERS	QQQ	ESI	SRM	[80]
30.	Fruits and vegetables	20	Modified QuEChERS	QQQ	ESI	MRM	[95]
31.	Paprika	168	Modified QuEChERS	QQQ	ESI	MRM	[68]
32.	Fruits and vegetables	54	LEE	QQQ	ESI	SRM	[46]
33.	Fruits and vegetables	32	QuEChERS	QQQ	ESI	MRM	[12]
34.	Fruits and vegetables	14	QuEChERS	QQQ	ESI	SRM	[96]
35.	Onion	5	MSPD	QQQ	ESI	MRM	[49]
36.	Olives	104	(I) QuEChERS, (ii)MSPD	QQQ	ESI	MRM	[97]
37.	Eggplant, citrus fruits	10	(I) QuEChERS, (ii) CHEMAC, (iii)STEMIT	QQQ	ESI	MRM	[98]
38.	Fruits and vegetables	11	LLE	QQQ, Q-TOF	ESI	SRM	[99]
39.	Grape	150	QuEChERS	Q-trap	ESI	MRM	[100]
40.	Fruits and vegetables	69	QuEChERS	QQQ	ESI	MRM	[101]

fruit samples are summarized in Table 3. The highest level of carbendazim (3.4 mg kg^{-1}) was recorded in a papaya sample [77].

The most frequently detected pesticide residues in the vegetables group included: acetamiprid, imidacloprid, carbendazim, azoxystrobin, chlorpyrifos, acephate, cypermethrin, dimethoate, metalaxyl, cyprodinil, imazalil, methomyl, and thiabendazole. The presence of the most frequently occurring acetamiprid was also confirmed by eight authors as was the case with fruits (Fig. 4). The results of the survey for acetamiprid are presented in Table 4. Monitoring data showed that the mean acetamiprid concentrations varied from 0.01 to 0.86 mg kg^{-1}. The highest acetamiprid level was found in a lettuce sample with the concentration of 0.86 mg kg^{-1} [89].

The results shown in Tables 3 and 4 were compared with the current maximum residue levels permitted by the European Union as of 5 January 2018 [3]. It should

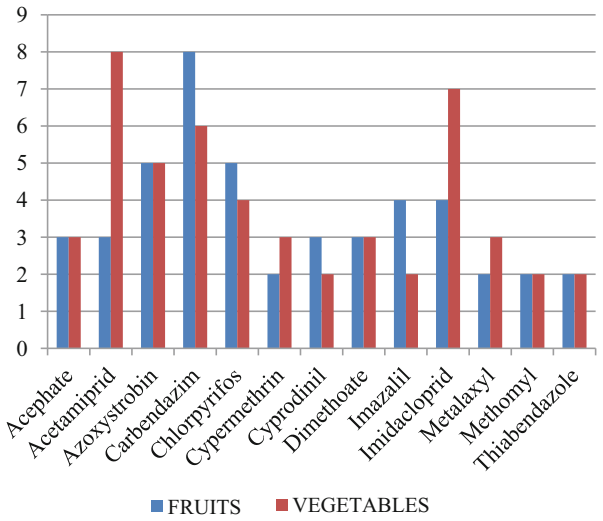


Fig. 4 The frequency of the most commonly detected pesticides in fruits and vegetables, based on the reviewed papers from Table 1

Table 3 Residues of carbendazim in fruit samples, based on the reviewed papers from Table 1

Matrix	Total	Positive	MRL[a] (mg kg^{-1})	Range min–max (mg kg^{-1})	Refs.
Tamarillo	No define	1	0.1	~0.05	[93]
Lulo	2	2	0.1	0.019–1.34	[77]
Carambolo	2	1	0.1	0.0021	
Granadilla	2	1	0.1	0.66	
Mangostan	2	1	0.1	0.0035	
Tamorillo	2	2	0.1	0.08–0.29	
Gulupa	2	2	0.1	0.0026–0.0072	
Maracuya	2	2	0.1	0.03–0.21	
Papaya	2	1	0.2	3.4	
Feijoa	2	1	0.1	0.0032	
Citrus	120	5	0.1	0.01–0.02	[98]
Peaches	No define	No define	0.2	0.02–0.178	[30]
Apple	No define	1	0.2	0.088	
Black currant	36	9	0.1	0.07–0.51	[29]
Red currant	32	5	0.1	0.06–0.13	
Banana	128	1	0.1	0.024	[90]
Apple	32	No define	0.2	1.304–1.325	[11]
Guava	12	No define	0.1	0.011–1.445	

(*continued*)

Table 3 (continued)

Matrix	Total	Positive	MRL[a] (mg kg^{-1})	Range min–max (mg kg^{-1})	Refs.
Apple	64	4	0.2	0.02–0.09	[89]
Apricot	9	2	0.2	0.04–0.1	
Banana	17	1	0.1	0.63	
Cherry	16	5	0.5	0.011–0.068	
Grape	72	9	0.3	0.03–0.215	
Kiwi	5	1	0.1	0.03	
Lemon	28	6	0.7	0.014–0.334	
Orange	29	3	0.2	0.01–0.124	
Peaches	20	3	0.2	0.024–0.151	
Pear	22	1	0.2	0.235	
Strawberry	14	1	0.1	0.02	

[a]The current maximum residue levels permitted by the European Union as of 5 January 2018 [3]

Table 4 Residues of acetamiprid in vegetables samples, based on the reviewed papers from Table 1

Matrix	Total	Positive	MRL[a] (mg kg^{-1})	Range min-max (mg kg^{-1})	Refs.
Arugula	11	1	3.0	0.102	[89]
Aubergine	26	11	0.2	0.018–0.23	
Bean	15	1	0.15	0.01	
Cucumber	49	9	0.3	0.016–0.069	
Lettuce	29	6	3.0	0.01–0.86	
Mushroom	58	1	0.01	0.017	
Pepper	59	29	0.3	0.01–0.79	
Purslane	5	3	3.0	0.028–0.08	
Tomato	118	58	0.5	0.01–0.29	
Zucchini	8	5	0.3	0.02–0.241	
Green pepper	2	1	0.1	0.024	[34]
Broccoli	8	2	0.4	0.014–0.023	[29]
Green chili	1	1	0.3	0.015	[92]
Paprika	50	1	0.3	<MRL	[68]
Tomato	60	6	0.5	0.0–0.03	[88]
Tomato	1471	1	0.5	0.03	[101]
Tomato	345	23	0.5	0.015–0.37	[60]

[a]The current maximum residue levels permitted by the European Union as of 5 January 2018 [3]

be remembered that these levels are constantly updated, and different levels may apply to a fruit or vegetable depending on the date of the analysis. The changes of the maximum residue levels from since the time Regulation (EC) No 396/2005 came into force, using the example of carbendazim in orange and lemon samples as well as acetamiprid in broccoli and tomato samples, are presented in Tables 5 and 6, respectively.

Table 5 Evolution of MRLs for carbendazim in orange and lemon [37]

	Reg. (EU) No 559/2011	Reg.(EU) No 893/2010	Reg. (EU) No 839/2008	Reg. (EU) No 149/2008
Lemon	0.7	0.7	0.5	0.5
Orange	0.2	0.5	0.5	0.5

Table 6 Evolution of MRLs for acetamiprid in broccoli and tomato [37]

	Reg. (EU) No 625/2017	Reg.(EU) No 1902/2016	Reg. (EU) No 401/2015	Reg. (EU) No 500/2013	Reg. (EU) No 978/2011	Reg. (EU) No 508/2011	Reg. (EU) No 149/2008
Broccoli	0.4	0.4	0.4	0.4	0.3	0.01	0.01
Tomato	0.5	0.5	0.2	0.2	0.15	0.1	0.1

The differences of the results obtained in various papers suggest that further and continuous investigation of pesticide residues in fruits and vegetables is necessary to ensure consumer safety.

6 Conclusions

The analysis of pesticide residues in fruits and vegetables is a key issue that is strongly related to human health. For more than a decade, a lot of progress has been made in multiresidue LC-MS/MS methods for pesticide analysis in terms of the ease of preparation, analysis time, and number of targeted compounds with concomitant improvements of instruments performance. Today, high performance liquid chromatography coupled with triple quadrupole mass spectrometry in combination with QuEChERS extraction is the most used technique for pesticide residue analysis in fruits and vegetables. Operating in the multiple-reaction monitoring (MRM) mode provides a high degree of sensitivity and selectivity, and is highly suited for quantitative identification and determination of target analytes in samples. However, it does not allow the identification of nontarget compounds. Therefore, the use of nontargeted screenings employing high-resolution mass spectrometers (HRMS), e.g., quadruple-time-of-flight (QTOF) mass analyzers, is worth considering. Both (QQQ and QTOF) are complementary tools to develop large-scale screening methods. The conducted review of data provided in the literature reveals a considerable variation in the contamination of fruits and vegetables with pesticides. At the same time, it shows that monitoring and pesticide residue determination methods for various groups of fruits and vegetables should be continually developed and improved in order to fully protect the health of consumers.

Acknowledgments Author gratefully acknowledges the use of the facilities and services of the Center for Interdisciplinary Research at The John Paul II Catholic University of Lublin, Poland, co-funded by the European Union from the European Regional Development Fund in the frame of the Operational Programme Development of Eastern Poland 2007–2013 (POPW.01.03.00-06-003/09-00). This work was supported by the Polish National Science Centre via the research project DEC-2017/01/X/ST4/00722.I also acknowledge assistance in preparation of tables provided by Ms Agata Surma.

References

1. Szpyrka E, Kurdziel A, Matyaszek A, Podbielska M, Rupar J, Słowik-Borowiec M (2015) Evaluation of pesticide residues in fruits and vegetables from the region of South-Eastern Poland. Food Control 48:137–142
2. Łozowicka B (2009) Chemical contaminants in food of plant origin. Progr Plant Protect 49:2071–2080
3. Commission Regulation (EC) 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal orgin, Official Journal of the European Union, L 70/1
4. Dehouck P, Grimalt S, Dabrio M, Cordeiro F, Fiamegos Y, Robouch P (2015) Proficiency test on the determination of pesticide residues in grapes with multi-residue methods. J Chromatogr A 1395:143–151
5. Hanot V, Goscinny S (2015) Deridder M (2015) a simple multi-residue method for the determination of pesticides in fruits and vegetables using a methanolic extraction and ultra-high-performance liquid chromatography-tandem mass spectrometry: optimization and extension of scope. J Chromatogr A 1384:53–66
6. Goscinny S, Joly L, De Pauw E, Hanot V, Eppe G (2015) Travelling-wave ion mobility time-of-flight mass spectrometry as an alternative strategy for screening of multi-class pesticides in fruits and vegetables. J Chromatogr A 1405:85–93
7. Hird SJ, Lau BPY, Schuhmacher R, Krska R (2014) Liquid chromatography-mass spectrometry for the determination of chemical contaminants in food. Trends Anal Chem 59:59–72
8. Alder L, Greulich K, Kempe G, Vieth B (2006) Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS/MS? Mass Spectrom Rev 25:838–865
9. Anagnostopoulos C, Miliadis GE (2013) Development and validation of an easy multiresidue method for the determination of multiclass pesticide residues using GC-MS/MS and LC-MS/MS in olive oil and olives. Talanta 112:1–10
10. Deme P, Upadhyayula VVR (2015) Ultra performance liquid chromatography atmospheric pressure photoionization high resolution mass spectrometric method for determination of multiclass pesticide residues in grape and mango juices. Food Chem 173:1142–1149
11. Sivaperumal P, Anand P, Riddhi L (2015) Rapid determination of pesticide residues in fruits and vegetables, using ultra-high-performance liquid chromatography/time-of-flight mass spectrometry. Food Chem 168:356–365
12. Lehotay SJ, Son KA, Kwon H, Koesukwiwat U, Fu W, Mastovska K (2010) Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. J Chromatogr A 1217:2548–2560
13. Stachniuk A, Fornal E (2016) Liquid chromatography-mass spectrometry in the analysis of pesticide residues in food. Food Anal Methods 9:1654–1665
14. Raina R (2011) Chemical analysis of pesticides using GC/MS, GC/MS/MS, and LC/MS/MS. In: Pesticides strategies for pesticides analysis. InTech, Rijeka, pp 105–130
15. Fenik J, Tankiewicz M, Biziuk M (2011) Properties and determination of pesticides in fruits and vegetables. Trends Anal Chem 30:814–826
16. Biziuk M (2001) Pesticides [book in polish]. Wydawnictwo Naukowo Techniczne, Warszawa
17. Dich J, Zahm SH, Hanberg A, Adami HO (1997) Pesticides and cancer. Cancer Causes Control 8(3):420–443

18. Eddleston M, Bateman DN (2012) Pesticides. Medicine 40:147–150
19. Gilden RC, Huffling K, Sattler B (2010) Pesticides and health risks. J Obstet Gynecol Neonatal Nurs 39:103–110
20. Kamrin MA (2000) Pesticides profiles. Toxicity, environmental impact, and fate. Lewis Publishers, New York
21. de Albuquerque NCP, Carrão DB, Habenschus MD, de Oliveira ARM (2018) Metabolism studies of chiral pesticides: a critical review. J Pharm Biomed 147:89–109
22. Hayden KM, Norton MC, Darcey D, Østbye T, Zandi PP, Breitner JCS (2010) Occupational exposure to pesticides increases the risk of incident AD: the Cache County study. Neurology 74:1524–1530
23. Cremonese C, Freire C, De Camargo AM, De Lima JS, Koifman S, Meyer A (2014) Pesticide consumption, central nervous system and cardiovascular congenital malformations in the south and southeast region of Brazil. Int J Occup Med Env 27:474–486
24. VoPham T, Brooks MM, Yuan J-M, Talbott EO, Ruddell D, Hart JE (2015) Pesticide exposure and hepatocellular carcinoma risk: a case-control study using a geographic information system (GIS) to link SEER-Medicare and California pesticide data. Environ Res 143:68–82
25. Costa LG, Giordano G, Guizzetti M, Vitalone A (2008) Neurotoxicity of pesticides: a brief review. Front Biosci 13:1240–1249
26. Chevrier C, Warembourg C, Gaudreau E, Monfort C, Le Blanc A, Guldner L (2013) Organochlorine pesticides, polychlorinated biphenyls, seafood consumption, and time-to-pregnancy. Epidemiology 24:251–260
27. Evangelou E, Ntritsos G, Chondrogiorgi M, Kavvoura FK, Hernández AF, Ntzani EE (2016) Exposure to pesticides and diabetes: a systematic review and meta-analysis. Environ Int 91:60–68
28. Aktar W, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2:1–12
29. Stachniuk A, Szmagara A, Czeczko R, Fornal E (2017) LC-MS/MS determination of pesticide residues in fruits and vegetables. J Environ Sci Health B 52(7):446–457
30. Christia C, Bizani E, Christophoridis C, Fytianos K (2015) Pesticide residues in fruit samples: comparison of different QuEChERS methods using liquid chromatography-tandem mass spectrometry. Environ Sci Pollut Res Int 22:13167–13178
31. Golge O, Kabak B (2015) Determination of 115 pesticide residues in oranges by high-performance liquid chromatography-triple-quadrupole mass spectrometry in combination with QuEChERS method. J Food Compos Anal 41:86–97
32. Costa FP, Caldas SS, Primel EG (2014) Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in canned and fresh peach. Food Chem 165:587–589
33. Prodhan MDH, Papadakis EN, Papadopoulou-Mourkidou E (2016) Analysis of pesticide residues and their variability in cabbage using QuEChERS extraction in combination with LC-MS/MS. Food Anal Methods 9:3470–3478
34. Núñez O, Gallart-Ayala H, Ferrer I, Moyano E, Galceran MT (2012) Strategies for the multi-residue analysis of 100 pesticides by liquid chromatography-triple quadrupole mass spectrometry. J Chromatogr A 1249:164–180
35. Sinha SN, Vasudev K, Vishnu Vardhana Rao M (2012) Quantification of organophosphate insecticides and herbicides in vegetable samples using the "quick easy cheap effective rugged and safe" (QuEChERS) method and a high-performance liquid chromatography-electrospray ionisation-mass spectrometry (LC-MS/MS). Food Chem 132:1574–1584
36. Mahour R, Khan MF, Forbes S, Perez-Estrada LA (2014) Pesticides and herbicides. Water Environ Res 86:1545–1578
37. Pesticides Database https://ec.europa.eu/food/plant/pesticides/eu-pesticides-%C2%A0database/public/?event=pesticide.residue.selection&language=EN
38. Official Journal of the European Union L 221/8 (2002) Commission decision of 12 august 2002 implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

39. SANCO/12571/2013 (2013) Guidance document on analytical quality control andvalidation procedures for pesticide residues analysis in food and feed
40. Mol HGJ, Zomer P, García López M, Fussell RJ, Scholten J, de Kok A (2015) Identification in residue analysis based on liquid chromatography with tandem mass spectrometry: experimental evidence to update performance criteria. Anal Chim Acta 873:1–13
41. Botitsi HV, Garbis SD, Economou A, Tsipi DF (2011) Current mass spectrometry strategies for the analysis of pesticides and their metabolites in food and water matrices. Mass Spectrom Rev 30:907–939
42. Malik AK, Blasco C, Picó Y (2010) Liquid chromatography-mass spectrometry in food safety. J Chromatogr A 1217:4018–4040
43. Lambropoulou DA, Albanis TA (2007) Methods of sample preparation for determination of pesticide residues in food matrices by chromatography-mass spectrometry-based techniques: a review. Anal Bioanal Chem 389(6):1663–1683
44. Wilkowska A, Biziuk M (2011) Determination of pesticide residues in food matrices using the QuEChERS methodology. Food Chem 125:803–812
45. Stachniuk AM, Fornal E (2016) Extraction techniques applied to LC-MS determination of pesticide residues in food. Zywnosc Nauka Technologia Jakosc/Food Science Technology Quality 2
46. Fenoll J, Hellín P, Martínez CM, Flores P (2010) Multiresidue analysis of pesticides in vegetables and citrus fruits by LC–MS–MS. Chromatographia 72:857–866
47. Papadakis EN, Vryzas Z, Papadopoulou-Mourkidou E (2006) Rapid method for the determination of 16 organochlorine pesticides in sesame seeds by microwave-assisted extraction and analysis of extracts by gas chromatography-mass spectrometry. J Chromatogr A 1127:6–11
48. Jia Z, Mao X, Chen K, Wang K, Ji S (2010) Comprehensive multiresidue method for the simultaneous determination of 74 pesticides and metabolites in traditional Chinese herbal medicines by accelerated solvent extraction with high-performance liquid chromatography/tandem mass spectrometry. J AOAC Int 93:1570–1588
49. Rodrigues SA, Caldas SS, Primel EG (2010) A simple; efficient and environmentally friendly method for the extraction of pesticides from onion by matrix solid-phase dispersion with liquid chromatography-tandem mass spectrometric detection. Anal Chim Acta 678:82–89
50. Kamel A, Qian Y, Kolbe E, Stafford C (2010) Development and validation of a multiresidue method for the determination of neonicotinoid and macrocyclic lactone pesticide residues in milk, fruits, and vegetables by ultra-performance liquid chromatography/MS/MS. J AOAC Int 93:389–399
51. Wardencki W, Michulec M, Curyło J (2004) A review of theoretical and practical aspects of solid-phase microextraction in food analysis.aspects of solid-phase microextraction in food analysis. Int J Food Sci Tech 39:703–717
52. Herrero M, Mendiola JA, Cifuentes A, Ibáñez E (2010) Supercritical fluid extraction: recent advances and applications. J Chromatogr A 1217:2495–2511
53. Moeder M, Bauer C, Popp P, Van Pinxteren M, Reemtsma T (2012) Determination of pesticide residues in wine by membrane-assisted solvent extraction and high-performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 403:1731–1741
54. Rejczak T, Tuzimski T (2015) A review of recent developments and trends in the QuEChERS sample preparation approach. Open Chem 13:980–1010
55. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int 86:412–431
56. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ (2002) Quick, easy, cheap, effective, rugged and safe (QuEChERS) approach for the determination of pesticide residues, European pesticide residues workshop (EWPR). Book of Abstracts, Rome
57. Lehotay SJ, De Kok A, Hiemstra M, Van Bodegraven P (2005) Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. J AOAC Inter 88:595–614

58. AOAC (2007) AOAC official method, 2007.01, pesticide residues in foods by, acetonitrile extraction and partitioning with magnesium sulfate
59. European Standard EN 15662 (2008) Foods of plant origin – Determination of pesticide residues using GC–MS and/or LC–MS/MS following acetonitrileextraction/partitioning and clean-up by dispersive SPE-QuEChERS-method. European Committee for Standardization
60. Golge O, Kabak B (2015) Evaluation of QuEChERS sample preparation and liquid chromatography-triple-quadrupole mass spectrometry method for the determination of 109 pesticide residues in tomatoes. Food Chem 176:319–332
61. Huertas Pérez JF, Sejerøe-Olsen B, Fernández Alba AR, Schimmel H, Dabrio M (2015) Accurate determination of selected pesticides in soya beans by liquid chromatography coupled to isotope dilution mass spectrometry. Talanta 137:120–129
62. Ramadan G, Al Jabir M, Alabdulmalik N, Mohammed A (2016) Validation of a method for the determination of 120 pesticide residues in apples and cucumbers by LC-MS/MS. Drug Test Anal 8:498–510
63. Souza DF, Souza EL, Borges EM (2016) Determination of pesticides in grape juices by QuEChERS and liquid chromatography-tandem mass spectrometry. J Brazilian. Chem Soc 27:1626–1635
64. Takatori S, Okihashi M, Okamoto Y, Kitagawa Y, Kakimoto S, Murata H (2008) A rapid and easy multiresidue method for the determination of pesticide residues in vegetables, fruits, and cereals using liquid chromatography/tandem mass spectrometry. J AOAC Int 91(4):871–883
65. Jardim ANO, Mello DC, Goes FCS, Frota EF, Caldas ED (2014) Pesticide residues in cashew apple, guava, kaki and peach: GC-ECD, GC-FPD and LC-MS/MS multiresidue method validation, analysis and cumulative acute risk assessment. Food Chem 164:195–204
66. López MG, Fussell RJ, Stead SL, Roberts D, McCullagh M, Rao R (2014) Evaluation and validation of an accurate mass screening method for the analysis of pesticides in fruits and vegetables using liquid chromatography-quadrupole-time of flight-mass spectrometry with automated detection. J Chromatogr A 1373:40–50
67. Li W, Morgan MK, Graham SE, Starr JM (2016) Measurement of pyrethroids and their environmental degradation products in fresh fruits and vegetables using a modification of the quick easy cheap effective rugged safe (QuEChERS) method. Talanta 151:42–50
68. Lee SW, Choi JH, Cho SK, Yu HA, Abd El-Aty AM, Shim JH (2011) Development of a new QuEChERS method based on dry ice for the determination of 168 pesticides in paprika using tandem mass spectrometry. J Chromatogr A 1218:4366–4377
69. Han Y, Zou N, Song L, Li Y, Qin Y, Liu S (2015) Simultaneous determination of 70 pesticide residues in leek, leaf lettuce and garland chrysanthemum using modified QuEChERS method with multi-walled carbon nanotubes as reversed-dispersive solid-phase extraction materials. J Chromatogr B 1005:56–64
70. Han Y, Song L, Zou N, Chen R, Qin Y, Pan C (2016) Multi-residue determination of 171 pesticides in cowpea using modified QuEChERS method with multi-walled carbon nanotubes as reversed-dispersive solid-phase extraction materials. J Chromatogr B 1031:99–108
71. Qin Y, Zhao P, Fan S, Han Y, Li Y, Zou N (2015) The comparison of dispersive solid phase extraction and multi-plug filtration cleanup method based on multi-walled carbon nanotubes for pesticides multi-residue analysis by liquid chromatography tandem mass spectrometry. J Chromatogr A 1385:1–11
72. Zhao P, Wang L, Zhou L, Zhang F, Kang S, Pan C (2012) Multi-walled carbon nanotubes as alternative reversed-dispersive solid phase extraction materials in pesticide multi-residue analysis with QuEChERS method. J Chromatogr A 1225:17–25
73. Bruzzoniti MC, Checchini L, De Carlo RM, Orlandini S, Rivoira L, Del Bubba M (2014) QuEChERS sample preparation for the determination of pesticides and other organic residues in environmental matrices: a critical review. Anal Bioanal Chem 406(17):4089–4116
74. Kraj A, Drabik A, Silberring J (2010) Proteomika i metabolomika [book in Polish]. Wydawnictwo Uniwersytetu Warszawskiego, Warszawa
75. Suder P, Silberring J (2006) Spektrometria mas [book in polish]. Wydawnictwo Uniwersytetu Jagiellońskiego, Kraków

76. Tette PAS, Da Silva Oliveira FA, Pereira ENC, Silva G, De Abreu Glória MB, Fernandes C (2016) Multiclass method for pesticides quantification in honey by means of modified QuEChERS and UHPLC-MS/MS. Food Chem 211:130–139
77. Botero-Coy AM a, Marín JM, Serrano R, Sancho JV i, Hernández F (2015) Exploring matrix effects in liquid chromatography-tandem mass spectrometry determination of pesticide residues in tropical fruits. Anal Bioanal Chem 407:3667–3681
78. Rajski Ł, Gómez-Ramos Mdel M, Fernández-Alba AR (2014) Large pesticide multiresidue screening method by liquid chromatography-Orbitrap mass spectrometry in full scan mode applied to fruit and vegetables. J Chromatogr A 1360:119–127
79. Stachniuk A, Szmagara A, Czeczko R, Fornal E (2017) LC-MS/MS determination of pesticide residues in fruits and vegetables. J Environ Sci Health B 52(7):1–12
80. Kmellár B, Pareja L, Ferrer C, Fodor P, Fernández-Alba AR (2011) Study of the effects of operational parameters on multiresidue pesticide analysis by LC-MS/MS. Talanta 84:262–273
81. Dong F, Chen X, Liu X, Xu J, Li Y, Shan W (2012) Simultaneous determination of five pyrazole fungicides in cereals, vegetables and fruits using liquid chromatography/tandem mass spectrometry. J Chromatogr A 1262:98–106
82. Swartz ME (2005) UPLC™: an introduction and review. J Liquid Chromatgr Relat Technol 28:1253–1263
83. Agilent Technologies (2010) Conepts guide – Agilent 6100 series quadrupole LC/MS systems. Agilent Technologies, Santa Clara
84. Sivaperumal P, Salauddin A, Ramesh Kumar A, Santhosh K, Rupal T (2017) Determination of pesticide residues in mango matrices by ultra high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Food Anal Methods 10:2346–2357
85. Grimalt S, Dehouck P (2015) Review of analytical methods for the determination of pesticide residues in grapes. J Chromatogr A 1433:1–23
86. Fu Y, Yang T, Zhao J, Zhang L, Chen R, Wu Y (2017) Determination of eight pesticides in Lycium barbarum by LC-MS/MS and dietary risk assessment. Food Chem 218:192–198
87. Dasenaki ME, Bletsou AA, Hanafi AH, Thomaidis NS (2016) Liquid chromatography–tandem mass spectrometric methods for the determination of spinosad, thiacloprid and pyridalyl in spring onions and estimation of their pre-harvest interval values. Food Chem 213:395–401
88. Andrade GCRM, Monteiro SH, Francisco JG, Figueiredo LA, Botelho RG, Tornisielo VL (2014) Liquid chromatography-electrospray ionization tandem mass spectrometry and dynamic multiple reaction monitoring method for determining multiple pesticide residues in tomato. Food Chem 175:57–65
89. Bakırcı GT, Bengü D, Acay Y, Bakırcı F, Ötles S (2014) Pesticide residues in fruits and vegetables from the Aegean region, Turkey. Food Chem 160:379–392
90. Carneiro RP, Oliveira FAS, Madureira FD, Silva G, de Souza WR, Lopes RP (2013) Development and method validation for determination of 128 pesticides in bananas by modified QuEChERS and UHPLC-MS/MS analysis. Food Control 33:413–423
91. Peruga A, Hidalgo C, Sancho JV, Hernández F (2013) Development of a fast analytical method for the individual determination of pyrethrins residues in fruits and vegetables by liquid chromatography-tandem mass spectrometry. J Chromatogr A 1307:126–134
92. Yang A, Park JH, Abd El-Aty AM, Choi JH, Oh JH, Do JA (2012) Synergistic effect of washing and cooking on the removal of multi-classes of pesticides from various food samples. Food Control 28:99–105
93. Botero-Coy AM, Marín JM, Ibáñez M, Sancho JV, Hernández F (2012) Multi-residue determination of pesticides in tropical fruits using liquid chromatography/tandem mass spectrometry. Anal Bioanal Chem 402(7):2287–2300
94. Chung SWC, Tran JCH, Tong KSK, Chen MYY, Xiao Y, Ho YY (2011) Nitrate and nitrite levels in commonly consumed vegetables in Hong Kong. Food Addit Contam B 4:34–41

95. Liu GZ, Rong L, Guo B, Zhang MS, Li SJ, Wu Q (2011) Development of an improved method to extract pesticide residues in foods using acetontrile with magnesium sulfate and chloroform. J Chromatogr A 1218:1429–1436
96. Lehotay SJ, Mastovska K, Lightfield AR, Gates RA (2010) Multi-analyst, multi-matrix performance of the QuEChERS approach for pesticide residues in foods and feeds using HPLC/MS/MS analysis with different calibration techniques. J AOAC Inter 93:355–367
97. Gilbert-Lopez B, Garcia-Reyes JF, Lozano A, Fernandez-Alba AR, Molina-Diaz A (2010) Large-scale pesticide testing in olives by liquid chromatography-electrospray tandem mass spectrometry using two sample preparation methods based on matrix solid-phase dispersion and QuEChERS. J Chromatogr A 1217:6022–6035
98. Guo B, Huang Z, Wang M, Wang X, Zhang Y, Chen B (2010) Simultaneous direct analysis of benzimidazole fungicides and relevant metabolites in agricultural products based on multi-function dispersive solid-phase extraction and liquid chromatography-mass spectrometry. J Chromatogr A 1217:4796–4807
99. Grimalt S, Sancho JV, Pozoa ÓJ, Hernándeza FE (2010) Quantification, confirmation and screening capability of UHPLC coupled to triple quadrupole and hybrid quadrupole time-of-flightmass spectrometry in pesticide residue analysis. J Mass Spectrom 45:421–436
100. Afify AEMMR, Mohamed MA, El-Gammal HA, Attallah ER (2010) Multiresidue method of analysis for determination of 150 pesticides in grapes using quick and easy method (QuEChERS) and LC-MS/MS determination. J Food Agri Environ 8:602–606
101. Camino-Sánchez FJ, Zafra-Gómez A, Oliver-Rodríguez B, Ballesteros O, Navalón A, Crovetto G (2010) UNE-EN ISO/IEC 17025:2005-accredited method for the determination of pesticide residues in fruit and vegetable samples by LC-MS/MS. Food Addit Contam A 27:1532–1544
102. Kittlaus S, Kempe G, Speer K (2013) Evaluation of matrix effects in different multipesticide residue analysis methods using liquid chromatography-tandem mass spectrometry, including an automated two-dimensional cleanup approach. J Sep Sci 36:2185–2195
103. Uclés S, Lozano A, Sosa A, Parrilla Vázquez P, Valverde A, Fernández-Alba AR (2017) Matrix interference evaluation employing GC and LC coupled to triple quadrupole tandem mass spectrometry. Talanta 174:72–81
104. López-Blanco R, Nortes-Méndez R, Robles-Molina J, Moreno-González D, Gilbert-López B, García-Reyes JF (2016) Evaluation of different cleanup sorbents for multiresidue pesticide analysis in fatty vegetable matrices by liquid chromatography tandem mass spectrometry. J Chromatogr A 1456:89–104
105. Stahnke H, Alder L (2015) Matrix effects in liquid chromatography-electrospray ionization-mass spectrometry. In: Tsipi D, Botitsi H, Economou A (eds) Mass spectrometry for the analysis of pesticide residues and their metabolites. Wiley, NJ
106. Stahnke H, Kittlaus S, Kempe G, Alder L (2012) Reduction of matrix effects in liquid chromatography-electrospray ionization-mass spectrometry by dilution of the sample extracts: how much dilution is needed? Anal Chem 84:1474–1482
107. Rahman MM, Abd El-Aty AM, Shim JH (2013) Matrix enhancement effect: a blessing or a curse for gas chromatography? – a review. Anal Chim Acta 801:14–21

Encapsulation to Protect Different Bioactives to Be Used as Nutraceuticals and Food Ingredients

73

Jacqueline Ruiz Canizales, Gustavo R. Velderrain Rodríguez, J. Abraham Domínguez Avila, Alejandra M. Preciado Saldaña, Emilio Alvarez Parrilla, Mónica A. Villegas Ochoa, and Gustavo A. González Aguilar

Contents

Abstract

Consumers' awareness of the relationship between diet and health is increasing the demand for nutraceuticals and functional food products. They usually involve the incorporation of bioactive compounds extracted from plant tissues, or, in some cases, beneficial microorganism species known as probiotics. Incorporation of these compounds as functional ingredients has to overcome various challenges related to their stability during food processing or gastrointestinal tract, in order to

J. Ruiz Canizales · G. R. Velderrain Rodríguez · J. A. Domínguez Avila · A. M. Preciado Saldaña · M. A. Villegas Ochoa · G. A. González Aguilar (✉)
Centro de Investigación en Alimentación y Desarrollo A.C. Carretera a La Victoria km 0.6, Hermosillo, Sonora, Mexico
e-mail: jaruca89@gmail.com; tavo_velderrain@hotmail.com; abrahamdominguez9@yahoo.com; nutalejandrapreciado@gmail.com; mvillegas@ciad.mx; gustavo@ciad.mx

E. Alvarez Parrilla
Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez. Anillo Envolvente del PRONAF y Estocolmo s/n. Cd. Juárez, Chihuahua, Mexico
e-mail: ealvarez@uacj.mx

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_84

guarantee that they exert health benefits after intake. An attractive strategy is the use of encapsulation technologies. Appropriate selection of encapsulation methods or core and carrier materials may influence most of the desired properties of the final food product. This chapter summarizes the main aspects to consider prior to developing nutraceutical or functional food products using encapsulated bioactive compounds.

Keywords
Stability · Encapsulation · Biological activity · Bioactive compound · Probiotic

1 Introduction

The design of functional foods and nutraceutical products is an active research area of the food industry. The addition of bioactive compounds to food products has increased in the last decades, as studies confirm their association with health-promoting benefits. It has also been shown that several bioactive compounds may be unstable under various environmental and processing conditions, which limits their industrial application [1]. Efforts have been made to facilitate their incorporation into food products, such as encapsulation, which is a useful technology that protects labile compounds by covering them in a resistant layer, such as polymeric matrix. This technology improves shelf life and masks undesirable taste, odor, and color [2]. Different encapsulation techniques can protect specific compounds, such as phenolic compounds (polyphenols) [3], carotenoids, phytosterols [4], and probiotics [5]. An adequate selection depends on the active compound to protect, as formation and rheological properties of most encapsulation systems strongly depend on interactions between carrier and core materials [6]. The most used encapsulation techniques are spray drying, freeze-drying, emulsions, nanoprecipitation, liposomes, and niosomes, while the main polymeric matrices are polysaccharides, proteins, and lipids. The objective of this chapter is to review the state-of-the-art encapsulation systems, focusing on the most common techniques and materials used to protect bioactive compounds from adverse conditions, such as food processing, storage, and physiological digestion conditions.

2 Overview of Encapsulation Methods and Matrices

Encapsulation is defined as entrapment of a core material (bioactive compound, inner phase, or payload phase) within another immiscible substance (carrier or wall), which can be solid or liquid [7]. The choice of the most suitable encapsulation method depends on the core material and desired characteristics of the final product [8]. It should be noted that carrier composition has a great influence on final properties, encapsulation efficiency, and release of the entrapped compounds. The

most relevant properties influenced by carrier composition are water solubility, shelf life, and bioavailability. As size and nature (hydrophilic or lipophilic) of an encapsulated particle are key factors to control these properties, nanoencapsulation provides further control by reducing particle size [9].

In spite of the benefits of nanoencapsulation, the most widely used methods are based on microencapsulation. This is achieved by physical methods like spray drying, fluid bed coating, extrusion, co-extrusion, molecular encapsulation, and emulsions, although chemical techniques are also employed. In terms of size, nanoparticles range from 1 nm–1 μm, whereas microparticles range from 1–800 μm. This makes nanoparticles more difficult to produce with common techniques used for microparticles; nanosize ranges are typically achieved through either bottom-up or top-down approaches (see Fig. 1).

A bottom-up approach refers to materials constructed through self-assembly and self-organization of molecules, by controlling factors like pH, temperature, concentration, and ionic strength. Common techniques for nanoencapsulation through a bottom-up approach are coacervation, nanoprecipitation, inclusion complexation, and supercritical fluid technique [9]. The opposite approach, top-down, involves the application of energy that allows size reduction and shaping of the material comprising the nanoencapsulation carrier. The most common top-down approach techniques are emulsification and emulsification-solvent evaporation [10]. Selecting the most adequate approach depends on the properties of the carrier and core materials, as well as their stability during encapsulation.

Current commercial nanoencapsulated products involve top-down approach, even if it usually requires increased temperature due to the high-energy shear rates applied during encapsulation. As bottom-up approach is becoming a promising

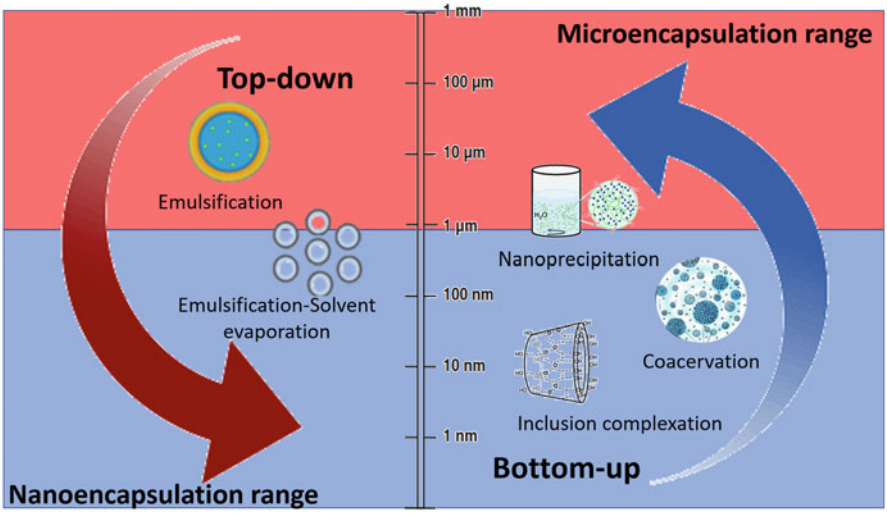


Fig. 1 Top-down and bottom-up approaches in nanoencapsulation technology (Adapted from [9])

option for nanoencapsulation, it is expected that future developments in nanotechnology lead to increased application.

2.1 Encapsulation Techniques

2.1.1 Spray Drying

High water content of food matrices, such as fruits and vegetables, is considered one of the main problems of preservation during packaging and storage. Spray drying allows the reduction of water content and microorganism proliferation because of conversion of liquids into solids. With the addition of carrier materials into the feed solution, labile compounds can be protected from light and oxygen, prolonging their shelf life. At the same time, smells and flavors are masked [6, 11]. This technique is based on atomizing a liquid/emulsion into a dryer chamber with a hot gas, that immediately removes water, producing a fine powder [12]. Carbohydrates and proteins are the main carrier materials used with this technology, and they noticeably prolong shelf life of bioactive compounds such as anthocyanins [13], carotenoids, and nut oils [14], among others. Spray drying has also been used to protect antigens [15], probiotics [5], and bacteriophages [16].

There are some processing parameters that can be optimized to obtain a final product with the highest quality; inlet/outlet temperature, flow rate, and wall-to-core ratio are the most important. Elez Garofulić and Zorić [6] found that maltodextrin with 13–17 dextrose equivalents (DE) in a 3:1 ratio and inlet temperature of 200 °C, got the highest retention of anthocyanins and phenolic acids, while anthocyanins from purple corncob were less affected when using 5% maltodextrin 16–19 DE and 150 °C [17]. Researchers can also use response surface methodology (RSM) to establish operational conditions that maximize the intended response, such as phenolic acid and anthocyanin retention from sour cherry juice by full factorial design [6], betalains from beetroot using Box-Behnken design [18], and total phenolic compounds from *Nigella sativa* oil with central composite design [19]. The main advantages of spray drying are its low cost, high reproducibility, low water content, high retention of bioactive compounds, and small and smooth particles; it is also one of the most used in food and pharmaceutical industries.

2.1.2 Freeze-Drying

Freeze-drying consists in drying the sample by sublimating its water content. This allows it to be widely used to dry thermosensitive compounds like essential oils, pigments, proteins, and microorganisms. Different authors report that freeze-drying prevents browning reactions and higher losses of vitamin C and polyphenols from grapefruit and kimchi (a Korean fermented food prepared from cabbage), in comparison with hot air drying, because this technique requires low temperature and absence of oxygen in the drying chamber [11, 20]. Ballesteros and Ramirez [2] observed higher retention of anthocyanins, phenolic compounds and antioxidant activity from spent coffee grounds, when encapsulating in maltodextrin through freeze-drying than using spray drying, similar results were obtained when bayberry

polyphenols were evaluated [3]. This behavior could be associated with low temperatures used during freeze-drying that minimize chemical changes of labile molecules.

Among the disadvantages of its use is that it requires crystallization of water, and mainly water-soluble materials can be used as core materials, such as maltodextrin, starch and gums [21]. Its high energy consumption and long processing time, also limits its industrial application. In order to reduce processing times and increase quality, some pretreatments have been evaluated, such as osmotic dehydration [22], or ultra-sonication [23].

2.1.3 Emulsions

Emulsification combines immiscible compounds in a mixture, through dispersions stabilized using surfactants. According to which solution is dispersed in a continuous phase, simple water-in-oil or oil-in-water (W/O, O/W) emulsions or even double water-in-oil-in-water and oil-in-water-in-oil ($W_1/O/W_2$, $O_1/W/O_2$) emulsions can be achieved. Ydjedd and Bouriche [24] found that encapsulation of phenolic compounds from carob (*Ceratonia siliqua* L.) pulp into $W_1/O/W_2$ emulsions protected them from chemical conditions of gastrointestinal tract, and released the bioactive compounds during intestinal phase, possibly enhancing their absorption into systemic circulation. Absorption of tea catechins was improved when they were incorporated into nanoemulsions; epigallocatechin gallate increased its plasma concentration, as compared to a non-encapsulated solution [25]. Encapsulation in coated emulsions could enhance the antimicrobial activity of essential oils, due to interactions between the positively charged surface coating and the negative surface potential of bacteria, by attractive electrostatic forces [26].

Some parameters directly affect the physicochemical properties of the emulsion, such as oil phase, emulsifier, and operational conditions. Addition of whey protein increases stability of O/W emulsions, while addition of calcium chloride promotes particle aggregation [27]. Concentrations of emulsifier and bioactive compounds were optimized to successfully produce stable emulsions with the incorporation of ascorbic acid and gallic acid. Addition of 4% Tween 20 and 1% bioactive compound produced emulsions with low polydispersity index <0.3, and emulsion stability index up to 98% [28]. Use of RSM can determine optimal process parameters that prevent coalescence, flocculation, and creaming of droplets [29].

2.1.4 Nanoprecipitation

Nanoprecipitation forms capsules and spheres by interfacial deposition of a polymeric matrix, with subsequent displacement of a polar organic solvent with a non-polar solvent. This technique is simple and reproducible, and yields small particles with unimodal size, in the range of 50–300 nm [30, 31]. High encapsulation efficiency (up to 80%) has been reported when using lipophilic or amphiphilic molecules, such as vitamin E [32], α-tocopherol [33], kaempferol [34], and lutein [35]. Release of bioactive compounds encapsulated by nanoprecipitation shows two steps, burst and prolonged release. Burst release is associated with adsorption of bioactive molecules onto the particles, while prolonged release is due to diffusion of

molecules trapped inside the polymeric matrix. Incorporation of quercetin into nanoparticles of polycaprolactone followed the Korsmeyer-Peppas kinetic release model, with burst release during the first 10 h and prolonged release until 48 h [36].

2.1.5 Liposomes

Liposomes are self-assembled bilayer vesicles of phospholipids with an aqueous core, which protect lipophilic and hydrophilic molecules. They can improve stability and bioavailability of bioactive compounds such as resveratrol, quercetin, lutein, β-carotene, and others. Addition of cholesterol during liposome formation is a key factor that directly affects the structural stability of the bilayer against environmental stress. Incorporation of cholesterol can maintain the bilayer's integrity under gastric conditions, but lipase and bile salts could change the phospholipids' assembly [37, 38]. Low stability, reproducibility, and encapsulation efficiency, as well as short shelf life and limited industrial scaling, are some of the main disadvantages of the implementation of liposomes in the food industry [39].

2.1.6 Niosomes

Niosomes are bilayer vesicles made from nonionic surfactants; they are a promising system due to their low cost, chemical stability, smaller size, and enhancement of bioavailability of lipophilic and hydrophilic molecules, among others [40]. There are many techniques to produce niosomes, such as film hydration, ethanol injection, microfluidization, and manual shaking. As with liposomes, cholesterol improves their stability, because it interacts with the surfactant's hydrophobic tail [41]. This system protected polyphenols under simulated gastrointestinal conditions, with a prolonged release for 24 h. The main problem during simulated digestion was an increase in size due to bile salts [42]. Niosome encapsulation enhances absorption and antiproliferative activity of curcumin and lycopene in cancer cell lines, with respect to free compound [43, 44]. It has been hypothesized that fusion, adsorption, or endocytosis could be the release mechanism from niosomes, making it suitable to deliver bioactive molecules through different routes (dermal, retinal, intravenous, and oral).

2.2 Encapsulating Matrices

Different matrices are available as options to protect bioactives, such as polysaccharides, proteins, and lipids. Selection of the adequate wall material is based on the desired properties and final application. Polysaccharides are the main polymeric matrix used; they are obtained from various sources, and are biodegradable, biocompatible, and nontoxic [45]. Some of the most used are maltodextrin, starch, pectin, chitosan, alginate, and gums. Bakowska-Barczak and Kolodziejczyk [46] found that maltodextrins with 11, 18, and 21 DE were more effective than inulin to protect anthocyanins, polyphenols, and antioxidant activity of blackcurrant extracts. At the same time, two or more combined carrier materials can improve physicochemical characteristics of particles, for example, maltodextrin/pectin particles of

herbal extracts maintained the antioxidant activity during 6 months of storage of various samples [47].

Proteins are also widely used as coating materials, mainly gelatin, zein (from maize), and whey protein. Bagheri and Madadlou [48] incorporated date palm pit extract (up to 70%) into whey protein particles. Quiroz-Reyes et al. (2014) evaluated gelatin nanoparticles as carrier for cocoa polyphenols, which resulted in narrow size distribution, spherical and uniform shape, with 77% encapsulation efficiency [49]. In both cases, infrared analyses showed that compounds extracted could be retained in protein particles through non-covalent interactions, such as hydrogen bonding between C=O groups of protein and OH groups of polyphenols, as well as van der Waals and hydrophobic interactions. Some polyphenols, such as epigallocatechin and quercetin, could covalently interact with zein, conferring thermal stability and higher antioxidant activity [50].

Different studies have focused on the use of lipid carriers to enhance transport and cellular absorption of lipophilic compounds. Because of their biocompatibility, low toxicity, and capability to entrap lipophilic and hydrophilic molecules, these systems have been widely studied. Phospholipids show good retention of quercetin and resveratrol, while a significantly reduced cytotoxicity against fibroblast cells, and higher efficacy against skin cancer cells was found when these molecules were incorporated into phospholipid vesicles, as compared to non-encapsulated molecules [51]. Incorporation of resveratrol into solid lipid nanoparticles improved oral bioavailability eightfold, with respect to free solution, with prolonged drug release up to 120 h [52].

As previously discussed, different matrices are available to encapsulate bioactive compounds, and an appropriate selection is crucial to obtain particles with desired qualities. It is important to also consider aspects like biodegradability, biocompatibility, and nontoxicity of coating materials to successfully use them in food applications.

3 Encapsulation of Polyphenols and Vitamins

Encapsulating a compound confers protection to it, so the most appropriate use of this methodology is on compounds whose chemical structure and/or biological activities are easily lost. Polyphenols and vitamins are thus excellent candidates to encapsulate, since they exert various health-promoting effects when consumed, but are highly sensitive to light, oxygen, pH, and temperature changes, which limits their shelflife when used as functional ingredients. The following paragraphs summarize the use of encapsulation on polyphenols and vitamins from different sources, in order to highlight their potential as nutraceutical products or functional ingredients.

Polyphenols have been considered as main contributors to the health effects of wine, and their incorporation into other edible products could allow them to exert the same effects. Sanchez and Baeza [53] used a maltodextrin matrix to encapsulate wine polyphenols by freeze-drying, a process that simultaneously eliminates the water and ethanol. Freeze-dried samples were milled to produce a powder, which

was then further studied. Polyphenols were concentrated in the dried samples (9.1 g L^{-1}), which only contained traces of ethanol (8.0 g L^{-1}, as compared to the initial 102.9 g L^{-1}). Furthermore, the stability of the polyphenols was well-preserved during storage, showing no degradation after 15 days of storage at 38 °C. This study suggests that wine polyphenols may be interesting functional ingredients that can be consumed in other products without an unnecessary alcohol intake.

Elez Garofulić and Zorić [6] used a spray drying method to encapsulate polyphenols from sour cherry juice, using maltodextrin (4–7 DE and 13–17 DE) or gum Arabic as wall material in varying concentrations. They determined that the ratio of carrier material to juice with the highest retention of polyphenols varied significantly. For example, a 2:1 ratio was optimal when the 4–7 DE maltodextrin was used, 3:1 was optimal with the 13–17 DE maltodextrin, and a ratio higher than 1:1 was optimal when gum Arabic was used. Furthermore, these particular ratios also varied when specific classes of polyphenols were analyzed, such as phenolic acids or anthocyanins. The authors of this study mention that selecting the proper wall material and its ratio is crucial to produce high-quality encapsulated sour cherry juice. This is likely to be the case for other polyphenol sources, since the concentration of sugars, proteins, and lipids may vary, along with physical characteristics such as viscosity, which suggests that working parameters should be optimized in order to use cherry polyphenols as functional ingredients.

Encapsulating specific polyphenols can be a useful approach when the most bioactive compounds from a particular source are known. For example, (+)-catechin and (−)-epigallocatechin-3-gallate (EGCG) have been intensely studied and have been recognized as highly bioactive compounds in teas and other sources. Song and Li [54] encapsulated both of these compounds in niosomes and analyzed their uptake in a Caco-2 cell model during 6 h. They determined that cellular uptake was 2.66-fold (catechin) and 2.13-fold (EGCG) higher when the compounds were encapsulated and administered in this form. The bioavailability of polyphenols is generally poor, which indicates that only a small fraction of the ingested amount will reach the systemic circulation and target organs. Interestingly, this study reveals that encapsulating certain polyphenolic compounds may increase their bioavailability, potentially requiring lower oral doses to reach pharmacologically-active concentrations in serum and in target organs. Similarly, Wang and Wang [55] encapsulated the polyphenolic compounds from *Cotinus coggygria* (a medicinal plant) in a polyvinylpyrrolidone K-30/sodium dodecyl sulfate and polyethylene glycol matrix. They administered free and encapsulated compounds to a brain glioblastoma cell line (DBTRG-05MG) and determined that the *C. coggygria* polyphenols exerted significantly stronger apoptosis-mediated antiproliferative effects when administered encapsulated. These results show that the encapsulation process played an important part in the treatments, by enhancing bioactivity of the compounds in this cell line model. Taken together, the results of Song and Li [54], and Wang and Wang [55] suggest that the encapsulation process may lower the dose necessary to exert a bioactive effect in a target organ by at least two mechanisms: first, increasing intestinal uptake, and second, enhancing their bioactivity.

Polyphenol encapsulation allows the use of different fruit by-products that would otherwise be discarded, as sources of bioactive polyphenols. For example, Saikia and Mahnot [56] extracted polyphenols from star fruit (*Averrhoa carambola*) pomace and compared freeze-drying or spray drying microencapsulation (using maltodextrin $\leq$20 DE). They showed that encapsulating efficiency varied according to the method used; it was higher when freeze-drying was used (78–97%), as compared to spray drying (63–79%). Other authors have used polyphenols from food by-product in various in vivo analyses, for example, Motilva and Macia [57] used grape pomace as a source of polyphenols, which were then encapsulated in a zein (protein) and L-lysine matrix. A randomized crossover study was performed (separated by 2 weeks of washout between treatments) to administer the polyphenols to healthy adult volunteers (six women, six men). The participants consumed either the vehicle (dealcoholized red wine), the vehicle with 1.3 g of non-encapsulated polyphenols, or the vehicle with 9 g of encapsulated polyphenols (equivalent to 1.3 g of free polyphenols). Subsequent analyses showed that different metabolites of phenolic acids, stilbenes, flavan-3-ols, phenyl alcohols, valerolactones, and anthocyanins were found in the urine of the volunteers, from which the authors were able to deduce the likely metabolic routes of these compounds after ingestion. In the experiments of Saikia and Mahnot [56], and Motilva and Macia [57], encapsulation was a valuable tool that allowed polyphenols from by-products to be thoroughly studied in different models, further highlighting the versatility of the methodology.

Vitamin encapsulation has the potential to increase their intestinal absorption, which can be used to prevent or treat deficiencies. Estevinho and Carlan [58] used chitosan, modified water-soluble chitosan, and alginate to encapsulate vitamin C and vitamin B12. They determined that the liberation of both vitamins was complete, but it varied according to the encapsulating matrix and with each vitamin. For example, liberation of vitamin C from the water-soluble chitosan and alginate matrices was complete in a short time (10 min for vitamin C, 20 min for vitamin B12), while complete liberation from the unmodified chitosan matrix required longer times (80 min for vitamin C and >140 min for vitamin B12). The significance of this study is emphasized by three facts: first, the encapsulation of vitamin B12 is of high interest because of its complex chemical structure that makes it highly susceptible to degradation; second, this form may be useful to patients with pernicious anemia (a potentially deadly vitamin B12 deficiency) by promoting its intestinal absorption; and third, the liberation of a vitamin can be tailored to be fast (< 10 min) or gradual (> 2 h), according to the encapsulating matrix.

Avinash and Purnima [59] used maltodextrin and modified starches to encapsulate (spray drying) vitamin A. They determined that inlet air temperature and concentration of solids were both critical factors that determine the quality of the resulting particles. These particles were then stored for up to 3 months under accelerated conditions (40 °C and 75% relative humidity, or 30 °C and 65% relative humidity), after which no significant changes were found, suggesting that vitamin A was well-preserved by the encapsulating matrix. These results suggest that vitamin A, encapsulated as described by the authors, may be incorporated into products with long shelf lives at room temperature.

Gamboa and Goncalves [60] used fully hydrogenated soybean oil and soybean oil (70:30%, w/w) to encapsulate α-tocopherol (vitamin E). They used a spray chilling methodology, varied the encapsulating material to α-tocopherol ratio (90:10, 80:20, 95:5, 85:15), and stored the microparticles at different temperatures (22 °C and −18 °C protected from light, and 25 °C exposed to light) for 180 days. Their findings showed very high encapsulation efficiency ≥90% and a near-complete retention (≥ 94.1%) after storage. Subsequent experiments showed that the physicochemical parameters of the capsules were unaltered by α-tocopherol, which was attributed in part to its solubility in the encapsulating matrix. Interestingly, the encapsulating matrix provided excellent protection even while storing the particles at room temperature and without shielding them from light. This particular methodology and encapsulating matrix could allow the use of vitamin E on edible products that can be stored at room temperature, without protection from light and for longer time.

Wagner and Spoth [61] encapsulated vitamin D_3 (also known as cholecalciferol) and ferrous sulfate ($FeSO_4$) within niosomes. The resulting particles had an average diameter of 1.4 μm and a 95.9% encapsulation efficiency for the vitamin. The particles were then stored at room (20 °C) or refrigerator (4 °C) temperature for 21 days, and interestingly, the particles retained their size and contents better at room temperature. Lower temperatures may have caused the Fe^{+2} to precipitate, oxidize, and crystalize, which may have then ruptured the niosomal membrane. According to these results, this system is useful to supplement edible products that are only stored at room temperature, in order to prevent rupture of the encapsulating matrix. Furthermore, the particles contained enough vitamin D_3 and Fe^{+2} ions to supply the recommended daily allowance (RDA) of both nutrients for men and women.

Ruiz-Rico and Perez-Esteve [62] encapsulated folic acid within silica particles (tetraethyl orthosilicate) that were then used to supplement commercial apple or orange juices. They showed that the low pH of the juices (approximately 3.5) and the various compounds they contain, did not promote liberation of the vitamin within the juice itself. This suggests that wall material would not dissolve during the juices' shelf life or in the acid environment of the stomach once ingested. At higher pH values (7.5), similar to those of the small intestine, the matrix does dissolve and allows complete liberation of the vitamin. Subsequent in vitro digestion studies confirmed complete liberation during the intestinal phase. Finally, encapsulated folic acid was resistant to temperatures and pressures of sterilization (121 °C, 100 kPa, up to 15 min), exposure to light (visible and UV, up to 24 h), and during refrigerated storage (4 °C, 28 days). Folic acid is an essential nutrient of particular importance for women of fertile age or pregnant, which is required for the synthesis of nucleic acids. This study shows that folic acid can be encapsulated and administered in a beverage (fruit juice), in order to prevent deficiencies.

In the case of polyphenols and vitamins, encapsulation is a suitable methodology to be applied to them. As summarized here, some of the benefits of encapsulating these compounds include protection during storage, protection during the digestive process against undesired interactions, controlled liberation, increased intestinal

uptake, enhanced bioactivity, and others. Encapsulation can therefore be used to incorporate polyphenols and vitamins in a stable form into various edible products, but choosing the most adequate encapsulating matrix, optimal core-to-carrier ratio, and operational parameters should be first performed in order to yield a high-quality product.

4 Nanoencapsulation

Encapsulation of bioactive compounds using a nanotechnological approach has been a goal of many scientists for the past decades [63]. Encapsulated particles can be classified into three different categories according to their size, macrocapsules (>500 μm), microcapsules (0.2–500 μm), and nanocapsules (<0.2 μm) [64]. Nanoparticles (NPs) are considered the most attractive delivery vehicles, product of nanoscience breakthroughs, which usually involve systems between 1–100 nm [65]. One of the most remarkable advantages of NPs, is that they have an induced homogeneity, which usually leads to improved physical and chemical properties, and, hence, a better encapsulation efficiency [64]. This section focuses on the state of art of nanoencapsulation of bioactive compounds, their possible application in food industry, and how it protects and enhances bioactive compound properties.

The encapsulation matrix must be suitable for food applications, and as previously mentioned, the most common carrier materials are carbohydrate-, protein-, and lipid-based systems. Carrier material will have a significant impact on its delivery and organoleptic properties it may confer to the food product. For example, carbohydrate-based carriers are suitable for most food applications, since they are biocompatible, biodegradable, and easy to incorporate within food products. In addition, carbohydrate-based systems are more resistant to high temperature processes, compared to lipid or protein systems, which is essential to consider in the development of certain food products.

Polyphenols are suitable to be nanoencapsulated, because their chemical structure allows them to exert various health effects (e.g., antioxidant, anti-inflammatory, or antibacterial) [66–69]. However, as mentioned by Fang and Bhandari [70], the effective concentration of polyphenols is higher on in vivo studies compared to those measured in vitro. In order to make bioactives, such as green tea catechins, as effective as they are on in vitro studies, researchers suggest increasing their stability and bioavailability [71]. The effectiveness of a bioactive compound strongly depends on preserving their chemical integrity and bioavailability.

One of the most studied and unstable polyphenolic molecules are anthocyanins. They are susceptible to pH changes, metal ions, exposure to light, temperature, oxygen, and enzymatic activities, which can modify their chemical structure, starting at the initial food product and continuing during digestion [72]. Ko and Lee [73] have suggested the use of nanoencapsulation in addition to copigmentation for anthocyanin protection. They found that copigmentated anthocyanin-loaded NPs, formed with chitosan and tripolyphosphate, had an increased half life and retention more than 2.6- and 7.4-fold greater than non-encapsulated anthocyanins stored at

high temperatures (25 and 50 °C). They also highlighted that the use of chitosan-based NPs is not affected at low pH, as has been observed with maltodextrins and other polymers.

In agreement, Han and Lee [74] showed that the antioxidant activity of chitosan NPs containing jujube phenolic extracts remains stable two-fold greater than non-encapsulated extract (3 days). In addition, the antioxidant decrease rate in chitosan NPs was slower than in non-encapsulated extract. As a stable antioxidant activity is related to the chemical stability of antioxidant molecules, it is essential to maintain it as long as possible at storage conditions, in order to be successfully applied in food products. Jeon and Lee [75] demonstrated that the use of chitosan NPs could increase the solubility of bioactive compounds, such as resveratrol, up to 4.2-fold compared to the non-encapsulated compound, while also preserving its antioxidant activity. Chitosan is one of the most suitable polymers to use in stable and effective NPs.

Protein-based nano-carriers confer desirable properties to nanoencapsulation systems, such as biodegradability. Some suggest that the use of proteins to encapsulate curcumin in nanoscale systems may improve its pharmacokinetic properties [76]. Zou and Zheng [77] found that protein-based nanosystems were able to incorporate the highest amounts of curcumin per unit mass of particles, which results in a lower amount required in food products in order to fortify them. In contrast to the economic advantages of this system, protein-based particles were not as effective to enhance curcumin potential absorption within micelles, as compared to lipid-based systems.

Bagheri and Madadlou [48] encapsulated an aqueous extract of date palm pit within whey protein NPs. They found that a heat treatment (60 °C, 30 min) decreased the mean size, and increased the monodispersity and particle yield of extract-free and extract-loaded particles. Combined nano-carriers can be made with protein-lipid mixtures. Sadeghi and Madadlou [78] nanoencapsulated date palm pit extract using microemulsification-cold gelation of whey proteins. They report a mean size of 23, 304, and 230 nm for heat-treated particles, extract-free particles, and extract-loaded capsules, respectively. They also suggest that a controlled release from extract-loaded NPs is achieved, based on the phenolic content of digestion experiments.

Belščak-Cvitanović and Bušić [79] report that combined carriers (alginate and whey proteins) are optimal delivery systems. They can promote NPs with regular spherical shape, high encapsulation efficiency of total polyphenols (77.35%), and very good retention of hydroxycinnamic acids (89.14%). The combination of alginate and hydroxypropyl methylcellulose enabled the best (prolonged) release profile of these compounds from the formulated microparticles in simulated digestion model. The use of combined systems (see Fig. 2) merges the advantages of individual nano-carriers, such as higher absorption rates of lipid carriers and health-related benefits of proteins. The addition of proteins to emulsion systems is the most common combination of these carriers, as whey peptides perform a dual-functional role in foods as both emulsifiers and bioactive compounds.

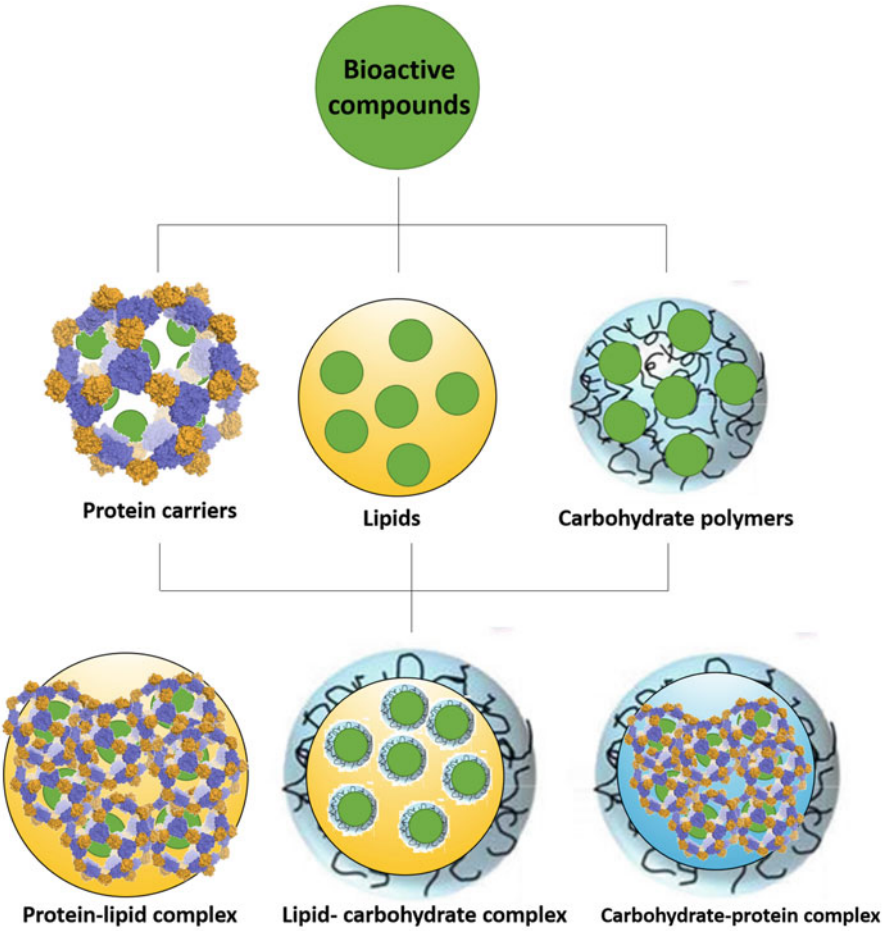


Fig. 2 Simple and combined encapsulation carrier materials

5 Encapsulation of Probiotics

Probiotics are living microorganisms that, when correctly administered, can have health benefits for the consumer [80]. One of the main advantages of probiotic consumption is its contribution to the restoration of intestinal homeostasis, as well as some anticancer, anti-bacterial properties and improvement of the nutritional status [81]. Most probiotics suffer rapid inactivation or degradation, because they are sensitive to environmental conditions, food processing, and storage. Once consumed, they are affected by physicochemical conditions of the gastrointestinal tract, such as pH bile salts [82]. In order to exert health benefits, several aspects must be considered, such as their survival rate, viability, and functionality [83].

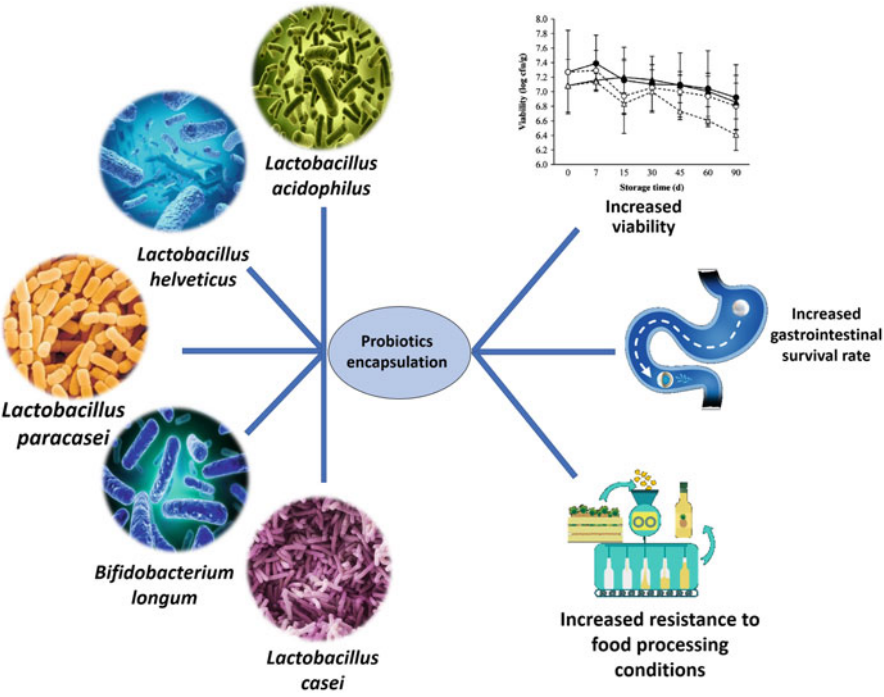


Fig. 3 Advantages of probiotic encapsulation

Encapsulation is a useful strategy to protect probiotics (see Fig. 3 [84]). This method stabilizes probiotic bacteria, improving their viability and stability and protecting them from adverse conditions [85]. Multiple methods are available to achieve this objective, such as spray drying, emulsions, and others [86].

Lactobacillus plantarum encapsulated in 3% alginate and coated with chitosan, showed lower viability loss (0.55 log) when added into yogurt and stored in refrigeration for 38 days, compared to free microorganisms incorporated in the same matrix [87]. González-Sánchez and Azaola [88] incorporated free and encapsulated *Bifidobacterium animalis* ssp. into kefir, and they observed that those encapsulated had higher survival rate than non-encapsulated. Addition of *Lactobacillus casei* and *Bifidobacterium lactis* encapsulated in calcium alginate beads into ice cream, significantly increases their survival rate. The increase of viability was 30% during 180 days of storage at −20 °C, and the addition of probiotics did not show significant changes in the sensory properties of the product [89].

Possemiers and Marzorati [90] incorporated *Lactobacillus helveticus* and *Bifidobacterium longum* as microencapsulated freeze-dried powder to different chocolate formulations, and evaluated the changes during digestion. Encapsulated probiotics showed 80% viability, which suggests that this technology is suitable to be used on probiotics as functional ingredients.

Probiotic encapsulation may increase their resistance to food processing conditions and other stressors. For example, viability of *L. casei*, *L. paracasei*, *L. acidophilus*, and *Bifidobacterium animalis*, encapsulated in alginate beads, were subjected to stress conditions (changes in pH, and temperature > 55 °C). *L. acidophilus* showed the highest survival rate, and showed greater tolerance to high temperatures, while free cells had low survival rates [91].

Sandoval-Castilla and Lobato-Calleros [92] encapsulated *L. casei* in pearls of sodium alginate and pectin, and the particles were incorporated into yogurt. Survival of *L. casei* under simulated digestion showed that they were better protected when encapsulated in a mixture of alginate and pectin. When encapsulating *L. plantarum* in alginate (2, 3, and 4%), the bacterium was protected from acidic conditions in the stomach, but were less resistant to in intestinal conditions [93]. Thantsha and Cloete [94] encapsulated *Bifidobacterium longum* in interpolymeric complexes formed with supercritical carbon dioxide. Once encapsulated, bacteria were subjected to simulated gastric and intestinal fluids, and with this, they obtained a limited reduction in the viability of the bacteria at the end of the exposure period. Encapsulation in the interpolymeric complex showed potential to protect probiotics.

L. acidophilus encapsulated in a W/O emulsion system with sodium alginate, showed that this microorganism survives at temperatures >70 °C and high salt concentrations (> 1%). Compared with free bacteria of the same strain, encapsulated ones were more stable, since they were reduced by 4.14 log, while the former were lost at 90 °C [95]. Papagianni and Anastasiadou [96] used W/O emulsions as an encapsulation system for *Pediococcus acidilactici*. They found that approximately 85% viability was conserved, while up to 92% of encapsulated bacteria could be released at the target site. This is in accordance with reports that state that emulsions can promote high survival rates [97]. Nag and Han [98] used a mixture of sodium caseinate and gellan gum to encapsulate *L. casei* in a W/O emulsion. Survival of encapsulated bacteria in simulated gastric fluid (30 min), was significantly higher than that of free bacteria, also protecting them from bile salts.

Sousa and Gomes [99] encapsulated four strains of probiotic bacteria (*L. paracasei*, *L. casei*, *L. acidophilus Ki*, and *Bifidobacterium animalis*) in alginate and alginate with L-cysteine. The capsules were stored at different temperatures (21, 4, −20, and −80 °C) for 6 months. Alginate encapsulation (−80 °C) protected all four strains, and L-cysteine improved protection of *B. animalis*. According to these results, encapsulation with alginate protects probiotics against freezing temperatures.

Encapsulating probiotics offers some advantages, but these depend on the materials and methods used. Encapsulation technologies allow probiotic bacteria to reach action sites and exert benefits along the digestive system. Commonly used encapsulation technologies have increased the number of food products in which probiotics could be incorporated without being affected by processing conditions. These methods still face various challenges, but consumer demand promotes continued work in the field.

6 Conclusion

Encapsulation technologies are viable options that can be used during development of functional food products. Nanoencapsulation is an improvement the basic method that allows precise and controlled release of bioactive compounds during digestion. It is essential to consider the desired properties of the final products, according to specific criteria like the nature of the core material and required release characteristics. Carrier materials are critical choices, and should be compatible with the method used and intended aplication.

Acknowledgments The authors are particularly grateful to the National Council on Science and Technology (CONACYT) for partial financial support through project 563: "Un Enfoque Multidisciplinario de la Farmacocinética de Polifenoles de Mango Ataulfo: Interacciones Moleculares, Estudios Preclínicos y Clínicos," CB-2012-01/179574 (basic science), CB-2015-01/254063 (basic science). Authors are grateful to academic authorities at CIAD, and to AlFaNutra research net.

References

1. Khalid N et al (2017) Formulation and characterization of monodisperse O/W emulsions encapsulating astaxanthin extracts using microchannel emulsification: insights of formulation and stability evaluation. Colloids Surf B Biointerfaces 157:355–365
2. Ballesteros LF et al (2017) Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chem 237:623–631
3. Cheng A-W et al (2017) Effects of storage time and temperature on polyphenolic content and qualitative characteristics of freeze-dried and spray-dried bayberry powder. LWT Food Sci Technol 78:235–240
4. Alexander M et al (2012) Incorporation of phytosterols in soy phospholipids nanoliposomes: encapsulation efficiency and stability. LWT Food Sci Technol 47(2):427–436
5. Eckert C et al (2017) Microencapsulation of lactobacillus plantarum ATCC 8014 through spray drying and using dairy whey as wall materials. LWT Food Sci Technol 82:176–183
6. Elez Garofulić I et al (2017) Retention of polyphenols in encapsulated sour cherry juice in dependence of drying temperature and wall material. LWT Food Sci Technol 83:110–117
7. Vinceković M et al (2017) Innovative technologies for encapsulation of Mediterranean plants extracts. Trends Food Sci Technol 69(Part A):1–12
8. Dias DR et al (2017) Encapsulation as a tool for bioprocessing of functional foods. Curr Opin Food Sci 13(Supplement C):31–37
9. Ezhilarasi P et al (2013) Nanoencapsulation techniques for food bioactive components: a review. Food Bioprocess Technol 6(3):628–647
10. Quintanilla-Carvajal MX et al (2010) Nanoencapsulation: a new trend in food engineering processing. Food Eng Rev 2(1):39–50
11. Agudelo C et al (2017) Phytochemical content and antioxidant activity of grapefruit (Star Ruby): a comparison between fresh freeze-dried fruits and different powder formulations. LWT Food Sci Technol 80:106–112
12. Gharsallaoui A et al (2007) Applications of spray-drying in microencapsulation of food ingredients: an overview. Food Res Int 40(9):1107–1121
13. Jafari SM, Ghalegi Ghalenoei M, Dehnad D (2017) Influence of spray drying on water solubility index, apparent density, and anthocyanin content of pomegranate juice powder. Powder Technol 311:59–65

14. Luna-Guevara JJ et al (2017) Microencapsulation of walnut, peanut and pecan oils by spray drying. Food Struct 12:26–32
15. Peabody J et al (2017) Characterization of a spray-dried candidate HPV L2-VLP vaccine stored for multiple years at room temperature. Papillomavirus Research 3:116–120
16. Leung SSY et al (2017) Effects of storage conditions on the stability of spray dried, inhalable bacteriophage powders. Int J Pharm 521(1–2):141–149
17. Lao F, Giusti MM (2017) The effect of pigment matrix, temperature and amount of carrier on the yield and final color properties of spray dried purple corn (Zea mays L.) cob anthocyanin powders. Food Chem 227:376–382
18. Bazaria B, Kumar P (2016) Optimization of spray drying parameters for beetroot juice powder using response surface methodology (RSM). J Saudi Soc Agric Sci
19. Mohammed NK et al (2017) Process conditions of spray drying microencapsulation of Nigella sativa oil. Powder Technol 315:1–14
20. Park H-J, Lee Y, Eun J-B (2016) Physicochemical characteristics of kimchi powder manufactured by hot air drying and freeze drying. Biocatal Agric Biotechnol 5:193–198
21. Evageliou V, Saliari D (2017) Limonene encapsulation in freeze dried gellan systems. Food Chem 223:72–75
22. Prosapio V, Norton I (2017) Influence of osmotic dehydration pre-treatment on oven drying and freeze drying performance. LWT Food Sci Technol 80:401–408
23. Cao X et al (2018) Effects of ultrasonic pretreatments on quality, energy consumption and sterilization of barley grass in freeze drying. Ultrason Sonochem 40:333–340
24. Ydjedd S et al (2017) Effect of in vitro gastrointestinal digestion on encapsulated and non-encapsulated phenolic compounds of carob (Ceratonia siliqua L.) pulp extracts and their antioxidant capacity. J Agric Food Chem 65(4):827–835
25. Peng Y et al (2018) Nanoemulsion delivery system of tea polyphenols enhanced the bioavailability of catechins in rats. Food Chem 242(Supplement C):527–532
26. Krogsgård Nielsen C et al (2016) Enhancing the antibacterial efficacy of isoeugenol by emulsion encapsulation. Int J Food Microbiol 229:7–14
27. Fan Q et al (2017) Partition and stability of resveratrol in whey protein isolate oil-in-water emulsion: impact of protein and calcium concentrations. Int Dairy J 73(Supplement C): 128–135
28. Katsouli M, Polychniatou V, Tzia C (2018) Optimization of water in olive oil nano-emulsions composition with bioactive compounds by response surface methodology. LWT 89(Supplement C): 740–748
29. Pérez-Mosqueda LM et al (2015) Formulation and optimization by experimental design of eco-friendly emulsions based on d-limonene. Colloids Surf B Biointerfaces 128(Supplement C): 127–131
30. Lepeltier E, Bourgaux C, Couvreur P (2014) Nanoprecipitation and the "Ouzo effect": application to drug delivery devices. Adv Drug Del Rev 71(Supplement C):86–97
31. Bilati U, Allémann E, Doelker E (2005) Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. Eur J Pharm Sci 24(1):67–75
32. Khayata N et al (2012) Preparation of vitamin E loaded nanocapsules by the nanoprecipitation method: from laboratory scale to large scale using a membrane contactor. Int J Pharm 423(2):419–427
33. Noronha CM et al (2013) Optimization of α-tocopherol loaded nanocapsules by the nanoprecipitation method. Ind Crop Prod 50(Supplement C):896–903
34. Tzeng C-W et al (2011) Enhancement of dissolution and antioxidant activity of Kaempferol using a nanoparticle engineering process. J Agric Food Chem 59(9):5073–5080
35. Silva JTDP et al (2017) Analytical validation of an ultraviolet–visible procedure for determining lutein concentration and application to lutein-loaded nanoparticles. Food Chem 230(Supplement C):336–342
36. Dinesh Kumar V, Verma PRP, Singh SK (2015) Development and evaluation of biodegradable polymeric nanoparticles for the effective delivery of quercetin using a quality by design approach. LWT Food Sci Technol 61(2):330–338

37. Liu W et al (2017) Kinetic stability and membrane structure of liposomes during in vitro infant intestinal digestion: effect of cholesterol and lactoferrin. Food Chem 230(Supplement C):6–13
38. Liu W et al (2012) Structure and integrity of liposomes prepared from milk- or soybean-derived phospholipids during in vitro digestion. Food Res Int 48(2):499–506
39. Sharma A, Sharma US (1997) Liposomes in drug delivery: progress and limitations. Int J Pharm 154(2):123–140
40. Basiri L, Rajabzadeh G, Bostan A (2017) Physicochemical properties and release behavior of Span 60/Tween 60 niosomes as vehicle for α-Tocopherol delivery. LWT Food Sci Technol 84:471–478
41. Ritwiset A, Krongsuk S, Johns JR (2016) Molecular structure and dynamical properties of niosome bilayers with and without cholesterol incorporation: a molecular dynamics simulation study. Appl Surf Sci 380(Supplement C):23–31
42. Liang R et al (2016) Niosomes consisting of Tween-60 and cholesterol improve the chemical stability and antioxidant activity of (−)-epigallocatechin gallate under intestinal tract conditions. J Agric Food Chem 64(48):9180–9188
43. Xu Y-Q et al (2016) Niosome encapsulation of curcumin: characterization and cytotoxic effect on ovarian cancer cells. J Nanomater 2016:9
44. Sharma P et al (2016) Novel encapsulation of lycopene in niosomes and assessment of its anticancer activity. J Bioequiv Bioavailab 8(5):224–232
45. Gavory C et al (2011) Polysaccharide-covered nanoparticles prepared by nanoprecipitation. Carbohydr Polym 84(1):133–140
46. Bakowska-Barczak AM, Kolodziejczyk PP (2011) Black currant polyphenols: their storage stability and microencapsulation. Ind Crop Prod 34(2):1301–1309
47. Sansone F et al (2011) Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts. J Food Eng 105(3):468–476
48. Bagheri L et al (2013) Nanoencapsulation of date palm pit extract in whey protein particles generated via desolvation method. Food Res Int 51(2):866–871
49. Quiroz-Reyes CN et al (2014) Development and characterization of gelatin nanoparticles loaded with a cocoa-derived polyphenolic extract. Fruits 69(6):481–489
50. Liu F et al (2017) A comparative study of covalent and non-covalent interactions between zein and polyphenols in ethanol-water solution. Food Hydrocoll 63(Supplement C):625–634
51. Caddeo C et al (2016) Effect of quercetin and resveratrol co-incorporated in liposomes against inflammatory/oxidative response associated with skin cancer. Int J Pharm 513(1):153–163
52. Pandita D et al (2014) Solid lipid nanoparticles enhance oral bioavailability of resveratrol, a natural polyphenol. Food Res Int 62(Supplement C):1165–1174
53. Sanchez V et al (2013) Freeze-drying encapsulation of red wine polyphenols in an amorphous matrix of maltodextrin. Food Bioprocess Technol 6(5):1350–1354
54. Song QX et al (2014) Enhanced uptake and transport of (+)-catechin and (−)-epigallocatechin gallate in niosomal formulation by human intestinal Caco-2 cells. Int J Nanomedicine 9(1):2157–2165
55. Wang G et al (2015) Role of SIRT1-mediated mitochondrial and Akt pathways in glioblastoma cell death induced by Cotinus coggygria flavonoid nanoliposomes. Int J Nanomedicine 10(1):5005–5023
56. Saikia S, Mahnot NK, Mahanta CL (2015) Optimisation of phenolic extraction from Averrhoa carambola pomace by response surface methodology and its microencapsulation by spray and freeze drying. Food Chem 171:144–152
57. Motilva MJ et al (2016) Human bioavailability and metabolism of phenolic compounds from red wine enriched with free or nano-encapsulated phenolic extract. J Funct Foods 25:80–93
58. Estevinho BN et al (2016) Soluble vitamins (vitamin B12 and vitamin C) microencapsulated with different biopolymers by a spray drying process. Powder Technol 289:71–78
59. Avinash G, Purnima A (2017) Microencapsulation by spray drying of vitamin a palmitate from oil to powder and its application in topical delivery system. J Encapsul Adsorpt Sci 7(1): 10–39

60. Gamboa OD, Goncalves LG, Grosso CF (2011) Microencapsulation of tocopherols in lipid matrix by spray chilling method. 11th international congress on engineering and food (ICEF11) 1:1732–1739
61. Wagner ME et al (2016) Stability of niosomes with encapsulated vitamin D-3 and ferrous sulfate generated using a novel supercritical carbon dioxide method. J Liposome Res 26(4):261–268
62. Ruiz-Rico M et al (2017) Protection of folic acid through encapsulation in mesoporous silica particles included in fruit juices. Food Chem 218:471–478
63. Anandharamakrishnan C (2014) Nanoencapsulation of food bioactive compounds. In: Techniques for nanoencapsulation of food ingredients. Springer New York, New York, pp 1–6
64. Jafari SM (2017) An overview of nanoencapsulation techniques and their classification. In: Jafari SM (ed) Nanoencapsulation technologies for the food and nutraceutical industries. Elsevier, p 1–34
65. Singh H, Ye A, Thompson A (2009) Nanoencapsulation systems based on milk proteins and phospholipids. ACS Publications, Washington, DC
66. Kim H et al (2016) Comparison of anti-inflammatory mechanisms of mango (Mangifera Indica L.) and pomegranate (Punica Granatum L.) in a preclinical model of colitis. Mol Nutr Food Res 60(9):1912–1923
67. Gawlik M et al (2017) Manganese neurotoxicity and protective effects of resveratrol and quercetin in preclinical research. Pharmacol Rep 69(2):322–330
68. Zamora-Ros R et al (2013) High concentrations of a urinary biomarker of polyphenol intake are associated with decreased mortality in older adults. J Nutr 143(9):1445–1450
69. Zamora-Ros R et al (2016) Urinary excretions of 34 dietary polyphenols and their associations with lifestyle factors in the EPIC cohort study. Sci Rep 6:26905
70. Fang Z, Bhandari B (2010) Encapsulation of polyphenols – a review. Trends Food Sci Technol 21(10):510–523
71. Wang X, Wang Y-W, Huang Q (2009) Enhancing stability and oral bioavailability of polyphenols using nanoemulsions. ACS Publications, Washington, DC
72. Chung C et al (2015) Enhanced stability of anthocyanin-based color in model beverage systems through whey protein isolate complexation. Food Res Int 76:761–768
73. Ko A et al (2017) Stabilization of black soybean anthocyanin by chitosan nanoencapsulation and copigmentation. J Food Biochem 41(2):e12316
74. Han HJ et al (2015) Extraction optimization and nanoencapsulation of jujube pulp and seed for enhancing antioxidant activity. Colloids Surf B Biointerfaces 130:93–100
75. Jeon YO, Lee J-S, Lee HG (2016) Improving solubility, stability, and cellular uptake of resveratrol by nanoencapsulation with chitosan and γ-poly (glutamic acid). Colloids Surf B Biointerfaces 147:224–233
76. Pan K et al (2014) pH-driven encapsulation of curcumin in self-assembled casein nanoparticles for enhanced dispersibility and bioactivity. Soft Matter 10(35):6820–6830
77. Zou L et al (2016) Food-grade nanoparticles for encapsulation, protection and delivery of curcumin: comparison of lipid, protein, and phospholipid nanoparticles under simulated gastrointestinal conditions. RSC Adv 6(4):3126–3136
78. Sadeghi S, Madadlou A, Yarmand M (2014) Microemulsification – cold gelation of whey proteins for nanoencapsulation of date palm pit extract. Food Hydrocoll 35(Supplement C):590–596
79. Belščak-Cvitanović A et al (2016) Emulsion templated microencapsulation of dandelion (Taraxacum officinale L.) polyphenols and β-carotene by ionotropic gelation of alginate and pectin. Food Hydrocoll 57(Supplement C):139–152
80. Rokka S, Rantamäki P (2010) Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. Eur Food Res Technol 231(1):1–12
81. Scully P et al (2013) Bifidobacterium infantis suppression of Peyer's patch MIP-1α and MIP-1β secretion during salmonella infection correlates with increased local CD4+ CD25+ T cell numbers. Cell Immunol 281(2):134–140

82. Dong QY et al (2013) Alginate-based and protein-based materials for probiotics encapsulation: a review. Int J Food Sci Tech 48(7):1339–1351
83. de Vos P et al (2010) Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int Dairy J 20(4):292–302
84. Sarkar S (2010) Approaches for enhancing the viability of probiotics: a review. Br Food J 112(4):329–349
85. Gbassi GK, Vandamme T (2012) Probiotic encapsulation technology: from microencapsulation to release into the gut. Pharmaceutics 4(1):149–163
86. Heidebach T, Först P, Kulozik U (2012) Microencapsulation of probiotic cells for food applications. Crit Rev Food Sci Nutr 52(4):291–311
87. Brinques GB, Ayub MAZ (2011) Effect of microencapsulation on survival of Lactobacillus plantarum in simulated gastrointestinal conditions, refrigeration, and yogurt. J Food Eng 103(2):123–128
88. González-Sánchez F et al (2010) Viability of microencapsulated Bifidobacterium animalis ssp. lactis BB12 in kefir during refrigerated storage. Int J Dairy Technol 63(3):431–436
89. Homayouni A et al (2008) Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. Food Chem 111(1):50–55
90. Possemiers S et al (2010) Bacteria and chocolate: a successful combination for probiotic delivery. Int J Food Microbiol 141(1):97–103
91. Borges S et al (2012) Effects of encapsulation on the viability of probiotic strains exposed to lethal conditions. Int J Food Sci Tech 47(2):416–421
92. Sandoval-Castilla O et al (2010) Textural properties of alginate–pectin beads and survivability of entrapped Lb. casei in simulated gastrointestinal conditions and in yoghurt. Food Res Int 43(1):111–117
93. Todorov SD, LeBlanc JG, Franco BD (2012) Evaluation of the probiotic potential and effect of encapsulation on survival for Lactobacillus plantarum ST16Pa isolated from papaya. World J Microbiol Biotechnol 28(3):973–984
94. Thantsha MS et al (2009) Supercritical carbon dioxide interpolymer complexes improve survival of B. longum Bb-46 in simulated gastrointestinal fluids. Int J Food Microbiol 129(1):88–92
95. Sabikhi L et al (2010) Resistance of microencapsulated Lactobacillus acidophilus LA1 to processing treatments and simulated gut conditions. Food Bioprocess Technol 3(4):586–593
96. Papagianni M, Anastasiadou S (2009) Encapsulation of Pediococcus acidilactici cells in corn and olive oil microcapsules emulsified by peptides and stabilized with xanthan in oil-in-water emulsions: studies on cell viability under gastro-intestinal simulating conditions. Enzym Microb Technol 45(6):514–522
97. Kailasapathy K (2009) Encapsulation technologies for functional foods and nutraceutical product development. CAB Rev: Perspect Agric Vet Sci Nutr Nat Resour 4(033):1–19
98. Nag A, Han K-S, Singh H (2011) Microencapsulation of probiotic bacteria using pH-induced gelation of sodium caseinate and gellan gum. Int Dairy J 21(4):247–253
99. Sousa S et al (2012) Encapsulation of probiotic strains in plain or cysteine-supplemented alginate improves viability at storage below freezing temperatures. Eng Life Sci 12(4):457–465

Part X

Miscellaneous Case Studies

Are *Myristica fragrans* L. (Myristicaceae) and Its Phytochemicals Useful for Human Health?

74

Monica Rosa Loizzo, Vincenzo Sicari, Jianbo Xiao, and Rosa Tundis

Contents

M. R. Loizzo (✉) · R. Tundis
Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Cosenza, Italy
e-mail: monica_rosa.loizzo@unical.it; rosa.tundis@unical.it

V. Sicari
Department of Agricultural Science, Mediterranean University of Reggio Calabria, Reggio, Calabria, Italy
e-mail: vincenzo.sicari@unirc.it

J. Xiao
Institute of Chinese Medical Sciences, State Key Laboratory of Quality Research in Chinese Medicine, Macau University, Taipa, Macau

Institut für Pharmazie und Lebensmittelchemie, Universität Würzburg, Würzburg, Germany
e-mail: jianboxiao@yahoo.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_23

Abstract

From *Myristica fragrans* L. (Myristicaceae) two spices, nutmeg and mace, were obtained. This plant is traditionally used in China, India, and African countries for the treatment of several diseases, such as decreased appetite, diarrhea, muscle spasm, rheumatism, etc. Recently, *M. fragrans* has been investigated for antioxidant, anticonvulsant, analgesic, anti-inflammatory, antidiabetic, hypolipidemic and hypocholesterolemic, antibacterial, and antifungal potential.

A perusal analysis of literature evidenced that *M. fragrans* deserves more attention by scientific community to explore its full range of bioactivity in the welfare of the society especially in emergent Countries.

Keywords

Myristica fragrans · Chemical profile · Bioactivity · Toxicity

List of Abbreviations

5-HT	Serotonin
AChE	Acetylcholinesterase
AMPK	AMP-activated protein kinase
BChE	Butyrylcholinesterase
DM	Diabetes mellitus
GABA	γ-Aminobutyric acid
IL	Interleukin
LDH-A	Lactate dehydrogenase
MCP	Monocyte chemotactic protein
MIP	Macrophage inflammatory protein
NO	Nitric oxide
PAS	Peripheral anionic site
THF	Tetrahydrofuran mixture

1 Introduction

Myristica fragrans L. (Myristicaceae) is an indigenous plant to India, Indonesia, and Sri Lanka. Now it is cultivated in many tropical countries of both hemispheres as well as in South Africa [1]. The tree is a small evergreen, not more than 40 feet in height, with smooth, greyish-brown bark, green on the younger branches. The alternate leaves are oblong-ovate, acute, entire, smooth, and dark-green. The flowers are very small and unisexual. The fruits, smooth and yellow, resemble a pear grooved by a longitudinal furrow and contain a single erect seed. The seed of the plant is known as “nutmeg” and the arillus of the seed is called “mace”.

Both kernel and arillus are rich in essential oil that imparts the characteristic aroma and taste to nutmeg as a unique culinary spice. Primary metabolites including carbohydrates, lipids/fatty acids, and proteins constitute up to 80% of the weight of dry nutmeg kernel while the remaining weight comprises secondary metabolites such as terpenes, phenolic acids, polyphenols, and pigments [2].

Since ancient times, *M. fragrans* nutmeg and its oil are used in Chinese and Indian traditional medicines for illnesses related to the digestive and nervous systems. Nutmeg is a rich source of essential oil, triterpenes, and various types of phenolic compounds that are responsible of the biological activities that may support its use in traditional medicine.

In this chapter, we reported and critically discussed data from literature published from 2000 to 2017 on the *M. fragrans* as source of bioactive compounds.

2 Bioactivity of Extract and Isolated Compounds

2.1 Effects on Metabolic Syndrome

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia with impaired metabolism of carbohydrates, fat, and proteins due to defects in insulin secretion, insulin action, or both [3]. Spices are extensively used to enhance the taste and flavor of foods and are known to possess several medicinal properties including hypoglycemic effect. In this context, Somani and Singhai [4] reported that *M. fragrans* petroleum ether extract decreased blood glucose levels in normal, glucose fed, and alloxan induced diabetic rats by a mechanism of action involving a promotion of release of insulin from β-cells. Moreover, after oral administration a decrease in the rate of intestinal glucose absorption or potentiation of pancreatic secretions or increasing the glucose uptake was observed. The dose-dependent insulin secretion on isolated islets of Langerhans was confirmed by Patil et al. [5]. Moreover, this spice showed α-amylase inhibitory activity with an IC_{50} value of 0.85 mg/mL. A moderate hypoglycemic effect was confirmed for *M. fragrans* fruits ethanol extract in streptozotocin-induced diabetic rats [6]. Mace dried powered extract showed carbohydrate-hydrolyzing enzyme inhibitory activity with IC_{50} values of 62.1 and 75.7 μg/mL against α-amylase and α-glucosidase, respectively [7]. Among *M. fragrans* isolated constituents, macelignan (**1**) (Fig. 1) exerted a promising antidiabetic activity since it enhanced the insulin sensitivity and improved lipid metabolic disorders by activating peroxisome proliferator receptor-alpha/gamma and reducing endoplasmic reticulum stress [8].

In addition, macelignan (**1**) possess an inhibitory action against advanced glycation end products, an effect that adds to its potential role in the management of diabetes and metabolic syndrome [9].

Frequently DM is associated to an impaired metabolism of fat. Ram et al. [10] showed that oral administration of nutmeg extract (500 mg/kg body weight) for 60 days to hyperlipidemic albino rabbits significantly reduced the lipoprotein lipids level. Several research studies evidenced that AMP-activated protein kinase (AMPK) activators mimic or potentiate the exercise-related effects. Therefore, the activation of AMPK could be used as drug target for the prevention and treatment of obesity and diabetes by regulating the glucose and lipid homeostasis [11]. Tetrahydrofuroguaiacin B, and nectandrin B at 5 μM produced strong AMPK stimulation in differentiated C2C12 cells. In addition, the preventive

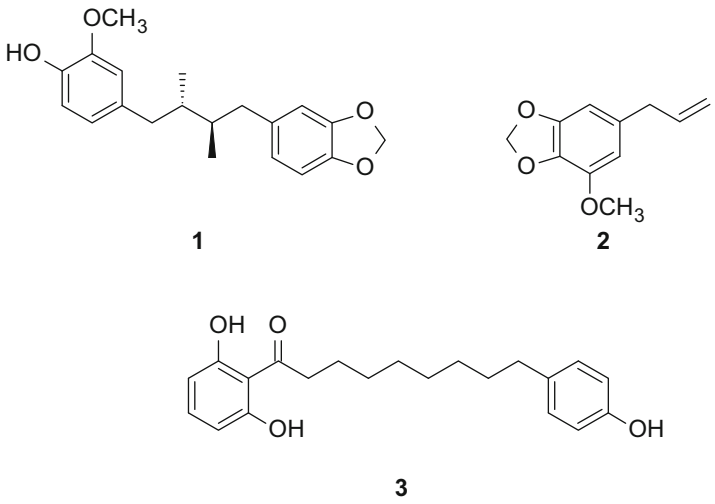


Fig. 1 Macelignan (1), myristicin (2) and malabaricone B (3)

effect of a tetrahydrofuran mixture (THF) containing tetrahydrofuroguaiacin B, saucernetindiol, verrucosin, nectandrin B, nectandrin A, fragransin C1, and galbacin on weight gain in a diet-induced animal model was examined. Results evidenced that final body weights and weight gain in the THF-treated mice were significantly lower than those of the control group. THF treatment prevented the increase in body weight and adipose tissue mass. However, increase in glucose and LDL levels in the THF-treated group was observed. These effects may be due partly by the AMPK activators in this extract [12].

2.2 Anti-Inflammatory and Analgesic Activity

Even though a number of steroidal or nonsteroidal anti-inflammatory drugs have been developed, the research of natural compounds as source of new anti-inflammatory agents with lower side effects is a topic of great interest. Nutmeg and mace essential oils are traditionally used to relief sprains and rheumatism. The oil exhibited an anti-inflammatory effect on acute inflammation. An analgesic effect was produced by the oil in the acetic acid-induced writhing model and in the late phase of the formalin-induced licking [13]. A similar effect was observed also with the chloroform extract in a model of inflammation such as carrageenan-induced edema in rats [14].

The anti-inflammatory effects of *M. fragrans* could be partially due to the presence of myristicin (**2**) (Fig. 1). This compound did not reduce the cell viability of mouse macrophages (RAW 264.7) but significantly inhibited the production of calcium, interleukin (IL)-6, IL-10, interferon inducible protein-10, monocyte chemotactic protein (MCP)-1, MCP-3, granulocyte-macrophage colony-stimulating

factor, macrophage inflammatory protein (MIP)-1α, MIP-1β, and nitric oxide (NO) [15]. Other promising anti-inflammatory agents from nutmeg are macelignans. These compounds suppressed lipopolysaccharide-induced activation of the Toll-like receptor 4 pathway. These actions are mediated by suppression of the nuclear factor NF-κB, reduction in cyclooxygenase type-2 (COX-2) expression, and inhibition of reactive oxygen species generation [16]. Moreover, macelignan (**1**) inhibited the calcium influx, degranulation, release of histamine, and other inflammatory mediators [17]. The anti-inflammatory activity was exerted also by malabaricone B (3) since it is able to attenuate the increased nitric oxide synthesis and enhancing the arginase pathway in mice [18]. Nutmeg is a traditional remedy for pain. Zhang et al. [19] demonstrated that nutmeg oil administration alleviate the induced joint swelling, mechanical allodynia, and heat hyperanalgesia in rats with a mechanism of action involving reduction of COX-2 expression and blood substance P level. An anti-pain action was demonstrated also for *M. fragrans* seed kernels alkaloids fraction [20]. At the dose of 1 g/kg this fraction significantly reduced the number of writhing responses in female but not male mice. However, a slightly toxic with LD_{50} value of 5.1 g/kg was found.

2.3 Antitumor

Several research articles evidenced the antitumor activity of *M. fragrans* with particular reference to its essential oil. Piras et al. [21] isolated volatile and fixed oils from nutmeg. Myristicin (**2**) (32.8%), sabinene (16.1%), α-pinene (9.8%), and β-pinene (9.4%) were identified as main compounds. In the fixed oil, the most represented fatty acids were myristic acid (14:0), oleic acid (18:1 n-9), and palmitic acid (16:0). Both oils and myristicin (**2**) displayed a significant growth inhibitory activity against the colon cancer cell line Caco-2.

A promising cytotoxic activity was observed with *M. fragrans* essential oil against human colorectal carcinoma (HCT-116) and human breast carcinoma (MCF-7) cell lines with IC_{50} values of 78.61 and 66.45 μg/mL, respectively [22]. *M. fragrans* essential oil showed antiangiogenic activity with IC_{50} of 77.64 μg/mL maybe due to the presence of some potential antiangiogenic compounds such as myristicin (**2**), limonene, eugenol, and terpinen-4-ol.

Myristicin (**2**) induced cytotoxicity in human neuroblastoma SK-N-SH with IC_{50} value $>$ or $=$ 0.5 mM at cells by an apoptotic mechanism since an accumulation of cytochrome-c and the activation of caspase-3 was displayed [23]. The apoptotic pathway with mitochondrial membrane potential alteration, cytochrome c release, caspase-3 activation, PARP-cleavage, and DNA fragmentation was observed, also, when myristicin (**2**) was applied to human leukemia K562 cell culture [24]. Previously, Chirathaworn et al. [25] observed that *M. fragrans* methanol extract at concentration of 10 μg/ml induces apoptosis in Jurkat leukemia T cell line through SIRT1 mRNA downregulation. Interestingly, *M. fragrans* antitumor activity could be mediated, also, by the inhibition of Lactate Dehydrogenase A (LDH-A). Tumor cells predominantly produce ATP by maintaining a high rate of lactate fermentation,

rather than by maintaining a comparatively low rate of tricarboxylic acid cycle. *M. fragrans* seeds extract suppressed cell growth and the overall Warburg effect in human colon cancer cells (HT29). Even though the expression of the enzyme was not changed, both lactate production and LDH-A activity were decreased [26].

Moreover recently, Li et al. [27] demonstrated that nutmeg-treated Apcmin/+ mice had decreased IL-6 levels and normalized dysregulated lipid metabolism, suggesting that uremic toxins are responsible, in part, for the metabolic disorders that occur during colon cancer tumorigenesis. Other *M. fragrans* essential oil representative compounds such as D-limonene, terpinen-4-ol, and eugenol showed cytotoxic activity through apoptosis-inducing effects on several cancer cells [28].

2.4 Antimicrobial and Antifungal Activity

The use of essential oils from spice is an attractive alternative method to control food/feed microorganisms as they should in principle not be toxic to man and could replace toxic synthetic compounds. The use of nontoxic natural compounds is attributed to growing problems encountered with microbial resistance towards conventional preservation with synthetic compounds and an increasing demand for minimal processed food along with "green" image policies of food industry. *M. fragrans* is known to exhibit strong antimicrobial activity against food poisoning and spoilage bacteria including *Bacillus subtilis*, *Escherichia coli, Saccharomyces cerevisiae*, and multi-drug resistant *Salmonella typhi* and *Helicobacter pylori* [29]. More recently, Firouzi et al. [30] reported the effect of nutmeg essential oil on growth and survival of *Yersinia enterocolitica* and *Listeria monocytogenes*. Nutmeg oil also had a significant effect on inhibiting the growth of *Escherichia coli* and *Staphyloccocus aureus* [31]. Several studies demonstrated also the antibacterial activity of nutmeg extracts. In particular, nutmeg acetone extract has shown the strongest antibacterial and antifungal activity with *Staphylococcus aureus* (13.8 mm) and *Aspergillus niger* (14.4 mm), respectively [32]. The nutmeg ethyl acetate extract showed a strong antibacterial activity against gram-positive and gram-negative bacteria with mean MIC value ranging from 0.625 to 1.25 mg/mL, and bactericidal effects at mean MBC value ranging from 0.625 to 20 mg/mL [33].

A good antibacterial activity was obtained also with seed and mace ethanol extracts. *M. fragrans* seed methanol extract had a strong MIC value of 12.5 μg/ml against the gram-negative bacterium *Helicobacter pylori* that is recognized as the primary etiological factor associated with the development of gastritis and peptic ulcer disease [34]. A promising activity against *H. pylori* was found also with dihydroguaiaretic acid (**4**) (Fig. 2) with MIC of 100 μg/ml and MBC of 125 μg/ml. This effect is comparable with that of clarithromycin [35]. Resorcinols, malabaricone B (**3**), and malabaricone C (**5**) from mace also showed strong antibacterial and antifungal activities while macelignan (**1**) is a potent natural antibiofilm agent against oral primary colonizers *Streptococcus sanguis* and *Actinomyces viscosus* [36, 37]. Nutmeg essential oil showed also antifungal activity against *Colletotrichum gloeosporoides, Colletotrichum musae, Fusarium*

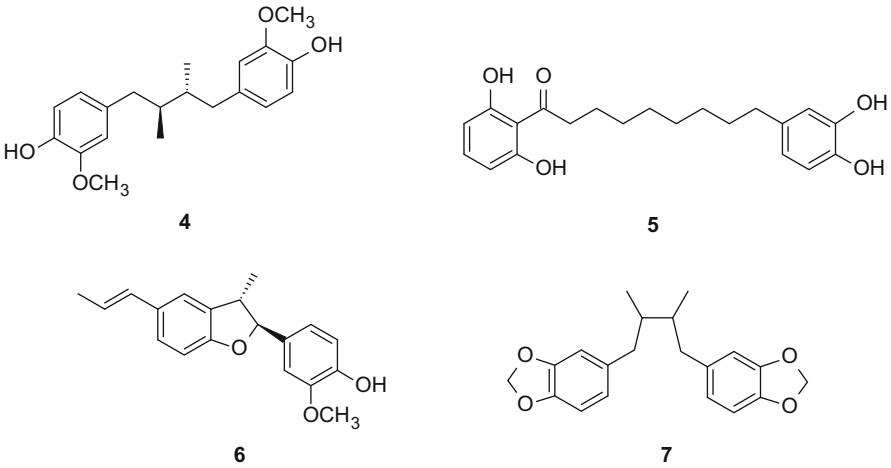


Fig. 2 Dihydroguaiaretic acid (4), malabaricone C (5), licarin A (6) and Machilin A (7)

oxysporum, Fusarium semitectum, Aspergillus niger, and *Aspergillus glaucus* [38]. Previously, Cho et al. [39] evidenced that erythro-austrobailignan-6, meso-dihydroguaiaretic acid and nectandrin-B isolated from the *M. fragrans* seeds methanol extract exhibited antifungal activities against *Alternaria alternata*, *Colletotrichum coccodes*, *Colletotrichum gloeosporioides*, *Magnaporthe grisea*, *Agrobacterium tumefaciens*, *Acidovorax konjaci*, and *Burkholderia glumae*. A significant antifungal activity was found also for mace methanol extract against *Candida albicans* and *A. niger* [40].

2.5 Effects on Central Nervous System

Several studies demonstrated different effects of *M. fragrans* in Central Nervous System (CNS).

Sonavane et al. [41] investigated the anxiogenic activity of the *M. fragrans* seed *n*-hexane extract seeds, acetone-insoluble part of the *n*-hexane extract, and trimyristin. Both extract and trimyristin reduced the number of head pock in the hole-board test. Authors suggests a nonspecific anxiogenic activity of TM and a mechanism, which involves serotonin (5-HT) and gamma-Aminobutyric acid (GABA). Successively, the same *n*-hexane extract at the dose of 10 mg/kg dose was found to have comparable potency to imipramine (15 mg/kg) and fluoxetine (20 mg/kg). The antidepressant-like effect of the *n*-hexane extract involved adrenergic, dopaminergic and serotonergic systems. The *n*-hexane extract inhibited both α1 and dopaminergic receptor antagonists as well as a serotonin synthesis inhibitor [42]. The memory enhancer activity of *M. fragrans* is confirmed by the in vitro and in vivo inhibition of acetylcholinesterase [43].

Cuong et al. [44] investigated the cholinesterase inhibitory activity of *M. fragrans* seeds MeOH extract and *n*-hexane, ethyl acetate, *n*-butanol, and water-soluble fractions. EtOAc showed the highest inhibitory potential was submitted to chromatographic purification led to the isolation of thirteen compounds. [(7S)-8′-(4′-hydroxy-3′-methoxyphenyl)-7-Hydroxypropyl]benzene-2,4-diol) exhibited the most effective activity with an IC_{50} value of 35.1 μM. Values of 42.1 and 44.0 μM were found for [(8R,8′S)-7′-(3′,4′-methylenedioxyphenyl)-8,8′-dimethyl-7-(3,4-dihydroxyphenyl)-butane] and malabaricone C (**5**), respectively.

More recently, Abdul Wahab et al. [45] evidenced that malabaricone B (**3**) (1.84 and 1.76 μM, respectively) and C (1.94 and 2.80 μM, respectively) are dual and mixed-type inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Molecular docking studies evidenced that both compounds interacts with the peripheral anionic site (PAS), the catalytic triad, and the oxyanion hole of the AChE. Differently Malabaricone B (**3**) interacted with the PAS, the catalytic triad, and the oxyanion hole of the BChE, while malabaricone C (**5**) interacted only with the catalytic triad and the oxyanion hole.

Anecdotal reports of *M. fragrans* use as a cheap marijuana substitute, coupled to previous studies reporting a cannabimimetic-like action, suggest that nutmeg may interact with the endocannabinoid system. *M. fragrans* seeds *n*-hexane extract administered orally in three doses 5–20 mg/kg p.o. enhanced learning and retention capacities of both young and aged mice. The effect of *M. fragrans* on endocannabinoid system was confirmed by El-Alfy et al. [46]. Dichloromethane and ethyl acetate fractions prepared from the methanol extract of powdered whole nutmeg interacts with the endocannabinoid system via inhibition of the endocannabinoid catabolizing enzymes fatty acid amide hydrolase and monoacylglycerol lipase. This mechanism provides insight into reported cannabis-like action as well as expands the potential therapeutic utility of nutmeg. Among nutmeg compounds, myristicin (**2**) demonstrated to possess anxiogenic effect and may antagonize the actions of benzodiazepine at the GABA A receptors level [47]. Moreover, trimyristin, the main triglyceride constituent of nutmeg, showed the depressant effect in mice [48]. On the other hand, Kiyofuji et al. [49] demonstrated that macelignan (**1**) treatment protected dopaminergic neurons against the interferon (IFN)-c and LP-induced degeneration by activation of the peroxisome proliferator activated receptor c, which in turns activated arginase 1 enzyme expression. For this reason, maceliganan can be used for the treatment of different neurodegenerative disorders. A neuroprotective effect was observed also for licarin A (**6**) against glutamate-induced toxicity of rat cortical cells [50]. The mechanism of action involves antioxidant effect, reduction of NO, and suppression of Ca^{2+} influx.

In order to clarify the potential central nervous system effects of nutmeg, Wu et al. [51] investigated the permeability of blood-brain barrier of twelve lignans and three phenolic malabaricones from the seeds of *M. fragrans*. Benzonfuran-type, dibenzylbutane-type, and arylnaphthalene-type lignans showed poor to moderate permeabilities; those of 8-O-4′-neolignan and tetrahydrofuran-lignan have moderate to high permeabilities, while the permeabilities of malabaricones were poor. Erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(3,4-dimethoxyphenyl)-propan-1-ol acetate,

verrucosin, and nectandrin B were transported by efflux way, while neolignans dehydrodiisoeugenol, myrislignan, and verrucosin permeabilities was passive diffusion.

2.6 Vascular Activity

In the treatment and prevention of cardiovascular disorders and strokes, the inhibition of platelet function plays a critical role. Erythro-(7S,8R)-7-acetoxy-3,4,3′,-5′-tetramethoxy-8-O-4′-neolignan (EATN), isolated from *M. fragrans*, inhibited thrombin- and platelet-activating factor (PAF)-induced platelet aggregation without affecting platelet damage with IC_{50} values of 3.2 and 3.4 μM, respectively. Moreover, this neolignan attenuated intracellular Ca^{2+} mobilization in thrombin-activated human platelets through increase cAMP levels [52]. A promising protective effect in vascular occlusive diseases was observed, also, with nectandrin B. This lignin activated AMPK in vascular smooth muscle cells (VSMC) and inhibited VSMC proliferation and neo-intima formation, events that are critical in the development of vascular occlusive diseases [53].

2.7 Other Activities

Nutmeg compounds showed other promising healthy effects. Machilin A (**7**) (Fig. 2) was able to stimulate, in a dose-dependent manner, osteoblast differentiation through activation of the p38 mitogen activated protein (MAP) kinase pathway [54]. The use of macelignan (**1**) as photo-aging protective agent is well documented. These compounds reduced the collagen synthesis and simultaneously upregulated the matrix metalloproteinases (MMPs) [55]. Moreover, these compounds act as natural depigmenting agent since at 10 μM inhibited melanosome transfer and dendrite formation in B16F10 melanoma cells [56]. Similarly acts licarin E that regulates the expression of MMP-1 and type-1 procollagen in UVB-irradiated human skin fibroblasts through MAPK and TGFβ signaling [57].

The hepatoprotective effect of nutmeg compounds was reported. Morita et al. [58] have reported that myristicin (**2**) showed a strong hepatoprotective effect with a mechanism of action that involves the inhibition of tumor necrosis factor-α release from macrophages while of macelignan (**1**) induced activation of the mitogen activated protein kinase (MAPK) signaling pathway, especially JNK and c-Jun [59]. The hepatoprotective effect demonstrated for nectandrin that protect hepatocytes against oxidative injury through the activation of Nrf2/ARE pathway mediated by ERK phosphorylation and AMPK-dependent inactivation of GSK-3β [60].

3 Conclusions

In traditional medicine, nutmeg has long been used as a remedy for several disease including gastrointestinal problems, cancer, infections, rheumatism, and psychological disorders.

Despite its traditional uses, the mechanisms underlying its effects remain unclear and further research studies are undoubtedly needed to properly assess the therapeutic potential of this plant and derived products. A perusal analysis of literature evidenced that myristicin (**2**), macelignan (**1**), nectandrin A and B, licarin A–E, and malabaricone B and C were well investigated especially for anxiolytic, anti-cancer, anti-inflammatory, and anti-infective effects. Although there is great interest in *M. fragrans* extract, oil and isolated constituents, limited preclinical studies on nutmeg not only for size but also for design and focus with no pure derived compounds included was observed. However, the evidence that nutmeg represent an in doubt font of secondary metabolites with significant potential as prototype agents for drug discovery, address the research interest on this plant. Further evaluation of bioactive constituents is warranted especially to determine in vivo pharmacokinetic parameters and to monitored nutmeg constituents toxicity. In fact, it is well documented that excessive doses have a narcotic effect, symptoms of delirium, and epileptic convulsions [61]. Moreover, one of the most promising compounds, myristicin (**2**) is hepatotoxic when ingested in large amounts [62].

Conflicts of Interest The authors declare no conflicts of interest.

References

1. Pal M, Srivastava M, Soni DK, Kumar A, Tewari SK (2011) Composition and anti-microbial activity of essential oil of *Myristica fragrans* from Andaman Nicobar Island. Int J Pharm Life Sci 2:1115–1117. https://doi.org/10.5897/AJB12.1043. ISSN 1684-5315
2. Abourashed EA, El-Alfy AT (2016) Chemical diversity and pharmacological significance of the secondary metabolites of nutmeg (*Myristica fragrans* Houtt.) Phytochem Rev 15:1035–1036. https://doi.org/10.1007/s11101-016-9469-x
3. American Diabetes Association. http://www.diabetes.org
4. Somani RS, Singhai AK (2008) Hypoglycaemic and antidiabetic activities of seeds of *Myristica fragrans* in normoglycaemic and Alloxan-induced diabetic rats. Asian J Exp Sci 7(2):675–80. https://doi.org/10.13040/IJPSR.0975-8232.7
5. Patil SB, Ghadyale VA, Taklikar SS, Kulkarni CR, Arvindekar AU (2011) Insulin secretagogue, alpha-glucosidase and antioxidant activity of some selected spices in streptozotocin-induced diabetic rats. Plant Foods Hum Nutr 66:85–90. https://doi.org/10.1007/s11130-011-0215-7
6. Ahmad R, Srivastava SP, Maurya R, Rajendran SM, Arya KR, Srivastava AK (2008) Mild antihyperglycaemic activity in *Eclipta alba*, *Berberis aristata, Betula utilis, Cedrus deodara, Myristica fragrans* and *Terminalia chebula*. Indian J Sci Technol 1:1–6. https://doi.org/10.17485/ijst/2008/v1i5/29348
7. Loizzo MR, Sicari V, Tenuta MC, Leporini MR, Falco T, Pellicanò TM, Menichini F, Tundis R (2016) Phytochemicals content, antioxidant and hypoglycaemic activities of commercial nutmeg mace (*Myristica fragrans* L.) and pimento (*Pimenta dioica* (L.) Merr.) Int J Food Sci Technol 51:2057–2063. https://doi.org/10.1111/ijfs.13178
8. Han KL, Choi JS, Lee JY, Song J, Joe MK, Jung MH, Hwang JK (2008) Therapeutic potential of peroxisome proliferator-activated receptor-alpha/gamma dual agonist with alleviation of endoplasmic reticulum stress for the treatment of diabetes. Diabetes 57:737–745. https://doi.org/10.2337/db07-0972

9. Poornima B, Anand Kumar D, Siva B, Venkanna A, Vadaparthi PR, Kumar K, Tiwari AK, Suresh Babu K (2016) Advanced glycation end-products inhibitors isolated from *Schisandra grandiflora*. Nat Prod Res 30:493–496. https://doi.org/10.1080/14786419.2015.1024117
10. Ram A, Lauria P, Gupta R, Sharma VN (1996) Hypolipidaemic effect of *Myristica fragrans* fruit extract in rabbits. J Ethnopharmacol 55:49–53
11. Misra P (2008) AMP activated protein kinase: a next generation target for total metabolic control. Expert Opin Ther Targets 12:91–100. https://doi.org/10.1517/14728222.12.1.91
12. Nguyen PH, Le TV, Kang HW, Chae J, Kim SK, Kwon KI, Seo DB, Lee SJ, Oh WK (2010) AMP-activated protein kinase (AMPK) activators from *Myristica fragrans* (nutmeg) and their anti-obesity effect. Bioorg Med Chem Lett 20:4128–4131. https://doi.org/10.1016/j.bmcl.2010.05.067
13. Olajide OA, Makinde JM, Awe SO (2000) Evaluation of the pharmacological properties of nutmeg oil in rats and mice. Pharm Biol 38:385–390. https://doi.org/10.1076/phbi.38.5.385.5976
14. Olajide OA, Ajayi FF, Ekhelar AI, Awe SO, Makinde JM, Alada AR (1999) Biological effects of *Myristica fragrans* fruits extract in rabbits. Phytother. Res 13:344–345. https://doi.org/10.1002/(SICI)1099-1573(199906)13:4<344::AID-PTR436>3.0.CO;2-E
15. Lee JY, Park W (2011) Anti-inflammatory effect of myristicin on RAW 264.7 macrophages stimulated with polyinosinic-polycytidylic acid. Molecules 16:7132–7142. https://doi.org/10.3390/molecules16087132
16. Ma J, Hwang YK, Cho WH, Han SH, Hwang JK, Han JS (2009) Macelignan attenuates activations of mitogen-activated protein kinases and nuclear factor kappa B induced by lipopolysaccharide in microglial cells. Biol Pharm Bull 32:1085–1090. https://doi.org/10.1248/bpb.32.1085
17. Ma CJ, Kim SR, Kim J, Kim YC (2005) Meso-dihyrdoguaiaretic acid and licarin A of *Machilus thunbergii* protects against glutamate-induced toxicity in primary cultures of a rat cortical cells. Br J Pharmacol 146:752–759. https://doi.org/10.1038/sj.bjp.0706380
18. Maity B, Banerjee D, Bandyopadhyay SK, Chattopadhyay S (2009) Regulation of arginase/nitric oxide synthesis axis via cytokine balance contributes to the healing action of malabaricone B against indomethacin-induced gastric ulceration in mice. Int Immunopharmacol 9:491–498. https://doi.org/10.1016/j.intimp.2009.01.028
19. Zhang WK, Tao SS, Li TT, Li YS, Li XJ, Tang HB, Cong RH, Ma FL, Wan CJ (2016) Nutmeg oil alleviates chronic inflammatory pain through inhibition of COX-2 expression and substance P release in vivo. Food Nutr Res 60:30849. 1–10. https://doi.org/10.3402/fnr.v60.30849
20. Hayfaa AA, Sahar AM, Awatif MA (2013) Evaluation of analgesic activity and toxicity of alkaloids in *Myristica fragrans* seeds in mice. J Pain Res 31:611–661. https://doi.org/10.2147/JPR.S45591
21. Piras A, Rosa A, Marongiu B, Atzeri A, Dessì MA, Falconieri D, Porcedda S (2012) Extraction and separation of volatile and fixed oils from seeds of *Myristica fragrans* by supercritical CO_2: chemical composition and cytotoxic activity on Caco-2 cancer cells. J Food Sci 77:C448–C453. https://doi.org/10.1111/j.1750-3841.2012.02618.x
22. Piaru SP, Mahmud R, Abdul Majid AM, Ismail S, Man CN (2012) Chemical composition, antioxidant and cytotoxicity activities of the essential oils of *Myristica fragrans* and *Morinda citrifolia*. J Sci Food Agric 92:593–597. https://doi.org/10.1002/jsfa.4613
23. Lee BK, Kim JH, Jung JW, Choi JW, Han ES, Lee SH, Ko KH, Ryu JH (2005) Myristicin-induced neurotoxicity in human neuroblastoma SK-N-SH cells. Toxicol Lett 157:49–56. https://doi.org/10.1016/j.toxlet.2005.01.012
24. Martins C, Doran C, Silva IC, Miranda C, Rueff J, Rodrigues AS (2014) Myristicin from nutmeg induces apoptosis via the mitochondrial pathway and down regulates genes of the DNA damage response pathways in human leukaemia K562 cells. Chem Biol Interact 218:1–9. https://doi.org/10.1016/j.cbi.2014.04.014
25. Chirathaworn C, Kongcharoensuntorn W, Dechdoungchan T, Lowanitchapat A, Sanguanmoo P, Poovorawan Y (2007) *Myristica fragrans* Houtt. methanolic extract induces apoptosis in a

human leukemia cell line through SIRT1 mRNA downregulation. J Med Assoc Thail 90:2422–2428. https://doi.org/10.1016/j.intimp.2008.01.012
26. Kim EY, Choi HJ, Park MJ, Jung YS, Lee SO, Kim KJ, Choi JH, Chung TW, Ha KT (2016) *Myristica fragrans* suppresses tumor growth and metabolism by inhibiting lactate dehydrogenase A. Am J Chin Med 44:1063–1079. https://doi.org/10.1142/S0192415X16500592
27. Li F, Yang XW, Krausz KW, Nichols RG, Xu W, Patterson AD, Gonzalez FJ (2015) Modulation of colon cancer by nutmeg. J Proteome Res 14:1937–1946. https://doi.org/10.1021/pr5013152
28. Huang M, JJ L, Huang MQ, Bao JL, Chen XP, Wang YT (2012) Terpenoids: natural products for cancer therapy. Expert Opin Investig Drugs 21:1801–1818. https://doi.org/10.1517/13543784.2012.727395
29. Jaiswal P, Kumar P, Singh VK, Singh DK (2009) Biological effects of *Myristica fragrans*. Ann Rev Biomed Sci 11:21–29. https://doi.org/10.5016/1806-8774.2009v11p21
30. Firouzi R, Shekarforoush SS, Nazer AH, Borumand Z, Jooyandeh AR (2007) Effects of essential oils of oregano and nutmeg on growth and survival of *Yersinia enterocolitica* and Listeria monocytogenes in barbecued chicken. J Food Prot 70:2626–2630. https://doi.org/10.4315/0362-028X-70.11.2626
31. Cui H, Zhang X, Zhou H, Zhao C, Xiao Z, Lin L, Li C (2015) Antibacterial properties of nutmeg oil in pork and its possible mechanism. J Food Saf 35:370–377. https://doi.org/10.1111/jfs.12184
32. Gupta AD, Bansal VK, Babu V, Maithil N (2013) Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt). J Genet Eng Biotechnol 11:25. https://doi.org/10.1016/B978-0-12-375688-6.10098-2
33. Shafiei Z, Shuhairi NN, Yap N, Sibungkil C-AH, Latip J (2012). Antibacterial activity of Myristica Fragrans against oral pathogens. J Evid Based Complement Alternat Med, Article ID 825362:7. doi:https://doi.org/10.1155/2012/825362
34. Mahady GB, Pendland SL, Stoia A, Hamill FA, Fabricant D, Dietz BM, Chadwick LR (2005) In vitro susceptibility of *Helicobacter pylori* to botanical extracts used traditionally for the treatment of gastrointestinal disorders. Phytother Res 19:988–991. https://doi.org/10.1002/ptr.1776
35. Bhamarapravati S, Juthapruth S, Mahachai W, Mahady G (2006) Antibacterial activity of *Boesenbergia rotunda* (L.) Mansf. and *Myristica fragrans* Houtt. against *Helicobacter pylori*. Songklanakarin J Sci Technol 28:157–163
36. Orabi KY, Mossa JS, El-Feraly FS (1991) Isolation and characterization of two antimicrobial agents from mace (*Myristica fragrans*). J Nat Prod 54:856–859. https://doi.org/10.1021/np50075a017
37. Yanti RY, Kim KH, Hwang JK (2008) In vitro anti-biofilm activity of macelignan isolated from *Myristica fragrans* Houtt. against oral primary colonizer bacteria. Phytother Res 22:308–312. https://doi.org/10.1002/ptr.2312
38. Valente VMM, Jham GN, Dhingra OD, Ghiviriga I (2011) Composition and antifungal activity of the Brazilian *Myristica fragrans* Houtt essential oil. J Food Saf 31:197–202. https://doi.org/10.1111/j.1745-4565.2010.00285.x
39. Cho JY, Choi GJ, Son SW, Jang KS, Lim HK, Lee SO, Sung ND, Cho KY, Kim JC (2007) Isolation and antifungal activity of lignans from *Myristica fragrans* against various plant pathogenic fungi. Pest Manag Sci 63:935–940. https://doi.org/10.1002/ps.1420
40. Pooja V, Goyal SH, Sandashwani AB, Srivastava AK (2012) Activity of *Myristica fragrans* and its effect against filamentous and nonfilamentous fungus. Int J Pharm Pharm Sci 4:17–20
41. Sonavane GS, Sarveiya VP, Kasture VS, Kasture SB (2002) Anxiogenic activity of *Myristica fragrans* seeds. Pharmacol Biochem Behav 71(1–2):239–244. https://doi.org/10.1016/S0091-3057(01)00660-8
42. Dhingra D, Sharma A (2006) Antidepressant-like activity of n-hexane extract of nutmeg (*Myristica fragrans*) seeds in mice. J Med Food 9:84–89. https://doi.org/10.1089/jmf.2006.9.84

43. Dhingra D, Parle M, kulkarni S (2006) Comparative brain cholinesterase-inhibiting activty of *Glycyrrhiza glabra, Myritica fragrans*, acorbic acid, and metrofinate in mice. J Med Food 9:281–283. https://doi.org/10.1089/jmf.2006.9.281
44. Cuong TD, Hung TM, Han HY, Roh HS, Seok JH, Lee JK, Jeong JY, Choi JS, Kim JA, Min BS (2014) Potent acetylcholinesterase inhibitory compounds from *Myristica fragrans*. Nat Prod Commun 9:499. https://doi.org/10.1016/j.bmcl.2016.05.046
45. Abdul Wahab SM, Sivasothy Y, Liew SY, Litaudon M, Mohamad J, Awang K (2016) Natural cholinesterase inhibitors from *Myristica cinnamomea* King. Bioorg Med Chem Lett 26:3785–3792. https://doi.org/10.1016/j.bmcl.2016.05.046
46. El-Alfy AT, Joseph S, Brahmbhatt A, Akati S, Abourashed EA (2016) Indirect modulation of the endocannabinoid system by specific fractions of nutmeg total extract. Pharm Biol 54:2933–2938. https://doi.org/10.1080/13880209.2016.1194864
47. Leiter E, Hitchcock G, Godwin S, Johnson M, Sedgwick W, Jones W, McCall S, Ceremuga T (2011) Evaluation of the anxiolytic properties of myristicin, a component of nutmeg, in the male Sprague-Dawley rat. AANA J 79(2):109. https://doi.org/10.1016/j.brainres.2008.06.042
48. Kasture SB, Gujar KN (2005) Depressant effect of trimyristin and its inhibition by some antidepressants in mice. In: Bernath J, Nemeth E, Cracker LE, Gardner ZE (eds) Proceedings WOCMAO III 2005, vol 1, Bioprospecting and ethnopharmacology. Acta Hort 675, ISHS, pp 147–152
49. Kiyofuji K, Kurauchi Y, Hisatsune A, Seki T, Mishima S, Katsuki H (2015) A natural compound maceligna protects midbrain dopaminergic neurons from inflammatory degeneration via microglial arginase-1 expression. Eur J Pharmacol 760:129–135. https://doi.org/10.1016/j.ejphar.2015.04.021
50. Ma CJ, Kim SR, Kim J, Kim YC (2005) Meso-dihydroguaiaretic acid and licarin A of *Machilus thunbergii* protect against glutamate-induced toxicity in primary cultures of a rat cortical cells. Br J Pharmacol 146(5):752–759. https://doi.org/10.1038/sj.bjp.0706380
51. Wu N, Xu W, Cao GY, Yang YF, Yang XB, Yang XW (2016) The blood-brain barrier permeability of lignans and malabaricones from the seeds of *Myristica fragrans* in the MDCK-pHaMDR cell monolayer model. Molecules 21:134–141. https://doi.org/10.3390/molecules21020134
52. Kang JW, Min BS, Lee JH (2013) Anti-platelet activity of erythro-(7S,8R)-7-acetoxy-3,4,3′,5′-tetramethoxy-8-O-4′-neolignan from *Myristica fragrans*. Phytother Res 27:1694–1699. https://doi.org/10.1002/ptr.4923
53. Ki SH, Lee JW, Lim SC, Hien TT, Im JH, Oh WK, Lee MY, Ji YH, Kim YG, Kang KW (2013) Protective effect of nectandrin B, a potent AMPK activator on neointima formation: inhibition of Pin1 expression through AMPK activation. Br J Pharmacol 168:932–945. https://doi.org/10.1111/j.1476-5381.2012.02228.x
54. Lee SU, Shim KS, Ryu SY, Min YK, Kim SH (2009) Machilin A isolated from *Myristica fragrans* stimulates osteoblast differentiation. Planta Med 75:152–157. https://doi.org/10.1055/s-0028-1112197
55. Lee KE, Mun S, Pyun HB, Kim MS, Hwang JK (2012) Effects of macelignan isolated from *Myristica fragrans* (Nutmeg) on expression of matrix metalloproteinase-1 and type I procollagen in UVB-irradiated human skin fibroblasts. Biol Pharm Bull 35:1669–1675. https://doi.org/10.1016/j.jdermsci.2009.10.005
56. Choi EJ, Kang YG, Kim J, Hwang JK (2011) Macelignan inhibits melanosome transfer mediated by protease- activated receptor-2 in keratinocytes. Biol Pharm Bull 34:748–754. https://doi.org/10.1248/bpb.34.748
57. Kwon YY, Kim D, Kim J, Hwang JK (2011) Effects of licarin E on expression of matrix metalloproteinase-1 and type-1 procollagen in UVB-irradiated human skin fibroblasts. Phytother Res 25(12):1891–1894. https://doi.org/10.1002/ptr.3521
58. Morita T, Jinno K, Kawagishi H, Arimoto Y, Suganuma H, Inakuma T, Sugiyama K (2003) Hepatoprotective effect of myristicin from nutmeg (*Myristica fragrans*) on lipopolysaccharide/d-galactosamine induced liver injury. J Agric Food Chem 51:1560–1565. https://doi.org/10.1021/jf020946n

59. Sohn JH, Han KL, Kim JH, Rukayadi Y, Hwang JK (2008) Protective effects of macelignan on cisplatin induced hepatotoxicity is associated with JNK activation. Biol Pharm Bull 31:273–277. https://doi.org/10.1248/bpb.31.273
60. Song JS, Kim EK, Choi YW, Oh WK, Kim YM (2016) Hepatocyte-protective effect of nectandrin B, a nutmeg lignan, against oxidative stress: role of Nrf2 activation through ERK phosphorylation and AMPK-dependent inhibition of GSK-3β. Toxicol Appl Pharmacol 307:138–149. https://doi.org/10.1016/j.taap.2016.08.003
61. Hang X, Yang XW (2007) GC-MS analysis of essential oil from nutmeg processed by different traditional methods. Zhongguo Zhong Yao Za Zhi 32:1669–1675. https://doi.org/10.1021/tx300239z
62. Beyer J, Ehlers D, Maurer HH (2006) Abuse of nutmeg (*Myristica fragrans* Houtt.): studies on the metabolism and the toxicologic detection of its ingredients elemicin, myristicin, and safrole in rat and human urine using gas chromatography/mass spectrometry. Ther Drug Monit 28:568–575. https://doi.org/10.1097/00007691-200608000-00013

Coriander (*Coriandrum sativum* L.): Bioactive Molecules and Health Effects

75

Muhammad Jawad Iqbal, Masood Sadiq Butt, and Hafiz Ansar Rasul Suleria

Contents

M. J. Iqbal · M. S. Butt
National Institute of Food Science and Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad, Pakistan
e-mail: mjkamboh@gmail.com; drmsbutt@yahoo.com

H. A. R. Suleria (✉)
UQ School of Medicine, Translational Research Institute, The University of Queensland, Brisbane, QLD, Australia

Department of Food, Nutrition, Dietetics and Health, Kansas State University, Manhattan, KS, USA
e-mail: hafiz.suleria@uqconnect.edu.au

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_44

Abstract

In recent times, the advancement in the knowledge regarding health perspectives of numerous micronutrients like carotenoids, anthocyanins, flavonoids, minerals, and vitamins at molecular level along with the findings of epidemiological studies has opened a new horizon in the field of nutrition. In this regard, various plant sources including herbs and spices exhibit high antioxidant activity owing to rich phytochemistry. Among herbs, coriander (locally known as "dhanya") is known for its therapeutic properties in the Indo-Pak subcontinent. It is one of the widely cultivated herbs and native to North Africa, Southern Europe, and southwestern Asia. Scientifically, coriander (*Coriandrum sativum* L.) belongs to the Umbelliferae (Apiaceae) family. The herb portion consists of leaves and stems. The herbs and seeds of coriander are being excessively used in the traditional culinary owing to its pleasant color and flavor. Coriander seeds are commonly used in spices, and its utilization is popular in the Mediterranean region. Furthermore, coriander seeds are added in the preparation of curry and traditional cuisines in south Asian region. Coriander leaves also possess unique aroma and commonly used to garnish the dish before serving. Leaves are also vastly utilized as a vital constituent in Vietnamese and Thai cuisine. Apart from appealing aroma, seeds and leaves are also known for their therapeutic potential in the Ayurvedic medicine since ages. Coriander has significant anti-inflammatory, hypoglycemic, and hypocholesterolemic potential. Alongside, it is also effective in mitigating gastrointestinal complications. Besides, essential oils extracted from coriander leaves and seeds are used in food applications, fish and meat products, pickles, beverages, and sweets owing to its pleasant aroma and high free radical scavenging activity. Coriander seeds and herbs also possess significant hepatoprotective and antioncogenic potential.

Keywords

Coriander · Flavonoids · Polyphenols · Hypoglycemia · Hepatoprotective · Hypocholesterolemic

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
BBD	Box-Behnken design
CAT	Catalase
CCl_4	Carbon tetrachloride
CE	Catechin equivalent
CS	*Coriandrum sativum*
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DW	Dry weight
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent

GI	Gastrointestinal
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
HO	Heme oxygenase
IC_{50}	Half maximal inhibitory concentration
LO	Lipid peroxidation
MRQ	Maharasnadhi Quather
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
PPM	Parts per million
Px	Peroxidase enzyme
ROS	Reactive oxygen species
RSM	Response surface methodology
SFE	Supercritical fluid extraction
SOD	Superoxide dismutase
SWE	Subcritical water extraction
TBARS	Thiobarbituric acid reactive substances
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity
TLC	Thin-layer chromatography
TPC	Total phenolic content

1 Introduction

Indispensable link between nutrition and health has diverted the focus of masses toward plant-based natural herbs and spices as a remedy to alleviate numerous lifestyle-related dysfunctions. Cognizance of consumer toward health-promoting foods has forced the scientists, researchers, and food designers to develop functional foods. Functional foods are the foods which contain numerous health-promoting and protective bioactive compounds along with the traditional nutritional value. Functional foods may be similar in appearance to the indigenous foods but have functional properties. The market of functional foods has been increasing on a rapid rate in recent years throughout the globe [1]. Undoubtedly, the medicines are incomparable for treating several physiological disparities; however, medicines have allied side effects along with imposing economic issues in developing countries like Pakistan. For the purpose, there is a dire need to establish a novel method to combat the diseases. In this scenario, coriander being an ancient herb and spice is imperative owing to its accessibility, low cost, and allied therapeutic claims [2]. Numerous scientific explorations have suggested that consumption of coriander (seeds/herb) provides copious health benefits owing to their enriched phytochemistry and health-promoting essential oil [3]. Categorically, these bioactive components have potential to mitigate lifestyle-related disorders like hyperglycemia, hypercholesterolemia, and inflammations along with oncogenic, hepatic, and renal modulating perspectives.

Considering the aforementioned evidences, the current research was designed to explore the therapeutic role of locally grown coriander variety against oxidative stress-mediated dysfunctions.

1.1 Coriander (*Coriandrum sativum* L.): At a Glance

Herbs and spices are being used from prehistoric times for their health-enhancing and disease-preventing potential owing to the presence of bioactive components. The bioactive moieties majorly include polyphenols also known as phenolic compounds and constitute a vast and complex array of phytochemicals which have significant antioxidant potential and impart beneficial physiological effects. Phytochemicals have the ability to delay lipid oxidation and also exhibit prophylactic activity. Keeping in view the bioactivity of phytochemicals and their wide occurrence in vegetables, these are considered as natural antioxidants. Bioactive moieties with considerable antioxidant activity include phenolic acids and flavonoids that are being extracted from numerous sources like sage, rosemary, oregano, pepper, thyme, and coriander [4].

The World Health Organization (2002) reported that a significant population of the world depends on traditional medicines prepared from herbs. Herbs and spices possess enriched antioxidant status and known for their therapeutic role from centuries. In recent decades, various research investigations have been conducted to explore the antioxidant and health-enhancing potential of herbs and spices. Coriander is one of the oldest herbs that have been used over 3,000 years for both culinary and medicinal purposes. Coriander is native to the Mediterranean region, while it has also been widely cultivated all over the globe as in Central Europe, Russia, Asia, and North Africa [5, 6]. It is considered as an annual herb and spice because its leaves and seeds both are used as condiment. It belongs to carrot family Umbelliferae (Apiaceae). It is also known by its scientific name as *Coriandrum sativum* L., and its seeds and leaves both are used as a condiment. The whole plant of coriander including fruits, leaves, stem, and roots possess pleasant aromatic odor and flavor. The plant coriander can acquire a height of 20–120 cm, it flowers after 45–60 days of sowing, and flowers get fully ripe in about 4 months. Leaves of coriander are mostly used as flavoring agent in preparation of various curries and soups as well as for garnishing purposes in salads owing to its attractive green color and sweet aroma in all over the globe, especially, in the region of subcontinent [7].

On the other hand, fruit/seed of coriander is made up of dry cremocarp, possessing an almost globular shape with a diameter of about 1.5–5.0 mm. The color of mature seeds is tan to yellow brown. Coriander seeds are being mostly employed in the preparation of curry powder, sausages, pickling spices, and seasonings. It has also been used in the bakery industry for the preparation of flavored pastry, buns, biscuits, cakes, etc. [2]. Coriander seeds have also been known for their therapeutic perspectives and are considered diuretic, carminative, antibilious, aphrodisiac, and refrigerant. Coriander leaves and seeds have been utilized for the preparation of Ayurvedic medicines and as an indigenous home therapy for

numerous dysfunctions. Conventionally, coriander and its products have been in use to cure gastrointestinal ailments like diarrhea, anorexia, flatulence, dyspepsia, vomiting, and pain [8]. Instant research has revealed that coriander is effective in modulating serum cholesterol level, blood sugar, and free radical production. In the recent era, research investigations have explored that coriander seeds and leaves also help in maintaining the serum glucose and cholesterol levels along with controlling the production of free radicals in the body. Furthermore, volatile oil and oleoresins extracted from coriander seeds have high demand globally and being used in formulating flavorings and also to mask the unpleasant odor of medicines. Likewise, coriander seed oleoresins have found their extensive application in seasonings for preparing beverages, sweets, and pickles [9].

Early physicians utilized coriander for its therapeutic characteristics, which include aromatic stimulant. In the Indo-Pak subcontinent, coriander leaves were known since the Vedic period, but seeds were first introduced by Muslims when they arrived in subcontinent, and it is obvious from the copious use of coriander seeds in numerous Mughlai cuisines. According to United Nations COMTRADE Database (2008), in the present world, India is the largest exporter of coriander followed by Bulgaria and Morocco. Additionally the other countries cultivating coriander are Romania, Spain, France, Italy, Pakistan, the Netherlands, Mexico, Myanmar, Turkey, Australia, Canada, and Argentina, though to a minor level, the USA and the UK are also producing coriander [2].

Furthermore, coriander comprises of variable quantities of fats, proteins, carbohydrates, minerals, fibers, and vitamins. However, due to usage in small quantities in foods, they nonsignificantly contribute to the nutritional requirements, although the essential oils of coriander seeds have significant importance in establishing the quality of spices. Proximate analysis of leaves indicated that they are comprised of moisture (87.9%), carbohydrates (6.5%), protein (3.3%), ash content (1.7%), and fat (0.6%), while dried fully ripen seeds of coriander have 6.3–8.0% moisture content, 0.3–2.06% essential oil, 13–18% fatty oil, 11.5–21.3% crude protein, 17.81–19.15% fat, 28.4–29.1% crude fiber, and 4.9–6.0% ash content [10].

Moreover, glycolipids were also detected in the seeds of coriander. The glycolipids include acylated steryl glucoside, glucocerebroside, and steryl glucoside [11]. Selenium content of about 23.53 ppm was also measured in the seeds of coriander which is higher than any other spices [12]. Previously, the presence of other minerals like aluminum, magnesium, phosphorus, silicon, potassium, sulfur, tin, calcium, copper, iron, manganese, and zinc was also reported [13].

It was noticed that the pleasant odor and taste of coriander seeds are owing to their essential/volatile oil which is colorless to slightly yellow in shade. The basic flavor of oil is spicy, aromatic, fruity, and a bit sweet. It was observed in a scientific exploration that essential oil content is more in immature fruits as compared to matured ones [14]. The essential oil is mainly made up of monoterpene hydrocarbons along with oxygenated monoterpenes which are about 20% of volatile oil [15]. The major and the most important oxygenated compound is linalool which is also known as coriandrol. Its concentration in essential oil varies from about 19.80% to 82%. Studies showed the presence of other components majorly in essential oil

including pinene, terpinene, geranyl acetate, camphor, and geraniol, present as 10.5%, 9.0%, 4.0%, 3.0%, and 1.9%, respectively [2].

Multiple scientific studies have reported numerous compounds present in coriander essential oil in different concentrations like thin-layer chromatographic (TLC) analysis ensured the presence of limonene, α-pinene, β-phellandrene, linalool, linalyl acetate, geraniol, borneol, citronellol, β-caryophyllene, thymol, citronellol, geranyl acetate, elemol, caryophyllene oxide, and methyl heptenol [16]. The volatile oil mainly contains linalool and terpenes in considerable concentrations as 50–60% and 20%, correspondingly [14]. Another group of scientists indicated 53 different components in essential oil of coriander and depicted that linalool is about 38% of oil [17].

Different factors affect the composition of coriander seeds, leaves, and stems which includes location, fertilization, and other cultural habits along with the season of sowing [18]. It has also been observed that the composition of coriander seeds and leaves varies with the maturity stage of the commodity [19]. Composition of coriander volatile oil also differs among various varieties owing to differences in geographic locations as Indian coriander differs from European coriander in linalool concentration which is less in the former. Fresh herbage of coriander has completely different odor and flavor as compared to mature seed. In the fresh herb oil, aliphatic aldehydes are predominant, while in seeds linalool content is predominant which results in more pleasant and appealing sweet odor [2]. Furthermore, it was explored that both leaf and seed oils contain linalool, cymene, pinene, phellandrene, borneol, and geraniol, but the composition of leaf oil is entirely different from that of seed oil [20].

1.2 Local and Traditional Uses

Coriander is well known for its use as a vital constituent of culinary formulations along with a traditional cure for the prevention and treatment of various dysfunctions in indigenous medicine systems of diverse societies/communities [21]. Among various herbs and spices, coriander has a distinct property that its all parts (leaves, stem, and seeds) are edible and have unique flavor and significance [22]. Nonetheless, seeds of coriander are being used as a famous spice in the Mediterranean region, while in subcontinent it has been used in curry powder in ground form. Moreover, the green leaves of coriander also exhibit a pleasant aroma and flavor. The fresh leaves are being predominantly used in Vietnamese and Thai cuisines [23].

Likewise, roots of coriander plant have a much deeper and strong flavor than its leafy part. Root portion of the plant has been generally used in a range of Asian dishes and cuisines. Nevertheless, the stem portion of coriander is mostly utilized in the preparation of stews and soups in chopped form [24]. Along with splendid aroma and taste, coriander is well known as medicinal herb owing to its therapeutic perspectives. For the purpose, coriander has been widely employed in the preparation of numerous indigenous medicinal formulation to alleviate digestive ailments. Locally, in the northern region of Pakistan, the whole plant of coriander has been

utilized in folk medicinal interventions for the treatment of ailments like dysentery, flatulence, cough, diarrhea, jaundice, vomiting, and stomach complications [25]. In Indian indigenous remedies, coriander is employed to cure respiratory, digestive, and urinary systems with diuretic, diaphoretic, stimulant, and carminative activities. In the region of Turkey, brewed seeds of coriander have been used as carminative and digestive agent as well as appetite enhancer [26].

Numerous meta-analyses have authenticated the beneficial therapeutic perspectives of coriander. It has been depicted that the digestive stimulant role of coriander is owing to the stimulation of hepatic cells to secret bile acids enriched bile in more quantity. Likewise, it also enhances the activities and functionality of both intestinal and pancreatic aided digestive enzymes. These stimulations and activated actions elevated the rate of overall digestion and ultimately reduced the passage time of food via gastrointestinal (GI) tract [27].

Coriander has also been traditionally used for the treatment of enhanced glucose level in few countries like Jordan, Saudi Arabia, and Morocco [28–30]. Though the exact mechanism of limiting hyperglycemia has not been well defined, coriander has been used in Persian folk medicine to cure hyperglycemia. In this regard, a scientific investigation was conducted to evaluate the hypoglycemic potential of coriander in 20 diabetic rats by dividing them into four groups based on diet given to them. Three groups of them were fed on diet enriched with 15 g (60 g per kg body weight per day) of leaves for a period of 15 days, while the fourth diabetic and nondiabetic untreated group received a standard diet and served as positive and negative control, respectively. It was noticed that coriander leaves lower the serum glucose level nonsignificantly [31]. Ayurvedic literature has shown that continuous use of coriander seeds effectively lowers the serum lipid profile. Furthermore, the utilization of coriander as traditional treatment of urinary infections and as diuretic medicine has been traced back to pharmacopeia of Morocco and Palestine [32, 33]. Latterly, it was demonstrated that the administration of aqueous extract of coriander seeds via intravenous infusion for the period of 2 h at two selected doses of 40 and 10 mg/kg body weight, to anesthetized rats, can enhance the diuresis, rate of glomerular filtration, and excretion of electrolytes in a dose-dependent way [34].

Moreover, in traditional and Ayurvedic curative approaches, arthritis and inflammations have long been treated with coriander. For the treatment of arthritic condition, a traditional medicine has generally been recommended by the Ayurvedic practitioners, named as Maharasnadhi Quather (MRQ). MRQ is a polyherbal formulation having coriander seeds, one of the principle constituent. Study conducted to estimate the analgesic and anti-inflammatory perspectives of MRQ showed that it significantly prevents the rat paw edema induced by carrageenan and formulation also improves the pain tolerance by 57% after an hour of treatment. It was suggested that the MRQ has analgesic activity carried out by a supraspinal effect. It was also observed that treatment with MRQ for the period of 3 months significantly lowers the pain and inflammation along with improving the mobility of joints in the patients. The possible mechanisms of MRQ antiarthritic effects may include changes in the synthesis of leukotrienes and prostaglandins, antioxidant activity, and membrane stabilization [35].

Coriander has been used for a long time in traditional Iranian medicine for averting anxiety, convulsions, and insomnia. These primeval characteristics of aqueous coriander extracts have been validated by a number of pharmacological studies. It was observed that extracts possess sedative hypnotic and anticonvulsant activities [36, 37]. Traditionally, coriander has been used as an antimicrobial, antiseptic, and wound-healing agent in mouth, although coriander aqueous decoction has shown no antibacterial potential against almost 176 isolates of bacteria originated from 12 distinct genera of bacterial colonies obtained from the oral cavity of 200 individuals by means of disc diffusion technique [38].

Coriander has also been conventionally used as a curative measure against airborne ailments like bronchitis and cough; nonetheless scientific evidences supporting this specific character of coriander are not available. This showed that novel plant-based medicines can be made in the future depending on the knowledge obtained from the ethnobotanical studies [26].

2 Phytochemistry of Coriander

2.1 Coriander Seeds

The medicinal properties and nutritional significances of coriander seeds are owing to the presence of numerous bioactive moieties like fatty acids, tocols, sterol, and volatile components.

2.1.1 Polyphenols

The methanolic decoction of coriander seeds was evaluated for total phenolic content, articulated as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) of sample. Previously, it was reported that the coriander extract of Syrian variety had the maximum total phenolic content followed by Tunisian and Egyptian as 1.09, 1.00, and 0.94 (mg GAE/g, dry weight), respectively [39]. According to another study, the total phenolic content in the Canadian variety was found to be very much different which was about 15.16 mg GAE/g DW, while it was 12.10 mg GAE/g DW for the Tunisian variety [40, 41]. However, ethyl acetate extracts obtained from coriander seeds of Norwegian variety exhibited total phenolic contents to about 1.89 g GAE/100 g of extract [42]. Actually, the variations in the total phenolic content of coriander seeds could be the outcome of using diverse extraction solvents as highlighted by Wangensteen et al. [42]; they reported significant variations in the level of total phenolic contents of the same extract by varying the polarity of the solvent.

In literature, the total flavonoids and total tannin contents were estimated as catechin equivalents in mg per gram of dry weight (mg CE/g DW). Previously, total flavonoids were estimated in the methanolic extracts of coriander seeds, and they ranged from 2.03 to 2.51 mg CE/g DW. The total tannins were about 0.09–0.17 mg CE/g DW. It was noticed that the total flavonoids and total tannins were maximum in methanolic extract of Syrian variety [39].

Furthermore, the phenolic composition of coriander is still not completely understood and some data is conflicting. Overall, about 21 components were classified in various coriander varieties; these include about 11 phenolic acids named as chlorogenic, gallic, vanillic, caffeic, p-coumaric, rosmarinic, ferulic, o-coumaric, salicylic, trans-hydroxycinnamic, and trans-cinnamic acids, while almost 10 flavonoids were identified termed as quercetin-3-rhamnoside, luteolin, rutin trihydrate, resorcinol, quercetin dihydrate, kaempferol, apigenin, naringin, coumarin, and flavone. Furthermore, the researchers narrated that total phenolic acids were predominant in Tunisian variety (81.47%), while total flavonoids were maximum in Syrian variety (61.34%). Moreover, phenolic acids and flavonoids were about 49.17% and 50.83% in methanolic extracts of Egyptian variety of coriander [39]. In another scientific exploration, it was mentioned that coriander seeds only possess phenolic acids and its derivatives but lack flavonoids. Moreover, it was elucidated that among the derivatives, caffeoyl *N*-tryptophan hexoside was the most abundant phenolic derivative present at a concentration level of about 45.33 mg/kg, while caffeoyl *N*-tryptophan was among the least abundant derivatives as depicted from the concentration, i.e., 0.71 mg/kg, in the methanolic extracts of coriander seeds [43]. Additionally, the whole composition of polyphenol of seeds includes feruloyl *N*-tryptophan hexoside, p-coumaric acid, ferulic acid, feruloyl *N*-tryptophan, caffeic acid, di-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, and ferulic acid derivatives [43]. In addition, coriander possesses an appreciable amount of β-carotene and carotenoid, 160 and 1,010 μg/100 g, correspondingly [44].

2.1.2 Essential Oil

Extraction Yield

Extraction yield of essential oil is defined as the actual amount of essential oil obtained from the coriander seeds after the implementation of various extraction methods. Generally, extraction yield is expressed in terms of percentage. Commonly the extraction of volatile oil from coriander seeds is carried out by means of steam and hydrodistillation. Literature has suggested that the quantity and yield of coriander essential oil vary with several factors like climatic conditions, origin of cultivar, and geographic location of growing region, so it is not uniform across the countries. In this regard, it was reported that the yield of essential oil was about 0.18–0.39% from Indian coriander variety [45], while the yield obtained from Tunisian coriander was about 0.35% [46]. Likewise, various accessions suggested that the essential oil content of Iranian coriander seeds was about 0.1–0.36% [47]. A scientific study conducted in Bangladesh narrated that the essential oil in the coriander seeds was about 0.42% on the fresh weight basis [48]. Moreover, another study reported that the essential oil concentration of different cultivars of coriander sowed and grown in Canada under cool wet conditions ranged from 0.8% to 2.2%. Furthermore, two coriander varieties from Turkey named as vulgare and microcarpum were compared, and it was established that the content of essential oil was about 0.15–0.25% in vulgare, while in microcarpum it was about 0.31–0.43% [14].

Composition of Essential Oil

Chemical composition of different varieties of coriander seed essential oil has been comprehensively studied around the globe. All the scientific conclusions agreed that the main bioactive compounds of coriander were alcoholic monoterpene, and among monoterpene, the most predominant moiety is the (S)-(+)-linalool. According to an estimation, about 35 distinct components have been identified in the essential oil of Bangladeshi, Tunisian [19], Indian [5], and Pakistani [49] coriander varieties, whereas only 17 compounds have been detected in the coriander essential oil of Algerian origin [50].

Normally, chemical composition of essential oil procured from different regions was proved to be an excellent source of oxygenated monoterpenes, predominantly (S)-(+)- linalool. Certainly, the essential/volatile oil obtained from the Tunisian variety was majorly made up of linalool which was found to be about 87.54%, while cis-dihydrocarvone was only 2.36% [19]. Moreover, the linalool content in the essential oil of Algerian variety was about 73.1%, and the other components found were p-mentha-1,4-dien-7-ol, neryl acetate, and α-pinene present in the concentration of about 6.51%, 3.22%, and 3.41%, respectively [50]. Furthermore, bioactive molecules found in the volatile oil of Iranian variety of coriander seeds were linalool, neryl acetate, γ-terpinene, and α-pinene, present in the concentration of 40.9–79.9%, 2.3–14.2%, 0.1–13.6%, and 1.2–7.1%, respectively [47], whereas, in essential oil of Bangladeshi origin, linalool was about 37.65% followed by geranyl acetate and γ-terpinene as 17.57% and 14.42%, correspondingly [48].

The content of linalool in the essential oil of the coriander seeds of Pakistani origin was about 69.60%; other bioactive components were geranyl acetate α-pinene, γ-terpinene, anethole, and p-cymene found in considerable quantities as 4.99%, 1.63%, 4.17%, 1.15%, and 1.12%, respectively [49]. Basically, the chemical composition of essential oil from Pakistani and Indian varieties was somewhat comparable as in Indian seeds; linalool was 75.30%, geranyl acetate 8.12%, and α-pinene 4.09% [5]. The proportions of different components were not identical. It was reported that the linalool was 83.21%, geranyl acetate 5.74%, and α-pinene about 4.47%. Additionally, the coriander seeds from Atlantic Canada exhibited normal to high percentage of linalool as 64–85%, while the other components identified in the essential oil were camphor, α-pinene, phellandrene, linalyl acetate, limonene, para-cymene, and geranyl acetate [18]. Besides, a study conducted in Brazil elaborated that the major moieties in coriander essential oil were linalool (77.48%), γ-terpinene (4.64%), and α-pinene (3.97%) [51]. Furthermore, linalool content was 63.5–71.0% and 42.1–52.7% in the essential oils of two Turkish varieties, i.e., microcarpum and vulgare, respectively. Some other vital components isolated were geraniol, geranyl acetate, (Z)-isoapiole dillapiole, γ-terpinene, and p-cymene [14].

Furthermore, essential oil of immature Tunisian seeds exhibited a bit different trend as it possesses more amount of geranyl acetate as compared to linalool, i.e., 46.27% and 10.96%, respectively, although with maturation the linalool content elevated to about 87.54%, suggesting that the variations may be associated with alterations in secondary metabolism [52]. Numerous other investigations aiming to

explore the chemical composition of essential oil of coriander seeds emphasized on several factors like developmental stage [53], sowing time [18], maturity stage [52] and interaction among growing areas along with abiotic stress conditions such as salinity [53, 54] and drought [55, 56]. Furthermore, other studies focus on the effect of various extraction technologies like hydrodistillation [57], microwave-assisted hydrodistillation [58], and supercritical fluid extraction technique [59] using carbon dioxide as solvent, on the compositional profile of essential oil of coriander.

The essential oil of coriander seeds is highly perishable and sensitive to harsh storage conditions. The changes that occur in the essential oil of coriander mostly include the transformations/oxidation of terpenes in the presence of sunlight. These alterations in the chemical profile of essential oil may result in the modifications of organoleptic characteristics of the oil, and it may also result in the accumulation of components which negatively affect the oil flavor and could be harmful to human health [24]. Subsequently, polymerization and oxidative processes could result in the loss of pharmacological characteristics along with quality of essential oil [60].

2.1.3 Water-Soluble Constituents

The water-soluble moieties of coriander seeds were not well reported unlike essential oil, although these components have been studied by a few groups of researchers, and it was reported that about 33 different compounds exist in the water-soluble part of methanolic extract of coriander seeds, which include four novel monoterpenoid glycosides, two monoterpenoid glucoside sulfates, and two novel aromatic compound glycosides. Further spectral explorations clarified their structure. Besides, two glycosides were sequestered from coriander seeds [61].

2.1.4 Lipids

Fatty Acids

Several recent scientific studies have elaborated the compositional analyses of fatty acid profile of coriander seeds. The oil yield of German and Tunisian varieties of coriander seeds ranged from 19.24% to 28.4% of dry weight [11, 52, 62–64]. The predominant fatty acids highlighted in most lipid classes were petroselinic acid trailed by linoleic acids present in the concentration of about 65.70–80.9% and 13.05–16.70%, respectively. Other noticeable fatty acids determined were oleic, palmitic, and stearic acids. Furthermore, palmitoleic (0.4–1.1%), α-linolenic (0.15–0.50%), and arachidic (0.10–0.25%) were also identified in lesser quantities. Likewise, the same fatty acid profile was observed in the Tunisian coriander fruits, although gadoleic, docosahexaenoic, and erucic acids were also observed at a lesser level of about 0.1% [52].

It was observed in a study that the neutral lipids in the seed oil were about 94.88% of the total lipids and majorly composed of triglycerides (95.50%), diacylglycerols (1.88%), free fatty acids (2.05%), and diacylglycerols (0.57%). Additionally, polar lipids were predominant counterpart of major phospholipid subclass, i.e., phosphatidylcholine (35.98%), trailed by 33.83% phosphatidylethanolamine and 15.40% phosphatidylinositol. However, phosphatidic acid (8.11%) and phosphatidylglycerol

(6.68%) were detected in lesser levels, while the most dominant galactolipid observed was digalactosyldiacylglycerol trailed by monogalactosyldiacylglycerol as 62.32% and 37.68%, respectively [64].

Sterols

Seed oil of coriander has been recognized as an essential source of sterols, exhibiting cholesterol-lowering effect by inhibiting the absorption of cholesterol. According to previous literature, the total content of sterol is in a range of 36.93–51.86 mg per gram of oil. The most common sterols in coriander are β-sitosterol and stigmasterol which are about 24.8–36.8% and 36.93–51.86 mg per gram oil, respectively. Other characteristic sterols are Δ5- and Δ7-stigmasterol, campesterol, and 24-stigmastadienol, although Δ5-avenasterol and Δ7-avenasterol exist in lower quantities. A small amount of cholesterol was identified in Tunisian variety of coriander seeds, i.e., 1.02–2.18% [62]. On the other hand, this moiety is not identified in seeds obtained from Germany; nonetheless two other sterols were detected named as lanosterol and ergosterol [11].

Tocols

Coriander seeds have also been proved to be an enriched source of tocols which are about 327.47 μg/g. The chief tocopherol is γ-tocopherol trailed by δ-tocopherol and α-tocopherol as depicted by their concentration, i.e., 26.40, 3.50 and 11.70 μg/g. Furthermore, coriander seed possesses higher quantities of total tocotrienols; among tocotrienols, γ-tocotrienol is the predominant component (238.40 μg/g), trailed by α-tocotrienol (24.90 μg/g) and δ-tocotrienol (12.57 μg/g) [63].

2.2 Coriander Herb

Herbal portion of coriander plant is not well studied as compared to seeds or fruit portion. Nonetheless, phenolic acids, polyphenols, flavonoids, and essential oil are among the major components detected in the leaf portion [65, 66].

2.2.1 Polyphenols

In a scientific study, the methanolic extracts of coriander green part have been investigated for total flavonoid contents along with phenolic contents; outcomes showed that they were 5,259.52 and 1,013.95 mg/kg, respectively. The polyphenol profile of coriander green portion consists of quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucuronide, dimethoxycinnamoyl hexoside, quercetin-3-*O*-glucoside, and kaempferol-3-*O*-rutinoside present at about 3,296.16, 1,237.13, 406.39, 405.36, and 320.86 mg/kg, respectively. Among flavonoids, *p*-coumaroylquinic acid, 3-*O*-caffeoylquinic acid, ferulic acid glucoside, and caffeoylquinic acid were detected in the quantity of 303.83, 173.51, 122.29, and 7.92 mg/kg, correspondingly. It was also elucidated that the quercetin derivatives were the main bioactive commodities identified in the green part of coriander obtained from the Portugal [43].

Previously, about 21 phenolic components were recognized in the green part of coriander, and these were mainly the flavonoids, phenolcarboxylic acid, and coumarins. Some of the components were identified in the green part of coriander for the first time like luteolin, apigenin, hyperoside, vicenin, hesperidin, orientine, diosmin, dihydroquercetin, catechin, chrysoeriol, gallic acid, ferulic acid, dicoumarin, salicylic acid, esculin, 4-hydroxycoumarin, maleic acid, esculetin, arbutin, and tartaric acid [67].

Furthermore, it has also been described that the chief phenolic acids in coriander leaves of Indian variety were trans-ferulic, cis-ferulic, p-coumaric, and vanillic acids, while the flavonoids include acacetin, quercetin, 4′-OMe quercetin, 3′-OMe quercetin, and kaempferol. Furthermore, in green leaves of coriander, glycoflavones were not identified [66]. Nevertheless, aqueous extraction of Brazilian coriander leaves possesses phenolic acids like protocatechuic acid (6.43 μg/mL), caffeic acid (4.34 μg/mL), and glycitin (3.27 μg/mL) [4].

It has also been determined that the leaves of coriander possess anthocyanins. The biosynthesis of anthocyanins is increased by microelements and salicylic acid, particularly zinc application. On the contrary, anthocyanin content has a negative relation with nitrogen, phosphorous, and potassium [68]. Moreover, Barros et al. [43] performed a detailed analyses of phenolic components of coriander samples grown in vitro. It was reported that the samples possess more diverse polyphenolic profile and C-glycosylated apigenin as main component present in the concentration of 2,983 mg/kg. It was concluded that in vitro culture technique can be employed to explore new horizons of research in the field of industry, medicine, and pharmaceuticals along with synthesizing secondary metabolites like anthocyanins, flavonols, and flavones [43].

2.2.2 Essential Oil

Extraction Yield

It has been reported that coriander leaves possess lower amount of essential oil as compared to seeds [48]. It was observed that the air-dried leaves of Tunisian variety coriander contain 0.12% of essential oil, although the essential oil in root portion at the vegetative stage was about 0.06% [53].

Composition of Essential Oil

The studies revealed that the composition of essential oil of coriander leaves varies significantly in contrast to essential oil profile of seeds. Though the quantities differ, all the research investigations agreed that the major components of essential oil extracted from coriander leaf are aldehydes and alcohols. Essential oil from the leaves of Bangladeshi coriander variety exhibited the existence of about 44 moieties, which are mostly aromatic acids like 2-decenoic acid, E-11-tetradecenoic acid, and capric acid present about 30.8%, 13.4%, and 12.7%, respectively. Likewise, Kenyan coriander leaf essential oil encompasses chiefly aldehydes followed by alcohols, i.e., 56.1% and 46.3%, correspondingly. The main components were decanal (14.3%), (E)-2-decenal (15.9%), n-decanol (13.6%), and (E)-2-decen-1-ol (14.2%) [48].

In addition, compounds identified in trace quantities were (E)-2-dodecenal, (E)-2-tridecen-1-al, undecanol, dodecanal, undecanal, and alkanes [65]. Furthermore, the essential oil extracted from the coriander leaves from Fiji showed the presence of (E)-2-decen-1-ol in the quantity of about 26.0% as the predominant component. Besides, the chief moieties found in the Brazilian coriander essential oil were alcohols including 1-decanol (24.20%), (Z)-2-dodecenol (17.60%), and (E)-2-decenol (18.00%) along with aldehydes (89%) [69].

Moreover, Indian variety possesses (E)-2-decenal, decanal, dec-9-en-1-ol, (E)-2-dodecenal, n-tetradecanol, dodecanal, and decanol as the major essential components present about 18.02%, 14.36%, 11.66%, 8.72%, 6.09%, 5.81%, and 5.77% [70]. Furthermore, the leaf essential oil of Korean variety exhibited the existence of about 39 different compounds making about 99.62% of the total oil. The predominant compounds were cyclododecanol, tetradecanal, 2-dodecenal, 1-decanol, 13-tetradecenal, 1-dodecanol, dodecanal, 1-undecanol, and decanal with quantities about 23.11%, 17.86%, 9.93%, 7.24%, 6.85%, 6.54%, 5.16%, 2.28%, and 2.33%, respectively [71].

2.2.3 Lipids

The fatty acid profile of coriander green part was elaborated for the first time by Neffati and Marzouk [53]. In the research investigation, the impact of salinity was evaluated on the plasma membrane of fatty acid of coriander leaves of Tunisian variety grown in hydroponic culture. It was observed that the maximum content of fatty acids was found in the leaves present near to the base of coriander (61.21 mg/g DW), while in the leaves from the upper part, it was a bit low, i.e., 41.8 mg/g DW.

Furthermore, it has been found that the predominant fatty acids present in the coriander leaves were polyunsaturated fatty acids. Moreover, it was also reported that α-linolenic was the most abundant fatty acid in both basal and upper leaves in a percentage of about 41.1% and 39.4%, respectively, and in terms of mg/g dry weight, it was 17.1 and 24.1 mg/g DW, correspondingly. Next to α-linolenic acid, the predominant acids were linoleic, palmitic, and heptadecenoic acids. Nonetheless, stearic, oleic, stearidonic, trans-, and cis-palmitoleic acids were also found in negligible quantities in both basal and upper leaves, together making about 9.6% and 4.7% of total fatty acid content, respectively [53].

Besides, it was reported that the salinity lowered the content total fatty acids noticeably in basal and upper leaves. Elevating the level of NaCl leads to reduction in the ratio of unsaturated to saturated fatty acid, causing the development of rigid membrane. Moreover, five fractions were isolated from the ether extract of coriander leaves named as β-cryptoxanthin epoxide, β-carotene, violaxanthin, lutein-5,6-epoxide, and neoxanthin [72]. It was also elucidated that coriander leaves have proved to be a good source of β-carotene which is a precursor of vitamin A. Among carotenoids, β-carotene was about 61.14% in ether extract. It was observed that at the pre-flowering stage, β-carotene and total carotenoids content reached to about 73.64 and 217.50 mg/100 g, respectively, in coriander leaves [73].

3 Extraction of Bioactive Molecules

Coriander is being conventionally used as a cooking ingredient all around the globe in both seeds and leaves form. Moreover, it has also been used for its medicinal properties to treat various ailments from ancient times. Chemical analyses of seeds and leaves of coriander have revealed that the chief antioxidant constituent of seeds is linalool which is an oxygenated monoterpene, while quercetin derivatives are the predominant moieties of coriander leaves. These bioactive moieties possess high antioxidant activity and allied therapeutic perspectives [3]. Several methods have been employed for the extraction and isolation of active components from coriander leaves and seeds, i.e., hydrodistillation and Soxhlet extraction method using conventional organic solvents like ethanol, methanol, hexane, etc. along with some advance techniques like microwave-assisted extraction, ultrasonic extraction techniques, and supercritical fluid extraction techniques using carbon dioxide as supercritical fluid (Table 1).

3.1 Traditional/Conventional Extraction Techniques

Conventional extraction techniques include solvent extraction techniques, Soxhlet extraction, hydrodistillation, etc. Traditional methods of extraction with organic solvents and hydrodistillation are being utilized for the extraction of essential oils and healthy lipid fractions from various medicinal plants. Generally, for the efficient recovery of nonpolar moieties, organic solvents like methylene chloride or n-hexane are usually employed, but these solvents impart certain drawbacks like toxicity [73].

A scientific exploration was carried out to compare the extraction efficiency of several extraction methods using coriander as the raw material. In hydrodistillation technique the coriander seeds were grounded and extracted using 150 mL of steam for a period of 3 h, although in Soxhlet extraction technique, coriander sample was extracted by 200 mL hexane for a period of 12 h. In the study, about 18 major components were identified in the extracts by using gas chromatographic technique; among the components, linalool was the predominant one. It was about 77.97% in hydrodistillation and 73.62% was measured in Soxhlet extraction. It was also noticed that the yield of essential oil, in terms of cumulative area ratio of coriander seeds, was maximum in hydrodistillation (21.7) followed by Soxhlet extraction (19.4) [76].

Furthermore, in an investigation Kaiser et al. [76] used solvent extraction technique by extracting the coriander pasty product with aqueous methanol (methanol/water – 70:30 v/v) for a period of 1 h. The extract was filtered and prepared for further sophisticated analyses. It was observed that the maximum total phenolic content in coriander leaves blanched at 100 °C with water for the period of 1 min, i.e., 126.0 ± 9.1 mmol GAE/kg DM, compared to the unheated control sample (49.2 ± 3.4 mmol GAE/kg DM). Likewise, the maximum ferric reducing antioxidant power in terms of mmol TE/kg DM was observed at same conditions, i.e., 109.6 ± 14.1 mmol TE/kg DM. On the other hand, TEAC assay was highest for the

Table 1 Extraction rates of coriander bioactives by various extraction methods

Sr. No	Coriander component	Technique	Solvent	Conditions (time/temp/pressure)	Bioactive components/antioxidant assays	Concentration	References
1	Seeds	Hydrodistillation	Steam	3 h	Linalool	77.97%	[76]
2	Seeds	Soxhlet extraction	Hexane	12 h	Linalool	73.62%	[76]
3	Leaves	Solvent extraction	Aqueous methanol	1 h	TPC	49.2 ± 3.4–126.0 ± 9.1 mmol GAE/kg DM	[76]
4	Leaves	Solvent extraction	Aqueous methanol	1 h	FRAP	109.6 ± 14.1 mmol TE/kg DM	[76]
5	Leaves	Solvent extraction	Aqueous methanol	1 h	TEAC	19.0 ± 0.8 TE/kg DM	[76]
6	Fruits	Solvent extraction	Aqueous methanol	1 h	TPC and FRAP	55.4 ± 0.9 mmol GAE/kg DM and 51.2 ± 2.6 mmol TE/kg DM	[76]
7	Seeds	Soxhlet extraction	Lipid extract	–	Linalool	785.05 mg/100 g	[74]
8	Seeds	Soxhlet extraction	Ethyl acetate	–	TPC	1.9 g GAE/100 g extract	[42]
9	Leaves	Soxhlet extraction	Ethyl acetate	–	TPC	5.5 g GAE/100 g extract	[42]
10	Seeds	Soxhlet extraction		–	Essential oil/lipid extracts	14.45 ± 0.32%	[74]
11	Seeds	Supercritical fluid extraction	CO_2	300 bar/40 °C	Essential oil/lipid extracts	8.88 ± 0.18%	[74]
12	Seeds	Subcritical water extraction	Water	30 bar/200 °C	Essential oil/lipid extracts	2.22 ± 0.26%	[74]

13	Seeds	Soxhlet extraction	–	–	Linalool and camphor	785 and 27 mg/100 g CS	[74]
14	Seeds	SFE	CO_2	300 bar/40 °C	Linalool and camphor	598.51 and 26.64 mg/100 g CS	[74]
15	Seeds	SFE	CO_2	300 bar/40 °C	γ-Terpinene and (+)-limonene	31.08 and 23.98 mg/100 g CS	[74]
16	Seeds	Hydrodistillation	Steam	–	Essential oil	21.7%	[75]
17	Seeds	Soxhlet extraction			Essential oil	19.4%	[75]
18	Seeds	Subcritical water extraction	Water		Essential oil	14.1%	[75]
19	Essential oil	Subcritical water extraction	Water		Linalool	82.91%	[75]
20	Essential oil	Soxhlet extraction			Linalool	79.62%	[75]
21	Essential oil	Hydrodistillation	Steam		Linalool	77.98%	[75]
22	Seeds	Hydrodistillation	Steam		Linalool	72.3% of essential oil	[59]
23	Seeds	SFE	CO_2	90 bars/50 °C	Linalool	78.8% of essential oil	[59]
24	Seeds	SFE	CO_2		Linalool	877.07 mg/100 g CS	[85]
25	Seeds	Soxhlet extraction			Linalool	68.66 mg/100 g CS	[85]
26	Seeds	Hydrodistillation	Steam		Linalool	543.33 mg/100 g CS	[85]
27	Seeds	SFE	CO_2	100 bars/55 °C	Linalool	717 mg/g	[86]
28	Seeds	SFE	CO_2	60 bars/100 °C	Polyphenols	942 mg/100 g DW	[87]
29	Seeds	SFE	CO_2	30 bars/100 °C	Linalool	59.90 mg/100 g DW	[87]

leaves blanched at 100 °C water for a period of 10 min as 19.0 ± 0.8 mmol TE/kg DM. In case of coriander fruits, maximum total phenolic contents and FRAP assay were measured at the conditions of 100 °C water temperature blanched for a period of 1 min as 55.4 ± 0.9 mmol GAE/kg DM and 51.2 ± 2.6 mmol TE/kg DM, respectively. Overall, it was observed that the steam and water blanching of coriander leaves and seeds enhanced the content of phenolics and antioxidant activity as compared to unheated control samples, although too long heat treatment can decrease the antioxidant potential of leaves as well as seeds.

Furthermore, methanolic extracts of three coriander fruits varieties were compared named as Syrian, Tunisian, and Egyptian for their antioxidant potential. Extraction was carried out using pure methanol for a time period of 30 min. It was observed that Syrian variety exhibited the maximum amount of total polyphenols as well as total flavonoid, but the Tunisian variety showed the highest DPPH scavenging activity. It was concluded that the antioxidant activity is independent of polyphenolic content, while it depends upon the composition of polyphenols [39].

Recently, a research was conducted to compare the extraction efficiency of different traditional and green technologies. The yield of lipid extract by using Soxhlet extraction technique was about 14.45%. Likewise, linalool concentration was also maximum in the extracts taken by Soxhlet technique i.e., 785.05 mg/100 g coriander seeds, though the results from green technologies were also comparable [73]. Furthermore, coriander seeds and leaves were extracted using different solvents like ethanol, butanol, ethyl acetate, and diethyl ether. It was observed that ethyl acetate extracts possessed maximum amount of total phenolic contents in both seeds and leaves, i.e., 1.9 and 5.5 g GAE/100 g extract, respectively [42].

3.2 Modern/Green Extraction Techniques

Generally, Soxhlet extraction techniques and hydrodistillation have widely been used for the recovery of bioactive components from various spices and herbs like coriander seeds and leaves, though it has been suggested by many scientific explorations that the conventional techniques impart several negative impacts on the human life and environment. Furthermore, demerits of conventional techniques include consumption of huge amount of energy which can damage the heat-labile components [77]; time-consuming, uneconomical, and organic solvent needs to be disposed of properly. Nevertheless, the drawbacks of traditional techniques can be overcome by implementation of novel green technologies, like subcritical water extraction (SWE) as well as supercritical fluid extraction technique (SFE).

The subcritical state of water is the state at which the temperature of water is above boiling point but lower than the critical temperature (374.15 °C) and pressure enough to maintain the liquid state of water. At normal conditions water is an excellent polar solvent, so it can be used to extract water-soluble components like polyphenols and flavonoids, but it cannot be used to isolate nonpolar components like essential oils or lipid fraction. Increasing the temperature and pressure of water

can alter its characteristics, and it can be made suitable for the extraction of nonpolar components. Increasing temperature and pressure lowers the dielectric constant of water which is about 80 at normal conditions but lowers to 27 at 250 °C temperature and 50 bar pressure, while it further drops to about 14.86 at 350 °C temperature and 250 bar pressure [78]. In subcritical water extraction (SWE), water exhibits lesser viscosity and more diffusivity, which enhances the diffusion into the sample matrix ultimately releasing more bioactive moieties [79].

Earlier, in a study subcritical water was applied for the extraction of essential oil from the coriander seeds. The parameters for subcritical extraction were water temperature 65–150 °C, pressure 46.5–68.9 bar, and extraction time 15 min. The extraction yield obtained was much higher as compared to hydrodistillation (0.06–0.1%) [80]. Later, it was narrated that the use of supercritical fluid extraction technology greatly solved the problems associated with conventional techniques, i.e., hydrolysis of compounds, thermal degradation, and use of toxic organic solvents [81]. Basically, in supercritical fluid extraction technique, carbon dioxide was used as solvent at critical conditions (temperature = 31.1 °C, pressure = 73.8 bar) to extract nonpolar commodities. It was also reported that supercritical fluid extraction technique provides more dissolvability of moieties associated with essential oil along with better rates of mass transfer. Temperature and pressure of SFE system control both the composition and yield of coriander seed extract, so mild amendments in these two parameters change the density of the supercritical solvent [59]. The advantages of supercritical fluid extracts over conventional techniques include ease to remove solvent from extract, mild extracting temperatures (avoid heat damaging), and nontoxic and environment-friendly technique [82].

Recently, Pavlic et al. [73] used different traditional as well as modern extraction techniques to compare the qualitative and quantitative characteristics of coriander seed essential oil and lipid fraction. The yield of essential oil and lipid extracts was maximum with Soxhlet extraction (14.45 ± 0.32%) followed by supercritical fluid extraction at 300 bar pressure and 40 °C temperature (8.88 ± 0.18%) trailed by subcritical water extraction at 30 bar pressure and 200 °C temperature (2.22 ± 0.26%). Regarding the yield of five important components from essential oil of coriander (linalool, γ-terpinene, camphor, (+)-limonene, and geraniol), maximum yield of linalool, camphor, and geraniol was observed in Soxhlet extraction, i.e., 785, 27, and 22 mg per 100 g of seeds, respectively. Moreover, γ-terpinene and (+)-limonene yield was maximum in supercritical fluid extracts at 40 °C and 300 bar pressure, i.e., 31.08 and 23.98 mg/100 g CS, correspondingly. In the same conditions, SFE showed a particularly high yield of linalool, camphor, and geraniol, i.e., 598.51, 26.64, and 19.54 mg per 100 g of coriander seeds, respectively. Besides lipid fraction and essential oil, polyphenolic components were also extracted by means of subcritical water extraction.

In another study, a comparison was carried out among subcritical water extraction with Soxhlet and hydrodistillation techniques. It was observed that the maximum essential oil yield from coriander seeds was obtained from hydrodistillation (21.7%) followed by Soxhlet extraction (19.4%) and SWE (14.1%). Contrary to lesser total extraction yield from SWE as compared to hydrodistillation and Soxhlet extraction,

the final extract obtained from SWE was of better quality and more valuable owing to predominant presence of oxygenated compounds and very low hydrocarbons. In the essential oil of coriander, the linalool concentration extracted from SWE was between 82.91% followed by Soxhlet extraction (79.62%) and hydrodistillation (77.98%) [75].

Afterward, Grosso et al. [59] extracted essential oil from coriander seeds of Italian variety by employing supercritical fluid extraction technique to evaluate the effect of different extracting parameters on the yield and composition of essential oil. In the exploration, the best conditions are found to be 50 °C temperature, 90 bar pressure, 1.10 kg/h flow rate of carbon dioxide, and particle size of 0.6 mm. The highest amount of linalool recovered by hydrodistillation was 72.3% of essential oil, while linalool extracted by SFE was 78.8% of essential oil at best conditions mentioned above. Other vital components were c-terpinene, geranyl acetate, camphor, geraniol, a-pinene, and limonene, present in a range of 4–7%, 2–4%, 3%, 1–3%, 1–3%, and 1–2%, respectively.

Earlier, extraction of coriander seeds was carried out using supercritical and subcritical conditions (35 °C, 100–350 bar, and 25 °C, 100 bar, respectively) employing carbon dioxide as solvent. In supercritical conditions the complete extraction was achieved at 200–300 bar pressure in 2.0 and 1.5 h, respectively, while under subcritical conditions it took more time, i.e., ~6 h. It was observed that with the increase in pressure, the solubility of nonvolatile oil increases and the highest quantity of 15.3 g was extracted from 100 g coriander seeds at 200 bar and 16.4 g at 300 bar keeping the temperature constant at 35 °C. On the other hand, the concentration of essential oil decreased with an increase in the amount of recovered oil. Essential oil was about 70–80% in the oil obtained after 10–15% of yield, while it lowered to about 3–8% when 30% extraction was completed [83].

In a study, supercritical fluid extraction was employed to obtain volatile oil from coriander seeds under different conditions like temperatures, pressures, flow rates, and particle diameters. It was revealed that the major effect of particle size, flow rate, and pressure was imparted on the extraction yield, while the composition hasn't changed much. It was established that the best conditions to extract good quality and highest extraction ratio of oil were about pressure of 21 MPa, temperature of about 35 °C along with flow rate of 1 kg/h, and particle size of 0.5 mm. The amount of linalool extracted from coriander seeds using SFE was about 74.28% of essential oil [84].

Recently, a study was conducted to extract polar and nonpolar moieties from coriander seeds using modern technologies like supercritical fluid extraction. It was noticed that SFE gave certain advantages over traditional methods of extraction as substantiated by the yield of linalool (877.07 mg/100 g CS from SFE, 68.66 mg/100 g CS from Soxhlet extraction, and 543.33 mg/100 g CS from hydrodistillation extraction). In this exploration, the raffinate from supercritical fluid extraction was further subjected to ultrasound-assisted extraction for recovery of polar fractions, and it was reported that ethanolic extracts exhibited more antioxidant activity as compared to water extracts. It was concluded that along with essential oil, coriander seeds are also an enriched source of polyphenols with high antioxidant activity [85].

Considering the influence of operating conditions on the chemical composition of supercritical extracts, a study was performed using Box-Behnken experimental design (BBD) and response surface methodology (RSM) to optimize the conditions of supercritical extraction by varying pressure, temperature, and carbon dioxide flow rate. The outcome of the study suggested that the optimum conditions were pressure = 99.5 bar, temperature = 40.15 °C, and flow rate of carbon dioxide = 0.396 kg/h. It was reported that linalool was the predominant bioactive component obtained followed by methyl chavicol, camphor, (+)-limonene, eugenol, eucalyptol, geraniol, α-terpineol, and γ-terpinene. The highest amount of linalool (717 mg/g) was extracted at a pressure of 100 bar, at a temperature of 55 °C, and at a flow rate of 0.4 kg/h [86]. In the same year, Zekovic and his colleagues used subcritical water extraction technique and suggested that highest polyphenolic compounds (942 mg/100 g DW) were extracted from coriander seeds at 60 bar pressure and 100 °C of temperature for a period of 10 min. On the other hand, maximum linalool content (59.90 mg/100 g DW) was obtained at 30 bar pressure, 100 °C temperature, and 20-min time period [87].

4 Antioxidant Potential of Bioactive Molecules

The antioxidant potential of coriander is owing to the presence of various bioactive moieties like polyphenols or phenolic compounds and secondary metabolites. These moieties consist of a complex and wide array of phytochemicals exhibiting antioxidant potential and health-enhancing physiological effect. Phytochemicals acting as antioxidant agents have the ability to inhibit lipid peroxidation in food items as well as biological membranes; additionally their tendency to perform as a prophylactic agent has divulged new horizons of research in the field of food, nutrition, and medicine. These substances have been considered as natural antioxidants owing to their health-enhancing characteristics and copious presence in eclectic range of vegetables, herbs, spices, and fruits. Moreover, the vegetable or fruit which possesses these bioactive components is generally known as functional food [88]. Phenolic substances like phenolic acids and flavonoids have been extracted from various raw materials of vegetable/herb origin like sage, rosemary, thyme, oregano, and pepper [4].

Previously, it was observed that coriander aqueous extract possesses total phenolics of about 2.734 mg/100 g DW, demonstrating significant antioxidant activity. In another study, the total polyphenolic contents measured in aqueous extract of coriander were about 17.08 μg CE/mL of extract. Moreover, the antioxidant activity of extracts was about 69.83% in the aqueous extract of coriander [4]. Furthermore, it was reported that the ethyl acetate extracts of leaves and seeds possess maximum amounts of phenolic compounds, i.e., 5.5 ± 0.09 and 1.9 ± 0.08 g GAE/100 g extract, correspondingly. It was noticed that the total phenolic contents were higher in leaves as compared to the seeds. In the case of DPPH scavenging assay, it was studied that the ethanolic extracts of seeds and leaves exhibited a concentration-dependent activity as depicted by IC_{50} values of 510 ± 12 and 389 ± 5 μg/mL,

correspondingly. On the other hand, it was explored that the lipophilic extracts and coriander oil fraction, i.e., diethyl ether extract of leaves and dichloromethane seed extract, do not show any activity in this assay owing to inability of donating hydrogen. Coriander seed essential oil exhibited weak DPPH scavenging activity, while during TBARS antioxidant assay, coriander oil showed high antioxidant potential [4].

Moreover, in 15-lipoxygenase inhibition assay, the ethanolic extracts revealed a concentration-dependent characteristic. It was observed that leaves have higher activity as compared to seeds with value of about 157 ± 9 and 193 ± 11 IC_{50} μg/mL, correspondingly. The maximum 15-LO inhibitory activity was seen in the case of coriander leaf ethyl acetate extract followed by diethyl ether extract, i.e., IC_{50} 45 ± 2 and 88 ± 5 μg/mL, respectively. The essential oil of coriander seeds exhibited lower 15-LO inhibitory effect, i.e., 199 ± 11 μg/mL (IC_{50}). Further investigations reported that the lipid fraction of coriander demonstrated better 15-LO inhibitory activity as compared to DPPH scavenging activity, which has confirmed the independence of 15-LO assay from proton donation. Literature unveiled that the flavonoids and terpenoids are among the most active 15-LO inhibitors [89].

Recently, three varieties of coriander named as Syrian, Tunisian, and Egyptian were compared for their antioxidant activity by using methanolic extracts. Significant variations were observed among the antioxidant potential of the varieties. The total phenolic content varies from 0.94 to 1.09 mg GAE/g DW, and total flavonoids ranged from 2.03 to 2.51 mg EC/g DW. High-performance liquid chromatographic analyses showed that gallic acids and chlorogenic acids were the major phenolics identified in coriander seeds. Furthermore, methanolic extracts demonstrated significant DPPH scavenging assay, i.e., IC_{50} = 27–36 μg/mL. Likewise, the values of β-carotene antioxidant activity assay in terms of IC_{50} ranged from 160.00 to 240.00 μg/mL. Hence, it has been perceived that coriander fruit is an enriched and novel source of bioactive moieties and antioxidants [39].

Moreover, DPPH scavenging activity of methanolic extracts and essential oil of coriander seeds was carried out, and it was reported that methanolic extracts of two varieties, i.e., Tunisian and Canadian, exhibited DPPH scavenging activity of about IC_{50} = 32 and 36 μg/mL of extracts. On the other hand, essential oil showed very low DPPH scavenging activity, i.e., IC_{50} was around 60,000 μg/mL of essential oil [41]. Nevertheless, ethanolic extracts of coriander obtained from Norway exhibited a concentration-dependent DPPH scavenging activity with IC_{50} values of about 510 μg/mL, although it was also reported that essential oils possessing rich non-phenolic components may possess significant antioxidant potentials [90]. The method of β-carotene bleaching assay is generally based on the formation of free radicals upon the oxidation of linoleic acids; the formed free radicals then react with β-carotene and vanished their yellow color. In the presence of antioxidants, the bleaching rate of β-carotene slowed down significantly [91]. In the investigation, the oxidation inhibition values of linoleic acid were measured as 640 and 730 μg/mL in Canadian and Tunisian samples, respectively. Similar to the case of DPPH scavenging assay, the radical scavenging activity of essential oil exhibited very

low anti-bleaching potential, i.e., 56,000 and 52,000 μg/mL IC_{50} for Tunisian and Canadian samples, correspondingly. Furthermore, the ferric reducing power of coriander extracts showed that Tunisian variety exhibited lower capacity as compared to Canadian variety, i.e., $EC_{50} = 780$ and 700 μg/mL, respectively [41].

Furthermore, Kaiser et al. [76] estimated the antioxidant potential of coriander by conducting FRAP assay, and it was observed that the coriander leaves blanched at 100 °C temperature in water for a time period of 1 min exhibited maximum FRAP activity of 109.6 ± 14.1 mmol TE/kg DM, while coriander fruits exhibited FRAP activity of 51.2 ± 2.6 mmol TE/kg DM when blanched at the same condition as used for coriander leaves.

5 Coriander-Based Designer Foods

The association of diet and health has increased the scope for diet-based therapy against various lifestyle-related disorders among people. Nowadays, demand for natural ingredients is mounting day by day due to public awareness about diet-linked health problems [92]. Herbs and spices like coriander plant and seeds have excellent nutritional profile, and they are a promising source of health-promoting compounds. There is a need for optimum utilization of these compounds that require product development as a tool to carry bioactive components to the targeted population [93].

Development of novel food products is a multifarious and uncertain task that depends on scientific obstacle, consumer satisfaction, convenience, price, age, and cultural habits [94]. A large community relies on plant-based foods to fulfill their dietary needs like carbohydrates, protein, fat, vitamin, and minerals. Among them cereal-based products along with various kinds of sauces occupy central position for people of different age groups to satisfy their nutritional requirements. All over the globe, the demand of numerous types of spreads and sauces is escalating with the passage of time.

Coriander seeds and herb have been traditionally used in the formulation of numerous food items like bakery products, sauces, and spices and in some local products of Indo-Pak regions like *chatni*, etc. Coriander has commonly been used as a flavoring agent in two forms, i.e., green herb and seed; the latter is generally used as a vital ingredient of spice. The odor and flavor of these two types of coriander product are evidently different. The herb is generally being used for the flavoring purposes of culinary in the Middle East, Asia, and continent of America. The whole green plant and leaves have been utilized for preparing various kinds of chutneys, sauces, curries, and soups. Leaves have also been used for the garnishing purposes. On the other hand, coriander seeds have been used as an important ingredient of curry powders, pickling spice, sausages, seasoning, buns, pastries, cakes, and other bakery products. Along with this coriander oil has been utilized for flavoring beverages, meat, candies, sauces, and even tobacco. Coriander juice has traditionally been utilized for the treatment of indigestion, dysentery, nausea ulcerative colitis, and hepatitis by adding it in fresh buttermilk. Chutney prepared from coriander seeds has been used as a cure of abdominal pain. Furthermore, coriander water prepared by

boiling coriander seeds helps in lowering the serum cholesterol level by upregulating the kidney function [2]. Nowadays, among various vegetable products, several kinds of sauces are in limelight. Generally, sauces are categorized by water activity and pH values which let their marketability for a small time under cool conditions, though storage for a longer period required the implementation of pasteurization/sterilization treatments.

6 Coriander Against Oxidative Stress-Mediated Dysfunctions

In modern life, a strong link has been recognized between oxidative stress and numerous lifestyle-related dysfunctions. Oxidative stress has been demarcated as a "condition in which the occurrence of oxidation reactions surpasses the natural antioxidant mechanisms of the body, ultimately losing balance among them." Oxidative stress has substantiated to cause perilous events like DNA damage, lipid peroxidation, and regulating the transduction of intracellular signals. Chemical compounds of *Coriandrum sativum* L. and their biological activities have been summarized in Table 2.

6.1 Oxidative Stress and Safety Concerns

Normally, an atom has a pair of electrons orbiting around a central nucleus. Although some atoms don't possess paired electrons, they instead have unpaired electrons and these atoms/molecules are known as free radicals. Free radicals proved to be very unstable and reactive owing to the presence of unpaired electrons which urge to make pair by accepting the electrons. When oxygen molecule metabolized in the body, it generates free oxygen radicals, which are more reactive as compared to original molecule and known as reactive oxygen species. Hydrogen peroxide, superoxide, singlet oxygen, and hydroxyl radicals are the forms of active oxygen species in a narrow sense. In aerobic organisms, highly reactive oxygen species have been removed by natural antioxidant defense system. The oxygen species generated in the body generally do not impose any threat owing to effective defense mechanism of the body. However, if these reactive species generated at unusual site or at a rate which surpasses the natural defense mechanism, then disturbance of balance among the generation of reactive species and their removal would be lost, resulting in oxidative stress. Ultimately, the free radicals and reactive oxygen species can invade the healthy tissues and cells, damaging their cell membranes and initiating numerous dysfunctions [95].

It has been established that vitamin E is among the most important agents required to protect the body against lipid peroxidation. Vitamin E acts as antioxidant as it can bind the lipid peroxyl radicals, preventing the propagation of free radical chain reaction. The exact scavenging mechanism includes the removal of hydrogen atom from the phenyl group of vitamin by lipid peroxyl radicals. The molecule that

Table 2 Chemical compounds in *Coriandrum sativum* L. and their biological activities

Sr. no.	Chemical constituents	Antioxidant	Antidiabetic	Anti-inflammatory	Antiaging	Antitumor	Anticancer	Antimicrobial	Antiulcer
1	Acetic acid (seeds)							✓	
2	α-Pinene			✓			✓	✓	
3	α-Phellandrene							✓	
4	α-Terpineol							✓	
5	α-Terpinene							✓	
6	Apigenin	✓		✓	✓	✓	✓	✓	
7	Ascorbic acid (leaf)		✓	✓	✓	✓	✓	✓	✓✓
8	Angelicin					✓		✓	
9	β-Pinene (seeds)			✓					
10	β-Carotene	✓			✓	✓	✓		✓
11	β-Sitosterol			✓		✓	✓	✓	
12	Bornyl acetate							✓	
13	Borneol			✓				✓	
14	Camphene	✓							
15	Caffeic acid	✓				✓	✓	✓	
16	Copper		✓	✓					
17	Carvone						✓		
18	Chromium		✓		✓				
19	Chlorogenic acid		✓			✓	✓	✓	✓
20	Citronellol							✓	
21	cis-Ocimene							✓	
22	Caryophyllene			✓		✓		✓	✓
23	Dipentene							✓	

(*continued*)

Table 2 (continued)

Sr. no.	Chemical constituents	Antioxidant	Antidiabetic	Anti-inflammatory	Antiaging	Antitumor	Anticancer	Antimicrobial	Antiulcer
24	Elemol								✓
25	Fructose		✓						
26	Fiber					✓	✓		✓
27	Geranial					✓	✓	✓	
28	γ-Terpinene	✓							
29	Isoquercitrin	✓				✓	✓	✓	
30	Linalool	✓	✓	✓		✓	✓	✓	
31	Linoleic acid			✓			✓		
32	Limone			✓		✓		✓	
33	Myristicin					✓		✓	
34	Magnesium		✓	✓					
35	Myristicin	✓		✓					
36	Myristic acid	✓							
37	Nerolidol							✓	
38	Nerol						✓	✓	
39	Niacin		✓				✓		
40	Oleic acid			✓			✓		
41	p-Hydroxy benzoic acid	✓				✓		✓	
42	p-Cymene							✓	
43	Psoralen					✓	✓		
44	Palmitic acid	✓							
45	Protocatechuic acid	✓	✓	✓		✓			
46	Pectin		✓			✓		✓	✓
47	Quercetin			✓		✓	✓	✓	

48	Rutin		✓	✓		✓	✓	✓	
49	Rhamnetin			✓			✓	✓	
50	Sabinene			✓				✓	✓
51	Terpinene-4-01	✓						✓	✓
52	Tannin					✓	✓	✓	✓
53	trans-Anethole	✓							
54	Terpinolene	✓							
55	Umbelliferone			✓		✓		✓	
56	Vanillic acid			✓		✓	✓		
57	Zinc		✓					✓	✓

receives the hydrogen of atom then becomes stabilized/nonreactive. This whole mechanism transforms the vitamin E into a free radical, but it would also be a less reactive and stable molecule.

Ultimately, this stabilized vitamin E molecule would not attack lipids, though it would react with another lipid peroxyl free radical and then becomes stable. This natural antioxidant mechanism protects the body from cellular membrane injuries caused by lipid peroxides and other free radicals. Interestingly, sometimes oxidative stress proves to be useful for the human body, i.e., during childbirth the oxidative stress initiated the apoptosis process to prepare the birth canal for a delivery; likewise, natural defense systems of the body have also been strengthened by oxidative stress during ischemia and appropriate exercise. It has been reported that oxidative stress induces many lifestyle-related disorders like inflammations, hyperglycemia, hypercholesterolemia, and even cancer, by imparting DNA damage and genetic mutations. The lifestyle-associated disorders have been categorized into three categories, i.e., habitual, environmental, and genetic [95].

In recent era, several genes have been identified which are linked with oxidative stress in the body. Furthermore, among various genes, heme oxygenase (HO) and NO synthase have been recognized as the candidate genes for inducing such lifestyle-related disorders/diseases, though it is difficult to point out the exact cause of maladies because they are multifactorial. Along with genetic malfunctioning, several daily habitual activities are closely linked with oxidative stress like alcohol drinking, tobacco smoking, and irregular dietary patterns. In Japan dietary patterns of masses have been changed dramatically over the years, as the energy intake from lipids is now over 25% of daily energy requirements. Furthermore, active oxygen species generated by environmental factors can cause the serious detrimental effects on the DNA, which can lead to the initiation and propagation of oncogenic events. Assessing the effectiveness of current biological antioxidant mechanism, by estimating the pertinent oxidative biomarkers, along with considering the genetic information of an individual, can be useful to prescribe curative interventions [95].

6.2 Hyperglycemia and Related Complications

Hyperglycemia is the condition in which the human body either synthesizes more pituitary hormone than normal or less insulin, resulting in the elevation of blood glucose to abnormal level. In hyperglycemic conditions the serum glucose level may shoot up to four times as compared to baseline value. Hyperglycemia is commonly used indicator of diabetes mellitus [96]. A strong association has been established between the elevation of oxidative stress and hyperglycemia, ultimately causing diabetic complications. Occurrence of hyperglycemia in the body catalyzes the formation of reactive oxygen species (ROS) from a number of chemical reactions as glucose auto-oxidation, oxidative phosphorylation, lipoxygenase, NADPH oxidase, nitric oxide synthase, and cytochrome P450 monooxygenases.

Globally, among the patients suffering from diabetes mellitus, only 10% are suffering from type 1 diabetes also known as insulin-dependent diabetes. Nonetheless, the most predominant diabetes is the type 2, also known as non-insulin-dependent diabetes. In type 2 diabetes, the decreased glucose uptake by the muscle and adipose tissue elevated the level of serum glucose level imparting some serious pathophysiological dysfunctions involving atherosclerosis, heart disease, peripheral nerve damage, cataract formation, retinopathy, etc. [97].

Indigenous foods of the Indo-Pak region like spices, herbs, vegetables, and fruits are considered a rich source of phytonutrients and antioxidants. These foods are known as "functional foods" as they provide additional health benefits along regular nutrition. The human body also possesses a natural antioxidant defense mechanism, including several enzymatic and nonenzymatic antioxidants. The enzymatic antioxidants include catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase; on the other hand, the nonenzymatic antioxidants are generally categorized into two subclasses like nutrient antioxidants including α-tocopherol, β-carotene, and ascorbic acid, while the other subclass is metabolic antioxidants, i.e., glutathione [98]. Coriander seeds are among the most commonly used spices possessing numerous medicinal characteristics. Coriander seeds in experimental subjects exhibited a significant antidiabetic behavior as depicted by the values of fasting glucose level which elevated to about 11% in control individuals, while it lowered to about 13% in coriander treated group. Hyperglycemia may cause the overload of glucose in mitochondria, enhance the electron transfer to oxygen, and generate oxygen free radicals. This reaction ultimately activates the pathways which cause diabetic complications [99].

Similarly, it was reported that a single-dose administration of coriander to obese, hyperglycemic, and hypercholesterolemic rats suppressed their hyperglycemia. In a sub-chronic study, reduction in plasma glucose and insulin resistance was observed. It was observed that the dose rate of about 40 mg/kg aqueous coriander extract lowers the plasma glucose level to about 41.63%, tested after 6 h of administration. Furthermore, it was reported that the daily dose of coriander seed extract lowers the plasma insulin level and insulin resistance to about 28 and 80% in a 30-day trial [100].

Furthermore, it was exposed by Deepa and Anuradha [101] that administration of streptozotocin negatively affects the β-cells on setting the diabetes mellitus in the experimental rats, while oral dose of about 1.2 g per rat per day of coriander significantly lowered the serum glucose level (280–124 mg/dL) and increased the plasma insulin level (3.89–9.82 μU/mL) to about 44% and 40%, respectively. Moreover, the effect of ethanolic extracts of coriander seeds on the release of insulin from beta cells of pancreas has been investigated in streptozotocin-induced diabetic rats. It was reported that the ethanol extracts given at a dose rate of 200–250 mg/kg by intraperitoneal injection showed a noticeable reduction in serum glucose, i.e., about 550–400 and 280–200 mg/dL, respectively. Application of streptozotocin lowered the number of β-cells as well as insulin secretory activity as compared to healthy animals, though treating with ethanolic extract of coriander momentously enhanced the β-cell activity [102].

Previously, it was reported that administration of diet and drink enriched with coriander to streptozotocin-induced diabetic rats significantly lowered the hyperglycemia (about 33%) in a 20-day trial. It was further narrated that water extract of coriander seeds at a dose rate of about 1 mg/mL elevated the glucose uptake, glycogenesis, and glucose oxidation to about 1.6-, 1.7-, and 1.4-fold, respectively. Furthermore, the aqueous coriander extract imparted positive effect on insulin secretion by pancreatic cells in a dose-dependent fashion [103].

6.3 Hepatoprotective Perspectives

Hepatic system in the human body referred to the functioning mechanism of the liver. The liver is one of the most vital internal organs of the body. It is regarded as the largest internal organ of the human body. The liver performs >500 distinct functions which are vital for sustaining healthy human life. The essential functions of the liver include assisting the digestion of lipids in food, filtering the wastes and harmful components from the blood, preserving the nutrient reserves, and synthesizing different kinds of proteins along with maintaining levels of various biochemicals in the bloodstream. One of the unique properties of the liver is its ability to regrow its cells, if damaged by some disease or injury. However, chronic conditions can cause irreversible changes in the integrity of liver cells.

The liver stores energy in the form of glycogen which is a polymer of glucose subunits. Furthermore, the liver synthesizes the bile which contains salts for the digestion of lipids. Moreover, it also stored fat-soluble vitamins, i.e., vitamins A, D, E, and K, alongside vitamin B complex. Additionally, hepatic system also helps the body in clearing toxic and harmful chemicals like alcohol and drugs from the bloodstream. Hepatic cells absorb the hazardous chemicals from the bloodstream and chemically modify and convert them into harmless components to be excreted from the body [104].

Reactive oxygen species generated during oxidative stress can attack the healthy hepatic cells as in the case of lipid peroxidation. It can damage the cells to such level that cells would become nonfunctional creating some serious health complications, ranging from inflammation to hyperglycemia, hypercholesterolemia, jaundice, and even oncogenesis. From ancient times, herbs and spice have been well known for their aromatic characteristics and medicinal properties. Over the decades, phytochemicals that exist in herbs and spices have shown their potential of lowering oxidative stress and preventing lipid peroxidation. In this regard, coriander is in limelight owing to presence of a number of health-enhancing components like polyphenols and flavonoids.

In vivo antioxidant assay of essential oil extracted from coriander seeds has been proved to regulate liver biochemical parameters. It was reported that the single-dose administration of CCl_4 momentously enhances the level of lipid peroxidation (LPx) along with increasing the activity of peroxidase enzyme (Px); on the other hand, it lowers the content of reduced glutathione (GSH) and limits the activity of glutathione peroxidase (GSH-Px) and catalase enzymes (CAT). Moreover, CCl_4 application

resulted in elevation of serum alanine transaminase (ALT) and aspartate transaminase (AST). It was further narrated that the introduction of coriander extracts lowers the lipid peroxidation and activity of peroxidase, i.e., 2.82 ± 1.0–1.57 ± 0.3 nmol/mg of protein and 2.96 ± 0.5–1.98 ± 0.4 nmol/mg of protein/min, respectively, while it increased the content of reduced glutathione and activity of glutathione peroxidase as depicted from the values, i.e., 0.80 ± 0.2–1.24 ± 0.2 nmol/mg of protein and 1.08 ± 0.3–1.24 ± 0.2 nmol/mg of protein/min, correspondingly [105].

Recently, a study was conducted to explore the modulatory effects of green part of coriander on CCl_4-induced hepatic toxicity. It was declared that the phenolic components of coriander leaves and stems fed to CCl_4-induced hepatotoxic rats resulted in a significant decrease in liver and kidney biomarkers, illuminating noticeable protective perspectives of coriander against hepatic and renal toxicity. Treatment of CCl_4 resulted in significant increase in plasma level of hepatic enzymes like ALT, AST, and ALP. In contrast, treatment of coriander extracts reduces the elevation of plasma levels of these enzymes as depicted by the measured values; i.e., the contents of AST, ALT, and ALP were about 67.0 ± 1.49, 36.4 ± 1.85 U/mL, and 266.0 ± 10.6 U/L which lowered to about 52.25 ± 2.40, 17.4 ± 0.28 U/mL, and 130 ± 2.57 U/mL, respectively. Likewise, coriander treatment also lowers the biomarkers of kidney damage. It lowered the level of urea, uric acid, and creatinine from 45.9 ± 0.41 to 26.9 ± 1.30, 4.35 ± 0.15 to 2.38 ± 0.12, and 1.16 ± 0.01 to 0.71 ± 0.02 mg/dL, respectively. The results confirmed the hepatic and renal protective potential of coriander herb plant [106].

Another scientific study was conducted to explore the hepatoprotective effects of coriander leaves and seeds. In the study, 48 male rats were divided into four groups named as control (group I), hepatotoxic rats (group II), hepatotoxic rats fed on coriander leaves (group III), and hepatotoxic rats fed on coriander seeds (group IV). It was observed that the level of serum ALT, AST, and ALP activities enhanced significantly in all three hepatotoxic groups, i.e., groups II, III, and IV, though the administration of coriander leaves and seeds lowered the level of these enzymes as well as of nitric oxide and thiobarbituric acid reactive substance as compared to positive control group (group II). It was concluded that the coriander leaves were proved to be more effective in reducing the hepatotoxic effects as compared to coriander seeds owing to the presence of more polyphenols. The hepatoprotective effects of leaves and seeds were obvious from the mean values of ALP, AST, and ALT, which were about 153.08 ± 21.81, 244.25 ± 58.10, and 182.25 ± 37.29 U/L, respectively, in hepatotoxic rats which lowered to 142.16 ± 15.47, 171.33 ± 22.64, and 143.91 ± 18.47 U/L by the administration of seeds and 131.58 ± 17.97, 161.66 + 21.22, and 133.5 + 24.44 U/L, respectively, by feeding rats on coriander leaves [107].

Furthermore, one of their peers conducted a study on CCl_4-induced hepatotoxic albino rats to evaluate the protective potential of coriander. The injection of carbon tetrachloride noticeably raises the serum hepatic markers and lowered the antioxidant enzymes. It was observed that pretreatment of animals with coriander plant extract lowered the level of serum ALT, ALP, AST, and TBARS as compared to the positive control group. Moreover, it also strengthens the biological antioxidant

mechanism by enhancing the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). It was also reported that the activity of 200 mg/kg body weight dose of coriander extract is comparable to the commercially available synthetic drug known as silymarin [108]. On the basis of aforementioned facts, it can be concluded that the coriander has the ability to ameliorate oxidative stress and protect the liver as well as renal cell from damage.

7 Conclusion

Generally, herbs and spices do not majorly contribute to nutrient supplementation of diet because of their use in lesser quantities and mostly utilized for the purpose of garnishing and flavoring. However, keeping in view the health-enhancing potential of these food components, they must be employed in the designing and formulating of functional foods. In this milieu, coriander seeds and herbs have been proved to be responsible for therapeutic effects against inflammation, diabetes, hypercholesterolemia, and hepatorenal toxicity. It can be abridged that coriander seeds and leaves are among the vital spices and herbs for health maintenance and disease prevention owing to their enriched phytochemistry which includes the predominant occurrence of linalool, flavonoids, etc., as the antioxidant potential of these bioactive moieties has been authenticated by a wide spectrum of in vitro and in vivo researches.

Acknowledgments The authors are thankful to Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. The project was partially supported by Higher Education Commission, Pakistan, under Pak-US Science and Technology Cooperation Program Phase IV with project entitled "Establishment of Functional and Nutraceutical Food Research Section at the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan."

References

1. Jayasekera H, Carter G, Clover K (2011) Comparison of the composite international diagnostic interview (cidi-auto) with clinical diagnosis in a suicidal population. Arch Suicide Res 15(1):43–55
2. Sharma MM, Sharma RK (2012) Coriander. In: Peter KV (ed) Handbook of herbs and spices, 2nd edn, vol 1. Woodhead Publishing series in food science, technology, and nutrition, no. 227. Woodhead Publishing Limited, Cambridge, UK, pp 216–249
3. Laribi B, Kouki K, M'Hamdi M, Bettaie T (2015) Coriander (*Coriandrum sativum* L.) and its bioactive constituents. Fitoterapia 103:9–26
4. Melo EA, Filho JM, Guerra NB (2005) Characterization of antioxidant compounds in aqueous coriander extract (*Coriandrum sativum* L.) LWT-Food Sci Technol 38:15–19
5. Singh G, Maurya S, De Lampasona MP, Catalan CAN (2006) Studies on the essential oils, Part 41. Chemical composition, antifungal, antioxidant and sprout suppressant activities of coriander (*Coriandrum sativum*) essential oil and its oleoresin. Flavour Frag J 21:472–479
6. Sriti J, Neffati M, Msaada K, Talou T, Marzouk B (2013) Biochemical characterization of coriander cakes obtained by extrusion. J Chem. https://doi.org/10.1155/2013/871631

7. Kamat A, Pingulkar K, Bhushan B, Gholap A, Thomas P (2003) Potential application of low dose gamma irradiation to improve the microbiological safety of fresh coriander leaves. Food Cont 4:529–537
8. Sahib NG, Anwar F, Gilani AH, Hamid AA, Saari N, Alkharfy KM (2012) Coriander (*Coriandrum sativum* L.): a potential source of high-value components for functional foods and nutraceuticals-a review. Phytother Res 27(10):1439–1456
9. Msaada K, Jemia MB, Salem N, Bachrouch O, Sriti J, Tammar S, Bettaieb I, Jabri I, Kefi S, Limam F, Marzouk B (2013) Antioxidant activity of methanolic extracts from three coriander (*Coriandrum sativum* L.) fruit varieties. Arab J Chem. https://doi.org/10.1016/j.arabjc.2013.12.011. (in press)
10. Coskuner Y, Karababa E (2007) Physical properties of coriander seeds (*Coriandrum sativum* L.) J Food Eng 80:408–416
11. Ramadan MF, Mörsel JT (2002) Oil composition of coriander (*Coriandrum sativum* L.) fruit-seeds. Eur Food Res Technol 215:204s–2049
12. Ozcan MM, Unver A, Ucar T, Arslan D (2008) Mineral content of some herbs and herbal teas by infusion and decoction. Food Chem 106:1120–1127
13. Al-Bataina BA, Masiat AO, Al-Kofahi MM (2003) Element analysis and biological studies on ten oriental spices using XRF and Ames test. Trace Elem Med Biol 17(2):85–90
14. Telci I, TO G, Sahbaz N (2006) Yield, essential oil content and composition of *Coriandrum sativum* varieties (var. vulgare Alef and var. microcarpum DC.) grown in two different locations. J Essent Oil Res 18:189–193
15. Bandoni AL, Mizrahi I, Juarez MA (1998) Composition and quality of essential oil of coriander (*Coriandrum sativum* L.) from Argentina. J Essent Oil Res 10:581–584
16. Rastogi RP, Mehrotra BN (1993) Compendium of Indian medicinal plants, vol II. CDRI, Lucknow
17. Nazrul M, Bhuiyan I, Begum J, Sultana M (2009) Chemical composition of leaf and seed essential oil of *Coriandrum sativum* L. from Bangladesh. Bangladesh J Pharmacol 4:150–153
18. Zheljazkov VD, Pickett KM, Caldwell CD, Pincock JA, Roberts JC, Mapplebeck L (2008) Cultivar and sowing date effects on seed yield and oil composition of coriander in Atlantic Canada. Ind Crop Prod 28:88–94
19. Msaada K, Hosni K, Ben Taarit M, Chahed T, Kchouk ME, Marzouk B (2007) Changes on essential oil composition of coriander (*Coriandrum sativum* L.) fruits during three stages of maturity. Food Chem 102:1131–1134
20. Ghani A (2003) Medicinal plants of Bangladesh: chemical constituents and uses, 2nd edn. Asiatic Society of Bangladesh, Ohaka
21. Sahib NG, Anwar F, Gilani AH, Abdul-Hamid A, Saari N, Alkharfy KM (2013) Coriander (*Coriandrum sativum* L.): a potential source of high-value components for functional foods and nutraceuticals – a review. Phytother Res 27(10):1439–1456
22. Bhat S, Kaushal P, Kaur M, Sharma HK (2014) Coriander (*Coriandrum sativum* L.): processing, nutritional and functional aspects. African J Plant Sci 8(1):25–33
23. Gil A, De La Fuente EB, Lenardis AE, López Pereira M, Suárez SA, Bandoni A (2002) Coriander essential oil composition from two genotypes grown in different environmental conditions. J Agric Food Chem 50:2870–2877
24. Verma A, Pandeya SN, Yadav SK, Singh S, Soni P (2011) A review on *Coriandrum sativum* (Linn.): an ayurvedic medicinal herb of happiness. J Adv Pharm Healthc Res 1(3):28–48
25. Khan SW, Khatoon S (2008) Ethnobotanical studies on some useful herbs of Haramosh and Bugrote valleys in Gilgit, northern areas of Pakistan. Pak J Bot 40(1):43–58
26. Ugulu I, Baslar S, Yorek N, Dogan Y (2009) The investigation and quantitative ethnobotanical evaluation of medicinal plants used around Izmir province, Turkey. J Med Plant Res 3(5):345–367
27. Platel K, Srinivasan K (2004) Digestive stimulant actions of spices: a myth or reality? Indian J Med Res 119:167–179
28. Al Rowais NA (2002) Herbal medicine in the treatment of diabetes mellitus. Saudi Med J 23(11):1327–1331

29. Otoom SA, Al-Safi SA, Kerem ZK, Alkofahi A (2006) The use of medicinal herbs by diabetic Jordanian patients. J Herb Pharmacother 6(2):31–41
30. Tahraoui A, El Hilaly J, Israili ZH, Lyoussi B (2007) Ethnopharmacological survey of plants used in traditional treatment of hypertension and diabetes in southeastern Morocco (Errachidia province). J Ethnopharmacol 110(1):105–117
31. Jelodar G, Mohsen M, Shahram S (2007) Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan induced diabetic rats. Afr J Tradit Complement Altern Med 4(3):299–305
32. Eddouks M, Maghrani A, Lemhadri ML, Ouahidi L, Jouad H (2002) Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). J Ethnopharmacol 82(2–3):97–103
33. Abu Rabia A (2005) Herbs as a food and medicine source in Palestine. Asian Pac J Cancer Prev 6(3):404–407
34. Aissaoui A, El-Hilaly J, Israili ZH, Lyoussi B (2008) Acute diuretic effect of continuous intravenous infusion of an aqueous extract of *Coriandrum sativum* L. in anesthetized rats. J Ethnopharmacol 115(1):89–95
35. Thabrew MI, Dharmasiri MG, Senaratne L (2003) Anti-inflammatory and analgesic activity in the polyherbal formulation Maharasnadhi Quathar. J Ethnopharmacol 85(2–3):261–267
36. Emamghoreishi M, Heidari-Hamedani G (2004) Anticonvulsant effect of extract and essential oil of *Coriandrum sativum* seed in conscious mice. Iran J Pharm Res 3(1):71
37. Emamghoreishi M, Heidari-Hamedani G (2006) Sedative-hypnotic activity of extracts and essential oil of coriander seeds. Iran J Med Sci 31(1):22–27
38. Chaudry NM, Tariq P (2006) Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates. Pak J Pharm Sci 19(3):214–218
39. Msaada K, Jemia MB, Salem N, Bachrouch O, Sriti J, Tammar S, Bettaieb I, Jabri I, Kefi S, Limam F, Marzouk B (2017) Antioxidant activity of methanolic extracts from three coriander (Coriandrum sativum L.) fruit varieties. Arab J Chem 10(2):S3176-S3183
40. Neffati M, Sriti J, Hamdaoui G, Kchouk ME, Marzouk B (2011) Salinity impact on fruit yield, essential oil composition and antioxidant activities of *Coriandrum sativum* fruit extracts. Food Chem 124:221–225
41. Sriti J, Wannes WA, Talou T, Vilarem G, Marzouk B (2011) Chemical composition and antioxidant activities of Tunisian and Canadian Coriander (*Coriandrum sativum* L.) Fruit. J Essen Oil Res 23:7–15
42. Wangensteen H, Samuelsen AB, Malterud KE (2004) Antioxidant activity in extracts from coriander. Food Chem 88:293–297
43. Barros L, Dueñas M, Dias MI, Sousa MJ, Santos-Buelga C, Ferreira ICFR (2012) Phenolic profiles of in vivo and in vitro grown *Coriandrum sativum* L. Food Chem 132(2):841–848
44. Kandlakunta B, Rajendran A, Thingnganing L (2008) Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin. Food Chem 106:85–89
45. Ravi R, Prakash M, Bhatt KK (2006) Aroma characterization of coriander (*Coriandrum sativum* L.) oil samples. Eur Food Res Technol 6:425–427
46. Msaada K, Ben Taarit M, Hosni K, Hammami M, Marzouk B (2009) Regional and maturational effects on essential oils yields and composition of coriander (*Coriandrum sativum* L.) fruits. Sci Hortic 122:116–124
47. Nejad ES, Hadian J, Ranjbar H (2010) Essential oil compositions of different accessions of *Coriandrum sativum* L. from Iran. Nat Prod Res 24(14):1287–1294
48. Bhuiyan NI, Begum J, Sultana M (2009) Chemical composition of leaf and seed essential oil of *Coriandrum sativum* L. from Bangladesh. Bangladesh J Pharmacol 4:150–153
49. Anwar F, Sulman M, Hussain AI, Saari N, Iqbal C, Rashid U (2011) Physicochemical composition of hydro-distilled essential oil from coriander (*Coriandrum sativum* L.) seeds cultivated in Pakistan. J Med Plant Res 5(15):3537–3544

50. Zoubiri S, Baaliouamer A (2010) Essential oil composition of *Coriandrum sativum* seed cultivated in Algeria as food grains protectant. Food Chem 122:1226–1228
51. De Figueiredo RO, Marques MOM, Nakagawa J, Ming LC (2004) Composition of coriander essential oil from Brazil. Acta Hortic 629:135–137
52. Msaada K, Hosni K, Ben Taarit M, Hammami M, Marzouk B (2009) Effects of growing region and maturity stages on oil yield and fatty acid composition of coriander (*Coriandrum sativum* L.) fruit. Sci Hortic 120:525–531
53. Neffati M, Marzouk B (2008) Changes in essential oil and fatty acid composition in coriander (*Coriandrum sativum* L.) leaves under saline conditions. Ind Crop Prod 28:137–142
54. Neffati M, Marzouk B (2009) Roots volatiles and fatty acids of coriander (*Coriandrum sativum* L.) grown in saline medium. Acta Physiol Plant 31:455–461
55. Ghamarnia H, Daichin S (2013) Effect of different water stress regimes on different Coriander (Coriander sativum L.) parameters in a semi-arid climate. Int J Agron Plant Prod 4(4):822–832
56. Hassan FAS, Ali EF (2014) Impact of different water regimes based on class-A pan on growth, yield and oil content of *Coriandrum sativum* L. plant. J Saudi Soc Agric Sci 13(2):155–161
57. Benyoussef EH, Saibi S (2013) Influence of essential oil composition on water distillation kinetics. Flavour Fragr J 28(5):300–308
58. Kosar M, Ozek T, Goger F, Kurkcuoglu M, Baser KHC (2005) Comparison of microwave assisted hydrodistillation and hydrodistillation methods for the analysis of volatile secondary metabolites. Pharm Biol 43(6):491–495
59. Grosso C, Ferraro V, Figueiredo AC, Barroso JG, Coelho JA, Palavra AM (2008) Supercritical carbon dioxide extraction of volatile oil from Italian coriander seeds. Food Chem 111 (1):197–203
60. Turek C, Stintzing FC (2012) Stability of essential oils: a review. Compr Rev Food Sci Food Saf 12:40–53
61. Kitajima J, Ishikawa T, Fujimatu E, Kondho K, Takayanagi T (2003) Glycosides of 2 C-methyl-D-erythritol from the fruits of anise, coriander and cumin. Phytochemistry 62: 115–120
62. Sriti J, Talou T, Wannes WA, Cerny M, Marzouk B (2009) Essential oil, fatty acid and sterol composition of Tunisian coriander fruit different parts. J Sci Food Agric 89:1659–1664
63. Sriti J, Wannes WA, Talou T, Mhamdi B, Handaoui G, Marzouk B (2010a) Lipid fatty acid and tocol distribution of coriander fruits' different parts. Ind Crop Prod 31:294–300
64. Sriti J, Wannes WA, Talou T, Mhamdi B, Cerny M, Marzouk B (2010b) Lipid profile of Tunisian coriander (*Coriandrum sativum*) seed. J Am Chem Soc 87:395–400
65. Matasyoh JC, Maiyo ZC, Ngure RM, Chepkorir R (2009) Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. Food Chem 113(2):526–529
66. Nambiar VS, Daniel M, Guin P (2010) Characterization of polyphenols from coriander leaves (*Coriandrum sativum*), red amaranthus (*A. paniculatus*) and green amaranthus (*A. frumentaceus*) using paper chromatography and their health implications. J Herb Med Toxicol 4(1):173–177
67. Oganesyan ET, Nersesyan ZM, Parkhomenko AY (2007) Chemical composition of the above-ground part of Coriandrum sativum. Pharm Chem J 41(3):30–34
68. Rahimi AR, Babaei S, Kambiz M, Asad R, Sheno A (2013) Anthocyanin content of coriander leaves as affected by salicylic acid and nutrients application. Int J Biosci 3(2):141–145
69. Begnami AF, Duarte MCT, Furletti V, Rehder VLG (2010) Antimicrobial potential of *Coriandrum sativum* L. against different Candida species *in vitro*. Food Chem 118:74–77
70. Padalia RC, Karki N, Sah AN, Verma RS (2011) Volatile constituents of leaf and seed essential oil of *Coriandrum sativum* L. J Essent Oil Bear Plants 14(5):610–616
71. Chung IM, Ahmad A, Kim SJ, Naik PM, Nagella P (2012) Composition of the essential oil constituents from leaves and stems of Korean *Coriandrum sativum* and their immunotoxicity activity on the *Aedes aegypti* L. Immunopharmacol Immunotoxicol 34(1):152–156
72. Guerra N, Melo E, Filho J (2005) Characterization of antioxidant compounds in etheric coriander extract (*Coriandrum sativum* L.) J Food Compos Anal 18:193–199

73. Divya P, Puthusseri B, Neelwarne B (2012) Carotenoid content, its stability during drying and the antioxidant activity of commercial coriander (*Coriandrum sativum* L.) varieties. Food Res Int 45(1):342–350
74. Pavlic B, Vidovic S, Vladic J, Radosavljevic R, Zekovic Z (2015) Isolation of coriander (*Coriandrum sativum* L.) essential oil by green extractions versus traditional techniques. J Supercrit Fluids 99:23–28
75. Eikani MH, Golmohammad F, Rowshanzamir S (2007) Subcritical water extraction of essential oils from coriander seeds (*Coriandrum sativum* L.) J Food Eng 80:735–740
76. Kaiser A, Kammerer DR, Carle R (2013) Impact of blanching on polyphenol stability and antioxidant capacity of innovative coriander (*Coriandrum sativum* L.) pastes. Food Chem 140:332–339
77. Pourmortazavi SM, Hajimirsadeghi SS (2007) Supercritical fluid extraction in plant essential and volatile oil analysis. J Chromatograph 1163:2–24
78. Brunner G (2014) Hydrothermal and supercritical water properties. In: Erdogan K (ed) Supercritical fluid science and technology series, vol 5. Elsevier, The Boulevard, Langford Lane, Kidlington, Oxford
79. Teo CC, Tan SN, Hong Yong JW, Hew CS, Ong ES (2010) Pressurized hot water extraction. J Chromatograph A 1217:2484–2494. (Review)
80. Saim N, Osman R, Yasin WAHM, Hamid RD (2008) Subcritical water extraction of essential oil from coriander (*Coriandrum sativum* L.) seeds. Malaysian J Anal Sci 12:22–24
81. Grosso C, Figueiredo AC, Burillo J, Mainar AM, Urieta JS, Barroso JG, Coelho JA, Palavra AMF (2009) Enrichment of the thymoquinone content in volatile oil from Satureja montana using supercritical fluid extraction. J Sep Sci 32:328–334
82. Vidovic S, Zekovic Z, Morasanovic B, Pandurevic M, Vladic J (2014) Influence of pretreatments on yield, chemical composition and antioxidant activity of Satureja montana extracts obtained by supercritical carbon dioxide. J Supercrit Fluids. https://doi.org/10.1016/j.supflu.10.019
83. Illes V, Daood HG, Perneczki S, Szokonya L, Then M (2000) Extraction of coriander seed oil by CO_2 and propane at super- and subcritical conditions. J Supercrit Fluids 17:177–186
84. Mhemdi H, Rodier E, Kechaou N, Fages J (2011) A supercritical tunable process for the selective extraction of fats and essential oil from coriander seeds. J Food Eng 105:609–616
85. Zekovic Z, Busic A, Komes D, Vladic J, Adamovic D, Pavlic B (2015) Coriander seeds processing: sequential extraction of non-polar and polar fractions using supercritical carbon dioxide extraction and ultrasound-assisted extraction. Food Bioprod Process 95:218–227
86. Zekovic Z, Pavlic B, Cvetanovic A, Durovic S (2016) Supercritical fluid extraction of coriander seeds: process optimization, chemical profile and antioxidant activity of lipid extracts. Ind Crop Prod 94:353–362
87. Zekovic Z, Kaplan M, Pavlic B, Olgun EO, Vladic J, Canli O, Vidovic S (2016) Chemical characterization of polyphenols and volatile fraction of coriander (*Coriandrum sativum* L.) extracts obtained by subcritical water extraction. Ind Crop Prod 87:54–63
88. McDonald S, Prenzler PD, Antolovich M, Robards K (2001) Phenolic content and antioxidant activity of olive extracts. Food Chem 73:73–84
89. Amagata T, Whitman S, Johnson TA, Stessman CC, Loo CP, Lobkovsky E, Clardy J, Crews P, Holman TR (2003) Exploring sponge-derived terpenoids for their potency and selectivity against 12-human, 15-human, and 15-soybean lipoxygenases. J Natur Prod 66:230–235
90. El-Massry KF, El-Ghorab AH, Farouk A (2002) Antioxidant activity and volatile components of Egyptian Artemisia judaica L. Food Chem 79:331–336
91. Kulisic T, Radonic A, Katalinic V, Milos M (2004) Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem 85:633–640
92. Pernice R, Borriello G, Ferracane R, Borrelli RC, Cennamo F, Ritieni A (2009) Bergamot: a source of natural antioxidants for functionalized fruit juices. Food Chem 112:545–550
93. Schieber A, Stintzing FC, Carle R (2001) By-products of plant food processing as a source of functional compounds – recent developments. Trends Food Sci Technol 12:401–413

94. Siró I, Kápolna E, Kápolna B, Lugasi A (2008) Functional food. Product development, marketing and consumer acceptance-A review. Appetite 51(3):456–467
95. Yoshikawa T, Naito Y (2002) What is oxidative stress? J Japan Med Assoc 45(7):271–276
96. Charles W, Paul K (2009) Sugar metabolism. Microsoft® Encarta® 2009 [DVD]. Microsoft Corporation, Redmond
97. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazura M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84
98. Mitchell RN, Cotran RS (2003) Cell injury, adaptation and death. In: Kumar V, Cotran RS, Robbins SL (eds) Robbins basic pathology, 7th edn. Harcourt (India) Pvt Ltd., New Delhi, pp 3–33
99. Rajeshwari CU, Andallu B (2011) Oxidative stress in NIDDM patients: influence of coriander (*Coriandrum sativum*) seeds. Res J Pharm Biol Chem Sci 2(1):31–41
100. Aissaoui A, Zizi S, Israili ZH, Lyoussia B (2011) Hypoglycemic and hypolipidemic effects of *Coriandrum sativum* L. in Meriones shawi rats. J Ethnopharmacol 137:652–661
101. Deepa B, Anuradha CV (2011) Antioxidant potential of *Coriandrum sativum* L. seed extract. Indian J Exp Biol 49:30–38
102. Eidi M, Eidi A, Saeidi A, Molanaei S, Sadeghipour A, Bahar M, Bahar K (2009) Effect of Coriander Seed (*Coriandrum sativum* L.) Ethanol Extract on Insulin Release from Pancreatic Beta Cells in Streptozotocin-induced Diabetic Rats. Phytother Res 23:404–406
103. Gray AM, Flatt PR (1999) Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant *Coriandrum sativum* (coriander). Br J Nutr 81:203–209
104. Redmond WA (2009) Liver. Microsoft® Encarta® 2009 [DVD]. Microsoft Corporation, Redmond
105. Samojlik I, Lakic N, Mimica-Dukic N, Dakovic-Svajcer K, Bozin B (2010) Antioxidant and hepatoprotective potential of essential oils of coriander (*Coriandrum sativum* L.) and caraway (Carum carvi L.) (Apiaceae). J Agric Food Chem 58:8848–8853
106. Kassem SS, Abdel-Kader MM, Al-Sayed EM, El-Din S, El-Hawary MHAZ, Haggag MM (2014) Modulatory effects of aerial parts of *Coriandrum sativum* L. On carbon-tetrachlorid induced hepatorenal toxicity. Global Veterinaria 12(4):523–531
107. Moustafa AHA, Ali EMM, Moselhey SS, Tousson E, El-Said KS (2012) Effect of coriander on thioacetamide-induced hepatotoxicity in rats. Toxicol Ind Health 30(7):621–9
108. Sreelatha S, Padma PR, Umadevi M (2009) Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced hepatotoxicity in rats. Food Chem Toxicol 47:702–708

Jackfruit (*Artocarpus heterophyllus*): Biodiversity, Nutritional Contents, and Health

76

Shrikant Baslingappa Swami and Sandeep Baban Kalse

Contents

Abstract

Jackfruit (*Artocarpus heterophyllus Lam.*) is an ancient fruit and is consumed either raw or processed into different value-added products. Jackfruit seeds are normally discarded or steamed and eaten as a snack or used in some local dishes;

S. B. Swami (✉) · S. B. Kalse
Department of Post Harvest Engineering, Post Graduate Institute of Post Harvest Management, Killa Roha, Maharashtra, India
e-mail: swami_shrikant1975@yahoo.co.in; sandeep.kalse@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_87

seed flour is used in some biscuit factories in various bakery products, etc. The use of jackfruit bulbs, seeds, and its other parts has also been reported since ancient times for their therapeutic qualities. The health benefits of jackfruit have been attributed to its wide range of physicochemical applications. It contains high levels of carbohydrates, protein, starch, calcium, vitamins, free sugar (sucrose), fatty acids, ellagic acid, and amino acids like arginine, cystine, histidine, leucine, lysine, methionine, theanine, and tryptophan. The jackfruit has diverse medicinal uses especially antioxidant, anti-inflammatory, antimicrobial, anticancer, and antifungal activity. This chapter describes an overview of the functional, medicinal, nutritional, and health aspects of jackfruit.

Keywords
Jackfruit · Antioxidant · Jacalin

Abbreviations

Caf-A	Caffeic acid
FA	Ferulic acid
FDA	Food and Drug Administration
FDT	Fast-dissolving tablets
GA	Gallic acid
GL	Glycemic load
HbA1c	Hemoglobin A1c
HDL-C	High-density lipoprotein cholesterol
IAUC	Incremental area under curve
LDL-C	Low-density lipoprotein cholesterol
NO	Nitric oxide
NSS	Normal serving size
TA	Tannic acid
UDL	Under detection limit

1 Introduction

Jackfruit (*Artocarpus heterophyllus*) belongs to the Moraceae family, native to India and seen abundant in Western Ghats, a biodiversity spot of India [1–5]. Besides India, jackfruit is commonly grown in home gardens of tropical and subtropical countries especially in Sri Lanka, Bangladesh, Burma, Philippines, Indonesia, Thailand, Malaysia, and Brazil [2, 6–10]. In India, it is widely distributed in the states of Assam, West Bengal, Uttar Pradesh, Maharashtra, Kerala, Tamil Nadu, and Karnataka [5] and considered to be the "poor man's food" [1, 4]. It is a medium-size tree typically reaching 28–80 ft. in height that is easily accessible for its fruit. The fruit is borne on side branches and main branches of the tree. The average weight of a fruit is 3.5–10 kg, and sometimes a fruit may reach up to 25 kg. The ripe jackfruits consisted 29% pulp, 12% seeds, and 54% rind [11]. Figure 1 shows the various parts of a

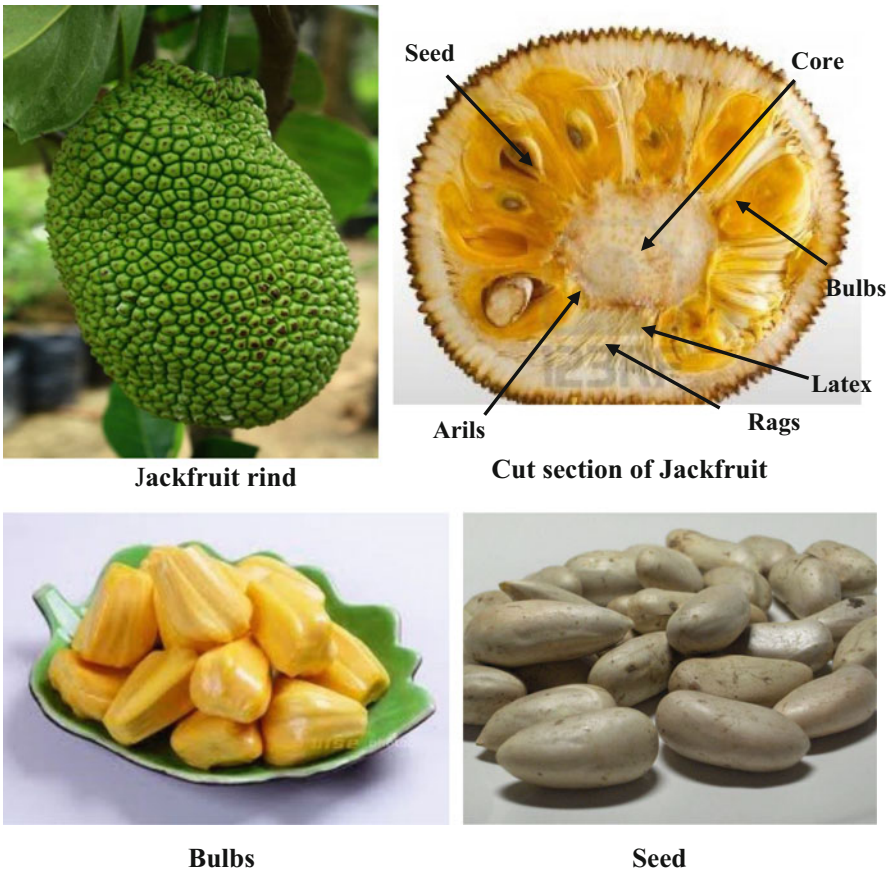


Fig. 1 Different parts of jackfruit

jackfruit. The jackfruit seed is 2–3 cm long and 1–2 cm in diameter, and each fruit contains 100–500 seeds.

There are two varieties of jackfruit in India: one is small, fibrous, soft, and mushy, with sweet carpels and a texture like that of raw oysters, and is called *Barka*, and the other variety is crisp and crunchy, but not very sweet, and is called *Kapa* [12]. In Bangladesh, *Khaja*, *Gala*, and *Durasha* are the main varieties [13]. *Khaja* is characterized by its hard and crispy bulb; *Gala* is soft and juicy and mostly melting bulb. On the other hand, *Durasha* is an intermediate between *Khaja* and *Gala* [14].

Jackfruit is reported to possess many medicinal properties. The phenolic compounds isolated from jackfruit are reported to exhibit anti-inflammatory effect [4]. The prenylflavonoids present in jackfruit had shown strong antioxidant properties [43] and is expected to act against lipid peroxidation of biological membranes [15]. The hot water extract of mature leaves are utilized in *Ayurvedic* treatment for

hyperglycemia and diabetes [4]. The flavonoids present in the extract have been identified to be responsible for the nontoxic hypoglycemic action [16]. Lectins present in the seeds have shown antifungal properties, while the crude methanolic extracts from root bark and stems have shown broad-spectrum antibacterial activity [17].

Resveratrol (trans-3,5,4-trihydroxystilbene, RES) is one of the polyphenols naturally present in jackfruit [18, 19] and is well-known for its health-promoting activities of antioxidant, cardioprotect, and anti-inflammatory [19]. Compounds that can inhibit angiogenesis have great potential for cancer treatment [20]. Jackfruit seeds contain secondary metabolites that display anticancer effects, especially anti-angiogenesis, and belong to the flavonoid group [21]. The jackfruit seed starch as superdisintegrant is suitable for the preparation of fast-dissolving tablets [22]. Extracts of jackfruit pulp show considerable anti-inflammatory activity by suppressing the production of nitric oxide (NO) and prostaglandin E2 (PGE2) [23], its leaf extracts also give remarkable antioxidant activity [43] and exhibit attenuation on hyperglycemia and hyperlipidemia [24]. Its wood was reported to be used as antioxidant, antiaging, anti-inflammatory, and skin care agents [25]. The leaf, root, bark, and fresh fruit of this plant have been certified to contain various compounds like flavonoids, phenolic acids, organic acids, carotenoids, stilbenes, triterpenes, and sterols, especially prenylflavonoids [2, 26, 27].

Jackfruit is also used for further processing. For instance, jackfruit leather and jackfruit chips can be made from dried jackfruit pulp [28]. Pureed jackfruit is also manufactured into baby food, juice, jam, jelly, and base for cordials [29]. Jackfruits are made into candies, fruit-rolls, marmalades, and ice cream [80]. Other than canning, advances in processing technologies too have pushed toward more new products [30]. Freeze-dried, vacuum-fried, and cryogenic processing are new preservation methods for modern jackfruit-based products. Various parts of the jackfruit tree have been used in medicine, and its wood is an important source in timber industries [29].

Nowadays, it is widely accepted that the beneficial health effects of fruits and vegetables in the prevention of disease are due to the bioactive compounds they contain [31]. In recent years, there has seen increased interest on the part of consumers, researchers, and the food industries into how food products can help maintain health; and the role that diet plays in the prevention and treatment of many illnesses has become widely accepted. This chapter describes an overview of the biodiversity of the tree and functional, medicinal, nutritional, and health aspects of jackfruit and its various parts.

2 Biodiversity

Jackfruit is an important crop of India, Burma, China, Sri Lanka, Malaysia, Indonesia, Thailand, and the Philippines. It is also grown in parts of Africa, Brazil, Suriname, the Caribbean, Florida, and Australia. Jackfruit has been cultivated

since prehistoric times and has been introduced to many Pacific islands since post-European contact and is of particular importance in Fiji [32].

Despite numerous advantages, the popularity of jackfruit as a commercial crop is very poor owing to wide variations in fruit quality, the long seed dormancy, and the widespread belief that excessive consumption of jackfruit bulbs leads to certain digestive ailments [33].

The jackfruit has innumerable types in the Western Ghats with varying fruit characteristics. The types differ among themselves in the shape and density of spikes on the rind, bearing, size, shape, latex, flake size, flake color, quality, and period of maturity. Innumerable variations in bulb sweetness, acidity, flavor, and taste are observed in jackfruit growing areas. Such a wide diversity among jackfruit types in Western Ghats offers tremendous scope for improvement of this crop by selection [33, 34]. Due to crosspollination and predominance of seed propagation over a long period of time, there is high degree of variability within the species.

Jagadeesh and others [1] selected 95 jackfruit types from the hilly (65 types) and coastal (30 types) zones of Karnataka situated in Western Ghats, a biodiversity spot of India. The Western Ghats falling in two agroclimatic regions of the state, viz., hilly and coastal, studied the physicochemical characters at edible ripe stage. It was apparent that the majority of selections (19), irrespective of their agroclimatic zone, were grouped in cluster "A," whereas clusters "B," "C," "D," and "E" were mono-tree type. It was found that the genetic drift and natural selection under different environmental conditions could cause considerable diversity than geographical distance. Highest values for TSS (34.33 °B), carotenoids (0.857 mg/100 g), total sugar (31.33%), and reducing sugars (13.37%) were observed in cluster "B," while cluster "D" exhibited highest values for TSS/acid ratio (123.29). Single-bulb mass (26.42 g) was the highest in cluster "A" with the majority of selections, whereas the solitary cluster "C" showed the highest edible portion (37.81%). With regard to fruit mass (14.86 kg) and flake mass (5.62 kg), cluster "C" exhibited the highest value, while titratable acidity (0.768%) was found highest in cluster "E." They conclude that jackfruit, being indigenous and a highly cross-pollinated crop, displays vast diversity in the Western Ghats of India. This wide range of variation existing in nature aids in the selection of superior desirable types.

2.1 Genetic Diversity

Jackfruit is a tetraploid; its somatic chromosome number is (4n) 56. Therefore, the basic chromosome number is 14 [35]. Only one study until now by Schnell and others [36] looked at the genetic diversity of 26 accessions from different parts of the world, using amplified fragment length polymorphism (AFLP) markers, and provided an actual picture of diversity and genetic relatedness in jackfruit. This study included only two accessions from India, and they scored a small number of markers (87), of which 92 (49.2%) were found to be polymorphic. The most recent study by Azad and others [37] looked at isozyme variation in jackfruit in Bangladesh. A total of 50 accessions were evaluated for four enzyme systems, and isozyme patterns were

determined on the basis of number and position of bands. They discovered that morphological traits such as weight, length, girth of the fruits, and percentage of pulp correlated poorly with environmental factors, suggesting that these characters are more likely genetically controlled. However, isozyme markers are also known to be affected by both environment and posttranslation modification, and their practical use is limited [38].

Jackfruit shows a considerable range of variation in morpho-agronomic characters, and this may be because jackfruit trees are cross-pollinated and are mostly propagated by seed. A considerable variation between trees has been observed for the traits such as growth habit, canopy structure, leaf size, fruit shape, size, color, fruit bearing (age and seasonality), and maturity (Table 1). The International Plant Genetic Resources Institute (IPGRI; now Biodiversity International) in 2000 issued a list of descriptor and descriptor states both for characterization of germplasm and for further evaluation. Variation also exists in density, size, and shape of spines on rind, fruit-bearing sensory quality, flesh types, sweetness, flavor, and taste [39].

3 Nutritional Characteristics of Jackfruit

Studies have proved that the nutritional and phytochemical composition among jackfruit varies depending on the cultivar as well as region [2, 30, 39–41]. It is a good source of vitamins (A, C, thiamine, riboflavin, niacin) and minerals (calcium, potassium, iron, sodium, zinc) (Swami and others) [12, 30, 39–41]. Protein and carbohydrate concentration also varied in seeds across India were some varieties contain 6.8% of protein in seeds [2]. The nutritional characteristics of jackfruit bulb, seed, and other part are discussed below.

3.1 Jackfruit Bulbs

Jackfruit is heavy and bulky, and actual recovery of bulbs or edible portion varies from 20 to 25% which is easily digestible. A 100 g portion of edible raw jackfruit provides about 95 calories and is a good source of the antioxidants and vitamin C, providing about 13.7 mg. The fruit is also rich in vitamin B_6, potassium, calcium, and iron.

The bulb of ripe jackfruit is eaten fresh and used in fruit salads. It possesses high nutritional value; every 100 g of ripe fruit pulp contains 18.9 g carbohydrate, 1.9 g protein, 0.1 g fat, 77% moisture, 1.1 g fiber, 0.8 g total mineral matter, 20 mg calcium, 30 mg phosphorus, 500 mg iron, 540 IU vitamin A, 30 mg thiamin, and 84 calories [33]. The jackfruit also contains useful antioxidant compounds [15]. Table 2 shows the composition of jackfruit edible portion of young fruit and ripe fruit. Figure 2 shows the nutraceutical characteristics of jackfruit bulb (pulp) and its effects on various diseases. Figure 3 shows principal functional and medicinal effects of jackfruit. The jackfruit could be considered a functional food because it has valuable compounds in different parts of the fruit that display functional and medicinal effects.

Table 1 Variation in morpho-agronomic characters [40]

Characteristic	Range of variation
Tree habit	Open, spreading, low spreading, sparse upright
Tree growth rate	Fast, moderate, slow
Canopy	Dense, mostly dome-shaped, slightly pyramidal, or flat-toped. It ranges from 3.5 to 6.7 m
Leaf shape	Elliptic, elliptic-obovate, obovate, oblong, lanceolate, oval
Leaf size	4–25 cm in length; 2–12 cm in width
Leaf petiole	1.2–4.0 cm long
Fruit maturity	Variable
Fruiting seasons	Variable
Fruit shape	Oblong, ellipsoid, triangular, spheroid, claviform, round
Number of fruits/tree	15–1450
Fruit weight (kg)	1.2–22.0
Fruit thickness	Thin, medium, thick
Fruit texture	Fibrous, firm, coarse, melting, crisp
Seed shape	Oblong, ellipsoid, irregular, reniform, elongated, spheroid
100 – seed weight (g)	250–1230
Flakes aroma	Mild, strong
Flakes color	Creamy white, light yellow, deep yellow, yellow, reddish, red golden
Flakes texture	Crisp, coarse, fibrous/coarse, fibrous, smooth
Quantity of fiber	Scarce, medium, abundant
Juiciness of pulp	Very juicy, juicy, medium juicy, less juicy, dry
Fruit weight (kg)	1.2–22.0
Fruit length (cm)	20.5–60.6
Fruit diameter (cm)	16.4–29.5
Fruit girth (cm)	50.5–95.8
No. of bulbs/fruit	24.2–580.2
Pulp (%)	18.3–60.9
Seed (%)	2.6–23.1
Rachis (%)	1.5–21.4
Rind (%)	20.6–72.0
TSS Brix (°)	13.8–25.3

3.1.1 Carotenoid Composition

The jackfruit bulb consists of 107.98 total carotenoids [27]. Jackfruit consists all-trans-β-carotene which is an important antioxidant for human health [40]. Jackfruit contains carotenoids that are important for prevention of several chronic degenerative diseases, such as cancer, inflammation, cardiovascular disease, cataract, and age-related macular degeneration [44, 45]. The total carotenoids present in jackfruit are shown in Table 3.

Table 2 Composition of jackfruit bulb (100 g edible portion) [30, 41, 42]

Sr.No	Composition	Young fruit	Ripe fruit
A	**Proximate analysis**		
1	Water (g)	76.2–85.2	72.0–94.0
2	Protein (g)	2.0–2.6	1.2–1.9
3	Fat (g)	0.1–0.6	0.1–0.4
4	Carbohydrate (g)	9.4–11.5	16.0–25.4
5	Fiber (g)	2.6–3.6	1.0–1.5
6	Total sugars (g)	–	20.6
B	**Minerals and vitamins**		
1	Total minerals (g)	0.9	0.87–0.9
2	Calcium (mg)	30.0–73.2	20.0–37.0
3	Magnesium (mg)	–	27.0
4	Phosphorus (mg)	20.0–57.2	38.0–41.0
5	Potassium (mg)	287–323	191–407
6	Sodium (mg)	3.0–35.0	2.0–41.0
7	Iron (mg)	0.4–1.9	0.5–1.1
8	Vitamin A (IU)	30	175–540
9	Thiamine (mg)	0.05–0.15	0.03–0.09
10	Riboflavin (mg)	0.05–0.2	0.05–0.4
11	Vitamin C (mg)	12.0–14.0	7.0–10.0

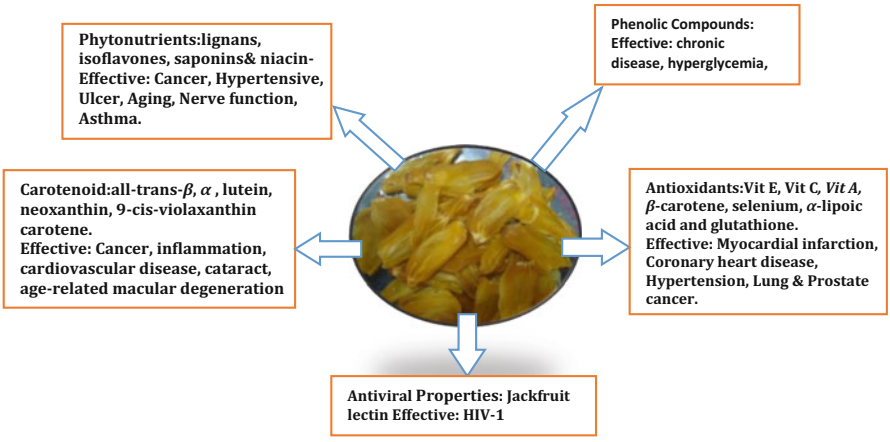


Fig. 2 Nutraceutical characteristics of jackfruit bulb (pulp) and its effect on various diseases

The main carotenoids in jackfruit were all-trans-lutein (24–44%), all-trans-β-carotene (24–30%), all-trans-neoxanthin (4–19%), 9-cis-neoxanthin (4–9%), and 9-cis-violaxanthin (4–10%). Jackfruit is a good source of provitamin A carotenoids, though not as good as papaya [46]. Thus increased consumption of ripe jackfruit could be advocated as part of a strategy to prevent and control vitamin A deficiency.

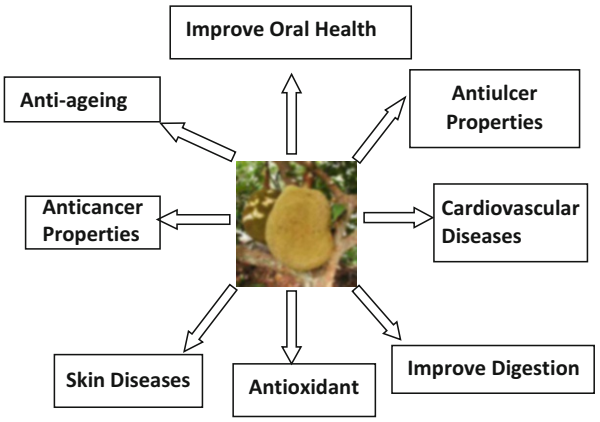


Fig. 3 Principal functional and medicinal effects of jackfruit

Table 3 Concentration (μg/100 g fresh weight) of different carotenoids in jackfruit [27]

Carotenoids	Values	Carotenoids	Values
All-trans-neoxanthin	8.85 ± 5.73	All-trans-zeinoxanthin	1.72 ± 1.20
9-cis-Neoxanthin	6.87 ± 4.25	9-cis-Zeinoxanthin	0.90 ± 1.12
All-trans-neochrome	0.88 ± 1.11	All-trans-α-cryptoxanthin	0.35 ± 0.60
All-trans-luteoxanthin	2.06 ± 0.90	All-trans-β-cryptoxanthin	1.21 ± 0.45
cis-Antheraxanthin	1.12 ± 0.36	15-cis-β-carotene	0.18 ± 0.31
9-cis-Violaxanthin	7.05 ± 5.97	13-cis-β-carotene	2.45 ± 1.40
cis-Luteoxanthin	0.34 ± 0.42	All-trans-α-carotene	1.24 ± 0.93
All-trans-lutein	37.02 ± 20.34	All-trans-β-carotene	29.55 ± 15.46
All-trans-zeaxanthin	0.96 ± 1.20	9-cis-β-carotene	0.79 ± 0.30
Total carotenoids			**107.98 ± 51.46**

3.2 Jackfruit Seed

The jackfruit seeds are around 10–15% of the total fruit weight and have high carbohydrate and protein contents [47]. There are 100–500 seeds in a single fruit. Seeds are normally discarded or steamed and eaten as a snack or used in some local dishes. The fresh seeds cannot be kept for a long time, seed flour can be an alternative product, which can be used in some food products.

The jackfruit seeds are a good source of starch (22%) and dietary fiber (3.19%) [48]. Jackfruit seeds contain lignans, isoflavones, and saponins that are called phytonutrients, and their health benefits are wide-ranging from anticancer to antihypertensive, antiaging, antioxidant, antiulcer, etc. [49].

The jackfruit seeds have medicinal properties. The oval, oblong, or oblong ellipsoid or rounded-shape, light brown color jackfruit seeds are nutritious and rich in potassium, fat, carbohydrates, and minerals. Manganese and magnesium elements have also been detected in seed powder [50]. Table 4 shows the composition of jackfruit seed. Seeds contain two lectins, namely, jacalin and artocarpin. Jacalin has

Table 4 Composition of jackfruit seed (100 g edible portion) [30, 39, 41, 42]

Sr. no.	Composition	Value	Sr. no.	Composition	Value
A	**Proximate analysis**		**B**	**Minerals and vitamins**	
1	Water (g)	51.0–64.5	1	Total minerals (g)	0.9–1.2
2	Protein (g)	6.6–7.04	2	Calcium (mg)	50.0
3	Fat (g)	0.40–0.43	3	Magnesium (mg)	54.0
4	Carbohydrate (g)	25.8–38.4	4	Phosphorus (mg)	38.0–97.0
5	Fiber (g)	1.0–1.5	5	Potassium (mg)	246
6	Total sugars (g)	–	6	Sodium (mg)	63.2
			7	Iron (mg)	1.5
			8	Vitamin A (IU)	10–17
			9	Thiamine (mg)	0.25
			10	Riboflavin (mg)	0.11–0.3
			11	Vitamin C (mg)	11.0

been proved to be useful for the evaluation of the immune status of patients infected with human immunodeficiency virus-1 [40].

Amylose content of jackfruit seed starch was 32% [51]. Jackfruit seed extract was found to inhibit the proteolytic activities of different animal pancreatic preparations effectively [52]. The fresh seed contains crude proteins (606 g), fat (0.4 g), carbohydrates (38.4 g), fiber (1.5 g), ash (1.25–1.50 g), and moisture (51.6–57.77 g), respectively [53].

4 Physicochemical Properties

There have been few studies on physicochemical properties of jackfruit seeds. The physicochemical properties of jackfruit seed is shown in Table 5. Jackfruit seeds are fairly rich in starch [54]. The pasting properties of the jackfruit seed starch against corn and potato starch were studied, and it is reported that the pasting temperature of jackfruit seed starch was higher than those of corn starch and potato starch. The jackfruit seed starch has lower swelling properties because of high amylose content. Jackfruit seed starch was more resistant to heat and mechanical shear and hence less prone to loss viscosity upon holding and shearing. Mukprasirt and Sajjaanantakul [55] also reported that the breakdown viscosity of jackfruit seed starch was lower than that of the commercial starch.

Fat absorption is an important property in food formulations because fats improve the flavor and mouthfeel of foods [57]. The jackfruit flour has 2.8 g/ml fat absorption characteristic. Jackfruit seed flour has a lot of potential in the food industry, especially its uses as thickener and binding agent in the food systems.

Kumar and others [58] studied the proximate compositions of two varieties of jackfruit seeds and reported considerable biochemical difference between the two varieties. The starch content of the seed increases with maturity [59].

Table 5 Some physicochemical and functional properties of jackfruit seed flour [56]

Sr. no.	Particular	Value (% dry matter)
	Physicochemical properties	
1.	Moisture	6.09 ± 0.01
2.	Crude fat	1.27 ± 0.01
3.	Ash	2.70 ± 0.02
4.	Protein	13.50 ± 0.06
5.	Fiber	3.19 ± 0.01
6.	Carbohydrate	79.34 ± 0.06
7.	Energy (Kcal/100 g)	382.79 ± 1.20
8.	pH	5.78 ± 0.01
	Functional properties	
9.	Titratable acidity (as, lactic acid)	1.12 ± 0.03
10.	Water absorption capacity (%)	25.00 ± 1.67
11	Fat absorption capacity (%)	17.00 ± 1.37
12.	Bulk density (g/cm^3)	0.80 ± 0.02
13.	Foaming capacity (%)	25.34 ± 0.02
14.	Foam stability (%)	33.00 ± 0.01
15.	Swelling power (g/g)	4.77 ± 0.10

5 Phytochemical Analysis

Gupta and others [60] analyzed phytochemical content of jackfruit seeds found high quantity of saponins (6.32 ± 0.098 g/100 g). Saponins have been known for their medicinal uses, including antispasmodic activity and toxicity to cancer cells. Some alkaloids function as spasmolytic, anticholinergic, and anesthetic agents. The alkaloid content in jackfruit seeds was found to be 1.16 ± 0.09 g/100 g. Polyphenolics are known to function as antioxidants through a number of mechanisms including radical scavenging by H-donation, prevention of chain initiation by donating electrons, or binding of transition metal ion catalysts. Flavonoids prevent platelet stickiness and hence platelet aggregation.

5.1 Primary Metabolites in Jackfruit Seed

Organic acids control acetic-alkali equilibrium, which affects human health and all the reactions in the body. Table 6 shows the various organic acid content of jackfruit seed kernel (SK) and seed coating membrane (SCM). In addition, they have a disinfecting function, and affect metabolism, etc. By interacting with other substances, organic acids affect the acetic-alkali balance, alkalizing the whole body. As a result, the person's health improves. Also the organic acids are involved in digestion, stimulate the stomach and pancreas, and increase intestine motor function.

Table 6 Organic acid content in jackfruit seed kernel (SK) and seed coating membrane (SCM) [61]

Sr. no.	Compounds (mg/kg dry matter)	SK	SCM
1	Oxalic	649.45 ± 26.38	122.38 ± 12.36
2	Aconitic	650.51 ± 6.93	96.52 ± 6.53
3	Citric	8086.95 ± 807.60	1745.72 ± 120.31
4	Pyruvic	–	59.32 ± 6.25
5	Malic	3539.64 ± 335.53	877.97 ± 137.97
6	Quinic	460.84 ± 10.50	230.38 ± 44.69
7	Shikimic	–	12.95 ± 0.30
8	Acetic	–	84.28 ± 5.82
9	Fumaric	535.88 ± 1.37	26.33 ± 1.47
	Total	**13923.26 ± 1188.30**	**3255.85 ± 335.70**

Oxalic acid that is a normal element in the blood has been reported to have a mean value of 288 mcg of anhydrous oxalic acid/100 ml of blood. Oxalic acid must be available for the immune system to fight the diseases such as cancer and viral, bacterial, and vascular conditions. When oxalic acid falls below an effective level, the immune system can no longer protect the body from various diseases. When the immune system can no longer eliminate abnormal cells, radical cells are allowed to develop and give rise to a detectable tumor.

Amino acids build proteins, and proteins are life-sustaining macronutrients. Table 7 shows the amino acid content in jackfruit seed kernel and seed coating membrane (SCM). When cells need protein, they follow instructions from DNA that define the specific amino acids and the order in which they must connect to build the protein. DNA depends on another macromolecule RNA to make the protein. RNA takes a copy of the code from your DNA, leaves the cell, finds the amino acids, and brings them back to the cell, where they bind into a chain. Each amino acid must be available at the time it's needed or the protein won't be synthesized. When the chain is complete, it twists and folds into a specialized shape. The chemical structure of each amino acid controls the final shape, and the shape determines the function of the protein. Several amino acids produce neurotransmitters, but two well-known examples are the amino acids tryptophan and tyrosine. Tryptophan is available in jackfruit seed in fair quantity which produces serotonin, regulates your moods, and makes the hormone melatonin. Food proteins vary depending on their amino acid content and contain varying concentrations of essential and nonessential amino acids [61].

Fernandes and others [61] identified 67 compounds and reported for the first time in jackfruit seed. Table 8 shows fatty acid content in jackfruit seed kernel (SK) and seed coating membrane (SCM).

The seed kernel is significantly richer in all metabolites. As expected, the accumulation of primary metabolites was higher, organic and amino acids being predominant in jackfruit seed kernel and seed coating material. Phenolic compounds allowed a more clear distinction of the two materials, being mainly accumulated in the seed kernel. Seed kernel and seed coating membrane showed antioxidant capacity.

Table 7 Amino acid content in jackfruit seed kernel (SK) and seed coating membrane (SCM) (mg/kg dry matter) [61]

Amino acids		SK	SCM
A. Essential			
1	Threonine	387.00 ± 1.40	48.20 ± 0.11
2	Valine	290.54 ± 3.62	37.43 ± 0.44
3	Isoleucine	157.01 ± 0.79	11.82 ± 0.29
4	Leucine	396.43 ± 7.61	71.39 ± 0.60
5	Tryptophan	94.69 ± 0.91	8.30 ± 0.14
6	Phenylalanine	210.92 ± 2.56	29.51 ± 0.10
7	Lysine	242.59 ± 0.63	29.04 ± 0.24
8	Histidine	104.90 ± 0.04	32.08 ± 0.25
	Total	**1884.08 (17.56)**	**267.76 ± 2.18**
B. Nonessential			
1	Aspartic acid	247.64 ± 20.35	25.83 ± 0.02
2	Glutamic acid	703.31 ± 30.18	20.39 ± 6.03
3	Asparagine	759.79 ± 14.07	158.01 ± 0.16
4	Glutamine	2670.13 ± 4.00	338.52 ± 0.73
5	Serine	309.86 ± 2.83	26.60 ± 0.13
6	Glycine	154.17 ± 4.81	18.46 ± 0.11
7	Alanine	241.32 ± 2.23	32.08 ± 0.07
8	Proline	2585.93 ± 10.58	194.38 ± 0.52
9	Arginine	1250.50 ± 3.64	63.36 ± 1.26
10	Cysteine	395.73 ± 0.64	147.36 ± 0.28
11	Ornithine	27.74 ± 0.67	9.18 ± 0.02
12	Tyrosine	504.10 ± 2.07	42.70 ± 0.14
	Total	**9850.21 ± 96.07**	**1076.87 ± 9.47**

The jackfruit leaves and stem show the presence of sapogenins, cycloartenone, cycloartenol, β-sitosterol, and tannins; they show estrogenic activity. A root contains β-sitosterol, ursolic acid, betulinic acid, and cycloartenone [62].

6 Health Benefits

The health benefits of jackfruit are still underway. The jackfruit bulb and jackfruit seeds are good sources of protein, starch, and minerals. Jackfruits also contain phytonutrients, i.e., lignans, isoflavones, and saponins, and they have numerous health benefits such as anticancer, antiaging, and antioxidant. Fig. 2 shows the nutraceutical characteristics of jackfruit bulb and its effect on various diseases.

6.1 Anticancer

Angiogenesis is the outgrowth of new blood vessels from preexisting vessels. It commonly occurs during the normal physiological process of blood vessel formation and during cancer growth [63].

Table 8 Fatty acid content in jackfruit seed kernel (SK) and seed coating membrane (SCM) (mg/kg dry matter) [61]

Sr. no.	Fatty acids	SK	SCM
1	Dodecanoic (C12:0)	–	12.69 ± 0.27
2	Tridecanoic (C13:0)	–	1.70 ± 0.06
3	Tetradecanoic (C14:0)	20.17 ± 0.22	53.38 ± 0.48
4	*cis*-10-Pentadecenoic (C15:1*n*-5c)	3.43 ± 0.10	–
5	Pentadecanoic (C15:0)	24.55 ± 0.26	31.93 ± 0.94
6	*cis*-9-Hexadecenoic (C16:1*n*-7c)	21.57 ± 0.41	40.41 ± 0.85
7	Hexadecanoic (C16:0)	836.70 ± 11.94	864.79 ± 15.42
8	*cis*-10-Heptadecenoic (C17:1 *n*-7c)	9.29 ± 0.08	11.17 ± 0.27
9	Heptadecanoic (C17:0)	23.42 ± 0.28	24.14 ± 0.33
10	*cis*-9,12-Octadecadienoic (C18:2*n*-6c)	801.19 ± 10.44	147.59 ± 1.02
11	*cis*-9-Octadecenoic (C18:1*n*-9c)	109.81 ± 2.48	189.42 ± 1.55
12	*trans*-9-Octadecenoic (C18:1*n*-9 t)	17.62 ± 1.92	23.31 ± 0.51
13	Octadecanoic (C18:0)	181.62 ± 4.52	254.75 ± 1.80
14	*cis*-9,12,15-Octadecatrienoic (C18:3*n*-3c)	2.17 ± 0.33	3.91 ± 0.11
15	Eicosanoic (C20:0)	74.71 ± 3.79	78.52 ± 1.03
16	Heneicosanoic (C21:0)	24.13 ± 0.68	20.79 ± 0.60
17	Docosanoic (C22:0)	112.41 ± 4.96	91.12 ± 1.58
18	Tricosanoic (C23:0)	20.65 ± 1.06	25.60 ± 0.07
19	Tetracosanoic (C24:0)	64.62 ± 4.30	85.98 ± 0.63
	TOTAL	**2348.06 ± 47.77**	**1961.20 ± 27.52**

The recent studies show all phytonutrients in jackfruit bulb shave anticancer benefits. The main role of these nutrients is to help prevent the harmful free radicals that have been known to develop cancer and many other chronic diseases. The phytonutrients prevent the very initial stage of cancer cell formation. Saponins are also strong anticancer agents. According to a study, saponins show colon cancer preventative properties. These phytonutrients have been found to induce mitotic arrest in the case of leukemia cells. The study also found that it helped in some cases to cause remission. Saponins were found to react to the outer layers of cancer cells. They bound the cells and prevented their further growth [64]. Swastika and others [21] reported that the effective dose of jackfruit seed methanolic extract for angiogenesis inhibition is 35.00 mg/ml.

Phytoestrogens are naturally occurring polycyclic phenols found in certain plants that may, when ingested and metabolized, have weak estrogenic effects. Two important groups of phytoestrogens that are present in jackfruit pulp are isoflavones and lignans (Swami and others) [12]. According to studies, these nutrients help in reducing the risk of endometrial cancer.

Jackfruit is rich in fiber. It also has a unique sticky form. Both these properties combine together to work as a great colon cleanser. It helps in removing toxins from your digestive tract. This further helps in reducing the risk of colon cancer. Three phenolic anticancer compounds of jackfruit were characterized as

artocarpesin [5,7,2′,4′-tetrahydroxy-6-βmethylbut-3-enyl) flavones] [65], norartocarpetin (5,7,2′,4′-tetrahydroxyflavone), and oxyresveratrol [trans-2,4,3′,5′tetrahydroxystilbene] [66]. Gowri and others [66] also reported that the reactive oxygen species (ROS) production is a common feature of tumor promotion. The Cressa critica aqueous extract (CCAE) showed higher antioxidant activity than single plant extracts of *P. zeylanica* (45%) [67], *L. acidissima* (19%) [68], and *A. heterophyllus* (36%) [69].

6.2 Diabetics

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin action, insulin secretion, or both. The most common type of diabetes mellitus is type 2 diabetes mellitus, which accounts for 85–95% of all cases and constitutes a major public health problem [70]. Hot water extract of mature jack leaves is recommended by Ayurvedic and traditional medical practitioners as a treatment for diabetes mellitus (Fernando et al. 1991 [71]. It is already indicated that an extract of jackfruit improves the glucose tolerance in normal human subjects and diabetic patients [72]. The leaves and stem show the presence of sapogenins, cycloartenone, ß-sitosterol, and tannins [73]. Jackfruit contains vitamin A, vitamin C, thiamin, riboflavin, niacin, calcium, potassium, iron, manganese, and magnesium among many other nutrients. It is good for diabetes as they improve insulin resistance.

Ajaiya Kumar and others [74] reported that consuming 100 g of the jackfruit meal per day for 4 months leads to quantitative reduction in fasting blood glucose (FBG), postprandial blood glucose (PBG), and hemoglobin A1c (HbA1c) compared with the baseline. The HbA1c decreased by 13.59%, FBG by 22.68%, and PBG by 25.69%. They have concluded that the dietary supplementation of the jackfruit raw fruit meal preparation has an impact in reducing type 2 diabetes.

Hettiaratchi and others [75] studied Nutritional assessment of a jackfruit meal. The total energy contribution of the jackfruit meal is 1370 kJ. Jackfruit meal provides 20% of daily energy requirement of a moderately active individual. Jackfruit seeds contained high amount of resistant starch (RS) (undigestible starch). Resistant starch is categorized into four types (RS_1–RS_4) [76], and jackfruit seeds may contain RS_1 type. The undigestible starch escapes digestion in the small intestine, passes into the colon, and is reported to act like dietary fiber (Hettiaratchi and others) [75]. The postprandial glycemic response and glycemic index (GI) of the jackfruit meal were determined. Jackfruit meal elicited a low GI (Table 9). This is the first reported data on GI of a jackfruit meal in spite of having 2487 data on GI of different foods in the recent "International Tables of Glycaemic Indices and Glycaemic Load Values" [77]. Jackfruit has beneficial nutritional parameters and a low GI. This could be due to the collective contributions of dietary fiber, slowly available glucose, intact starch granules in seeds, and influence of different sources of carbohydrates. Table 10 shows phenolic acids in different parts of jackfruit.

Table 9 Nutritional parameters of jackfruit flesh, seed, meal, and the standard [75]

Parameter	Jackfruit flesh	Jackfruit seeds	Jackfruit meal	Standard
Carbohydrate	10.0 ± 0.3	21.9 ± 0.8	50 g	50 g
Insoluble dietary fiber	1.5 ± 0.1	7.9 ± 0.5	13.5	0.8
Soluble dietary fiber	1.1 ± 0.1	3.2 ± 0.3	6.5	2.4
Total dietary fiber	2.6	11.1	20.0	3.2
Protein	0.9	4.7	6.8	8.2
Fat	0.8 ± 0.1	1.3 ± 0.3	11.5	3.2
Resistant starch	0.3	8.0	5.2	0.7
Slowly available glucose %	17%	33%	30%	16%
Amylose	29	54	31	15
Glycemic index (SEM)	–	–	75 ± 11	100
IAUC (SEM)	–	–	132 ± 19	181 ± 18
GL (NSS)	–	–	13	20

Table 10 HPLC analysis of various phenolic acids in different parts of jackfruit [54]

Plant parts	Phenolic acids (μg/g fresh wt.)			
	TA	**GA**	**FA**	**Caf – A**
Raw fruit skin	6.70 ± 0.05	22.73 ± 2.04	4.64 ± 0.02	UDL
Ripe fruit skin	5.73 ± 0.04	12.08 ± 1.03	13.41 ± 1.2	UDL
Raw fruit flesh	4.87 ± 0.05	9.70 ± 0.09	8.04 ± 0.07	UDL
Ripe fruit flesh	5.24 ± 0.06	19.31 ± 1.8	2.66 ± 0.06	UDL
Raw fruit pulp of seed	2.29 ± 0.01	11.05 ± 1.02	2.16 ± 0.05	UDL
Ripe fruit pulp of seed	UDL	6.26 ± 0.04	2.56 ± 0.02	UDL
Raw fruit seed	6.59 ± 0.07	11.3 ± 1.6	2.38 ± 0.01	2.84 ± 0.02
Ripe fruit seed	2.21 ± 0.01	11.30 ± 1.07	2.71 ± 0.01	UDL

6.3 Immune System

Jacalin, the major protein from the *Artocarpus heterophyllus* seeds, is a tetrameric two-chain lectin combining a heavy chain of 133 amino acid residues with a light β chain of 20–21 amino acid residues [78]. Jacalin's uniqueness in being strongly mitogenic for human CD4 + T lymphocytes has made it a useful tool for the evaluation of the immune status of patients infected with human immunodeficiency virus HIV-1 [79].

6.4 Improve Digestion

The presence of high fiber (3.6 g/100 g) in the jackfruit prevents constipation and helps in smooth bowel movements. These fibers also offer protection against colon mucous membrane by removing or driving away the carcinogenic.

6.5 Cardiovascular Health

One of the major risk factors for the development of coronary heart disease is dyslipidemia, which is mainly characterized by elevated levels of low-density lipoprotein cholesterol (LDL-C) and/or reduced high-density lipoprotein cholesterol (HDL-C) [81].

Epidemiological studies have shown that high concentrations of serum total cholesterol and LDL-C are independent risk factors for cardiovascular disease [82] and could produce atherosclerosis. Atherosclerosis, a major degenerative disease of the arteries, involves a series of inflammatory and oxidative modifications within the arterial wall [83]. Oxidative excess in the vasculature reduces levels of the vasodilator nitric oxide, causes tissue injury, promotes protein oxidation and DNA damage, and induces proinflammatory responses [84]. Oxidative stress induces inflammation by acting on the pathways that generate inflammatory mediators like adhesion molecules and proinflammatory cytokines [85].

6.6 Fast-Dissolving Tablets

The major storage carbohydrate in plants is starch. The annual worldwide production of starch is 66.5 million tons (FAOSTAT) [86]. Growing demand for starches in the industry has created interest in new sources of this polysaccharide, such as leaves, legume seeds, and fruits [87]. It has immense industrial use in the manufacture of products such as food, textile, paper, adhesives, and pharmaceuticals. Starch can also serve as a thickening, gelling, and film-forming properties [88, 89].

Jackfruit seed cotyledons are fairly rich in starch and protein. The recent investigation shows that the jackfruit seed starch has potential in pharmaceutical industries. The starches extracted from jackfruit seeds are used as superdisintegrants for the formulation of fast-dissolving tablets (FDT).

The FDT technology makes tablets dissolve or disintegrate in the mouth without additional water intake. The FDT formulation is defined by the Food and Drug Administration (FDA) as "A solid dosage form containing medical substances whish disintegrates rapidly, usually within a seconds, when placed upon the tongue." Fast-dissolving tablets are also called mouth-dissolving tablets, melt-in-mouth tablets, orodispersible tablets, rapidmelts, porous tablets, quick dissolving, etc. [90]. The basic approach in the development of FDT is the use of superdisintegrants, which provide instantaneous disintegration of tablet after putting on tongue, thereby releasing the drug in saliva [91]. The fast-dissolving tablets are rapidly dissolved or disintegrate by the use of superdisintegrants.

Vidyadhara and others [22] reported as Irbesartan (IRB), which is anangiotensin II type, receptor antagonist, is selected as a model drug. IRB and FDT formulations that contained various concentrations of jackfruit starch extracts and CCS (croscarmellose sodium) were prepared by wet granulation technique using IPA (Isopropyl alcohol) as granulating fluid. The evaluated pre-compression parameters indicated that the granules exhibited good flow properties. In vitro dissolution

studies were performed on all prepared matrix tablets using the USP apparatus II with 900 mL of 0.1 N HCl. From the results of dissolution studies, it was observed that the type of starch as superdisintegrant and the proportion of superdisintegrant have considerably influenced the dissolution parameters of various formulations. The tablets prepared from jackfruit seed starch as superdisintegrant were found to be suitable for preparation of fast-dissolving tablets.

Jackfruit is well known to have antibacterial property against 24 species of bacteria [92]. A jackfruit lectin, i.e., jacalin, inhibits DNA viruses such as herpes simplex virus type II (HSV-2), varicella-zoster virus (VZV), and cytomegalovirus (CMV) [93].

The jackfruit could be considered a functional food because it has valuable compounds in different parts of the fruit that display functional and medicinal effects (Fig. 2).

"Functional foods" are those that provide more than simple nutrition; they supply additional physiological benefit to the consumer. Because dietary habits are specific to populations and vary widely, it is necessary to study the disease-preventive potential of functional micronutrients in the regional diets.

6.7 Dental Health

In jackfruit tree, latex or resin are found on the trunk of tree as well as the fruit. All parts of jackfruit tree contain sticky white latex which produced from special secretory cells called laticifers. Latex is an aqueous emulsion containing many ingredients, for instance, lipids, rubbers, resins, sugars, and proteins including proteolytic enzymes [94].

Rao and others [95] reported that the jackfruit latex extract which is rich in flavonoids and alkaloids was checked for antibacterial and antifungal properties which shows fairly well and significant comparison with standard antibacterial and antifungal drugs. They concluded that this information gives about the several important uses of jackfruit latex or resin, or both can be utilized as the cementing medium, irrigation solution (washing of a body cavity or wound by a stream of fluid), denture cleaning solution, resin, and other future dental filling material in terms of cost-effectiveness.

7 Conclusion

There is a need for commercial utilization of the jackfruit in developing countries and can serve as a possible alternative of many vitamins in the body. An activity of certain phytochemicals along with their antioxidant properties further supports the cause of commercial utilization of the fruit. The antioxidant constituents present in the fruits play important role in scavenging free radicals and reactive oxygen species which are responsible for a number of human disorders. The jackfruits and fruit

products hold potential in the diet as they possess not only pleasant taste but also source of naturally and readily available source of instant energy.

In Ayurveda the jackfruit is used as a cooling tonic and pectorial, roots in diarrhea and fever, leaves to activate milk in women and animals, as a source to treat antisyphilic and vermifuge, leaf ash applied to ulcers wounds and the warmed leaves have healing properties if pasted on the wounds. The richness of jackfruit in bioactive natural metabolites encourages their consumption. Furthermore, the aqueous extracts activity suggests that it may be useful for food and pharmaceutical industries. The valued jackfruit material, which nowadays is largely discarded by the population, might have an important economic impact for the producers.

References

1. Jagadeesh SL, Reddy BS, Basavaraj N, Swamy GSK, Kirankumar G (2007) Inter tree variability for fruit quality in jackfruit selections of Western Ghats of India. J Sci Hortic 112(4):382–387
2. Baliga MS, Shivashankara AR, Haniadka R, Dsouza J, Bhat HP (2011) Phytochemistry, nutritional and pharmacological properties of *Artocarpusheterophyllus Lam* (jackfruit): a review. Food Res Int 44(7):1800–1811
3. Reddy BMC, Patil P, Kumar SS, Govindaraju LR (2014) Studies on physico-chemical characteristics of jackfruit clones of South Karnataka. Karnataka J Agric Sci 17(2):279–282
4. Prakash O, Kumar R, Mishra A, Gupta R (2009) *Artocarpusheterophyllus* (jackfruit): an overview. Pharmacogn Rev 3(6):353–358
5. Wangchu L, Singh D, SK M (2013) Studies on the diversity and selection of superior types in jackfruit (*Artocarpusheterophyllus Lam.*). Genet Resour Crop Evol 60(5):1749–1762
6. Dutta H, Paul SK, Kalita D, Mahanta CL (2011) Effect of acid concentration and treatment time on acid-alcohol modified jackfruit seed starch properties. Food Chem 128(2):284–291
7. Siti BZ, Rosma A (2011) *Artocarpus* integer leaf protease: purification and characterisation. Food Chem 129(4):1523–1529
8. Lin KW, Liu CH, Tu HY, Ko HH, Wei BL (2009) Antioxidant prenylflavonoids from *Artocarpus communis* and *Artocarpus elasticus*. Food Chem 115(2):558–562
9. Maia JGS, Andrade EHA, Zoghbi MDGB (2004) Aroma volatiles from two fruit varieties of jackfruit (*Artocarpusheterophyllus Lam.*). Food Chem 85(2):195–197
10. Hameed BH (2009) Removal of cationic dye from aqueous solution using jackfruit peel as nonconventional low-cost adsorbent. J Hazard Mater 162(1):344–350
11. Aziz A (2006) Development of an innovative ingredient from jackfruit seed flour in health bakery products. Universiti Sains Malaysia
12. Swami SB, Thakor NJ, Haldankar PM, Kalse SB (2012) Jackfruit and its many functional components as related to human health: a review. Compr Rev Food Sci Food Saf 11:565–576
13. Haque MA (1991) Village and forestry in Bangladesh. Joint Publication of Bangladesh Agricultural University and SAARC Documentation Center, New Delhi
14. Noor F, Rahman MJ, Mahomud MS, Akter MS, Talukder MAI, Ahmed M (2014) Physico-chemical properties of flour and extraction of starch from jackfruit seed. Int J Nutr Food Sci 3(4):347–354
15. Ko FN, Cheng ZJ, Lin CN, Teng CM (1998) Scavenger and antioxidant properties of prenylflavones isolated from *Artocarpusheterophyllus*. Free Radic Biol Med 25:160–168
16. Chandrika UG, Wedage WS, Wickramasinghe SMDN, Fernando WS (2006) Hypoglycaemic action of the flavonoid fraction of *Artocarpus heterophyllus* leaf. Afr J Tradit Complement Altern Med 3:42–50

17. Trindade MB, Lopes JL, Costa SCA (2006) Structural characterization of novel chitin-binding lectins from the genes *Artocarpus* and their fungal activity. Biochim Biophys Acta 1764:146–152
18. Lagouge M, Argmann C, Zachary G, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1a. Cell 127:1109–1122
19. Shen T, Wang XN, Lou HX (2009) Natural stilbenes: an overview. Nat Prod Rep 26:916–935
20. Ribatti D, Gulandris A, Bastaki M, Vacca A, Iurlaro M, Roncali L (1997) New model for the study of angiogenesis and antiangiogenesis in the chick embryo chorioallantoic membrane: the gelatin sponge/chorioallantoic membrane assay. J Vasc Res 34:455–463
21. Oktavia S, Wijayanti N, Retnoaji B (2017) Anti-angiogenic effect of *Artocarpusheterophyllus* seed methanolic extract in *ex ovo* chicken chorioallantoic membrane. Asian Pac J Trop Biomed 7(3):240–244
22. Vidyadhara S, Sasidhar RL, Lakshmi HD, P V, Vijetha K (2017) Studies on jackfruit seed starch as a novel natural superdisintegrant for the design and evaluation of irbesartan fast dissolving tablets. Integr Med Res 6(3):280–291. https://doi.org/10.1016/j.imr.2017.04.001
23. Fang SC, Hsu CL, Yen GC (2008) Anti-inflammatory effects of phenolic compounds isolated from the fruits of *Artocarpusheterophyllus*. J Agric Food Chem 56(12):4463–4468
24. Omar HS, El-Beshbishy HA, Moussa Z, Taha KF, Singab ANB (2011) Antioxidant activity of *Artocarpusheterophyllus Lam*. (Jack fruit) leaf extracts: remarkable attenuations of hyperglycemia and hyperlipidemia in streptozotocin-diabetic rats. Sci World J 11:788–800
25. Nguyen NT, Nguyen MHK, Nguyen HX, Bui NKN, Nguyen MTT (2012) Tyrosinase inhibitors from the wood of *Artocarpusheterophyllus*. J Nat Prod 75(11):1951–1955
26. Arung ET, Yoshikawa K, Shimizu K, Kondo R (2010) Isoprenoid-substituted flavonoids from wood of *Artocarpusheterophyllus* on B16 melanoma cells: cytotoxicity and structural criteria. Fitoterapia 81(2):120–123
27. De Faria AF, de Rosso VV, Mercadante AZ (2009) Carotenoid composition of jackfruit (*Artocarpusheterophyllus*), determined by HPLC-PDA-MS/MS. Plant Foods Hum Nutr 64(2):108–115
28. Nakasone HY, Paull RE (1998) Tropical fruits. CAB International, New York
29. Roy SK, Joshi GD (1995) Minor fruits-tropical. In: Salunkhe DK (ed) Handbook of fruit science and technology. Marcel Dekker, Inc., New York, pp 570–573
30. Narasimham P (1990) Breadfruit and jackfruit. In: Nagy S, Shaw PE, Wardowski WF (eds) Fruits of tropical and subtropical origin. Florida Science Source, Lake Alfred, pp 193–259
31. Galaverna G, Di Silvestro G, Cassano A, Sforza S, Doceana A, Drioli E, Marchelli R (2008) A new integrated membrane process for the production of concentrated blood orange juice: effect on bioactive compounds and antioxidant activity. Food Chem 106:1021–1030
32. Thaman RR, Ali I (1993) Agroforestry on smallholder sugarcane farms in Fiji. In: Clarke WC, Thaman RR (eds) Agroforestry in the Pacifi Islands: Systems for Sustainability. United Nations University Press, Tokyo
33. Sammadar HM (1985) Jackfruit. In: Bose TK, Mitra SK (eds) Fruits of India: tropical and subtropical. Naya Prokash, Calcutta, pp 638–649
34. Guruprasad TR (1981) Studies on systematic selection of jackfruit (*Artocarpus heterophyllus Lam.*) types. M.Sc.(Hort.)Thesis, University Agriculture Science, Bangalore, Karnataka, India
35. Darlington CD, Wylie AP (1956) Chromosome atlas of flowering plants. Allen and Unwin, London
36. Schnell RJ, Olano CT, Campbell RJ, Brown JS (2001) AFLP analysis of genetic diversity within a jackfruit germplasm collection. Sci Hortic 91:261–274
37. Azad AK, Jones JG, Haq N (2007) Assessing morphological and isozyme variation of jackfruit (*Artocarpusheterophyllus Lam.*) in Bangladesh. Agrofor Syst 71:109–125

38. Akashi Y, Fukuda N, Wako T, Masuda M (2002) Genetic variation and phylogenetic relationships in east and south Asian melons, *Cucumis melo L*., based on the analysis of fie isozymes. Euphytica 125:385–396
39. Azad AK (2000) Genetic diversity of jackfruit in Bangladesh and development of propagation methods. Ph.D Thesis, Universityof Southampton, UK. 200 p
40. Haq N (2006) Jackfruit, Artocarpusheterophyllus, Southampton Center for Underutilised Crops, University of Southampton, Southampton. 192 p
41. Arkroyd WR, Gopalan C, Balasubramanuyam SC (1996) The nutritive value of Indian food and the planning of satisfaction diet. September Report Series, New Delhi: Indian Council of medical Research, p 42
42. Gunasena HPM, Ariyadasa KP, Wikramasinghe A, Herath HMW, Wikramasinghe P, Rajakaruna SB (1996) Manual of Jack cultivation in Sri Lanka. Forest Information Service, Forest Department: 48
43. Cadenas E, Packer L (1996) Hand book of antioxidants. Plenum Publishers, New York
44. Krinsky NI, Landrum JT, Bone RA (2003) Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr 23:171–201
45. Stahl W, Sies H (2005) Bioactivity and protective effects of natural carotenoids. Biochim Biophys Acta Mol basis Dis 1740:101–107
46. Chandrika UG, Jansz ER, Warnasuriya ND (2005) Analysis of carotenoids in ripe jackfruit (*Artocarpusheterophyllus*) kernel and study of their bioconversion in rats. J Sci Food Agric 85:186–190
47. Bobbio FO, El-Dash AA, Bobbio PA, Rodrigues LR (1978) Isolation and characterization of the physicochemical properties of starch of jackfruit seeds (*Artocarpusheterophyllus*). J Cereal Chem 55:505–511
48. Hettiarchi UPK, Ekanayake S, Welihinda J (2010) Nutritional assessment of jackfruit (*Artocarpusheterophyllus*) meal. Ceylon Med J 56(2):54–58
49. James O, Friday E (2010) Phytochemical composition, Bioactivity and wound healing potential of euphorbia Heterophylla (euphorbiaceae) leaf extract. Int J Pharm Biomed Res 1(1):54–63
50. Barua AG, Boruah BR (2004) Minerals and functional groups present in the jackfruit seed: a spectroscopic investigation. J Food Sci Nutr 55:479–483. https://doi.org/10.1080/09637480400015810
51. Tulyathan V, Tananuwong K, Songjinda P, Jaiboon N (2002) Some physicochemical properties of jackfruit (Artocarpusheterophyllus lam) seed flour and starch. Sci Asia 28:37–41
52. Bhat AV, Pattabiraman TN (1989) Protease inhibitors from jackfruit seed (*Artocarpus integrifolia*). J Food Sci Technol 14(4):351–365
53. Morton J (1987) Jackfruit (Artocarpusheterophyllus). In: Fruits of warm climates. Creative Resource Systems, Winterville, pp 58–64
54. Singh A, Maurya S, Singh M, Singh U (2015) Studies on the phenolic acid contents in different parts of raw and ripe jackfruit and their importance in human health. Int J Appl Sci Res Rev 2:69–73
55. Mukprasirt A, Sajjaanantakul K (2004) Physico-chemical properties of flour and starch from jackfruit seeds. Int J Food Sci Technol 39(3):271–276
56. Ocloo FCK, Bansa D, Boatin R, Adom T, Agbemavor WS (2010) Physico-chemical, functional and pasting characteristics of flour produced from jackfruits (*Artocarpusheterophyllus*) seeds. Agric Biol J N Am 1(5):903–908
57. Kinsella JE (1976) Functional properties of proteins in food – a survey. Crit Rev Food Sci Nutr 7:219–280
58. Kumar S, Singh AB, Abidi AB, Upadhyay RG, Singh A (1988) Proximate composition of jack fruit seeds. J Food Sci Technol 25:308–309
59. Rahman MA, Nahar N, Mian AJ, Mosihuzzaman M (1999) Variation of carbohydrate composition of two forms of fruit from jack tree (*Artocarpus heterophyllus L*) with maturity and climatic conditions. Food Chem 65:91–97

60. Gupta D, Mann S, Sood A, Gupta RK (2011) Phytochemical, nutritional and antioxidant activity evaluation of seeds of jackfruit (*Artocarpus heterophyllus lam*.). Int J Pharm Bio Sci 2(1):336–345
61. Fernandes F, Ferreres F, Gil-Izquierdo A, Oliveira A, Valentão P, Andrade PB (2017) ccumulation of primary and secondary metabolites in edible jackfruit seed tissues and scavenging of reactive nitrogen species. J Food Chem 233:85–95
62. Dayal R, Seshadri TR (1974) Colourless compounds of the roots of Artocarpusheterophyllus. Isolation of new compound artoflavone. Indian J Chem 12:895–896
63. Spano D, Zollo M (2012) Tumor microenvironment: a main actor in the metastasis process. Clin Exp Metastasis 29:381–395
64. Sagar SM, Yance D, Wong RK (2006) Natural health products that inhibit angiogenesis: a potential source for investigational new agents to treat cancer – part 1. Curr Oncol 13:14–26
65. Rowe DP, Garner RJ, Chaudhri SA (1985) *Artocarpus Heterophyllus* jackfruit. The propagation of tropical fruit trees, commonwealth bureau of horticulture and plantation crops. FAO, Rome, pp 269–290
66. Gowri SK, Divya S, Ignacimuthu S, James AP, Albin TF (2017) In vitro and in silico anticancer effect of combined crude acetone extracts of *Plumbago zeylanica L*., *Limonia acidissima L*. and *Artocarpusheterophyllus Lam*. J Synergy 5:15–23
67. Eldhose B, Notario V, Latha MS (2013) Evaluation of phytochemical constituents and in vitro antioxidant activities of *Plumbago indica* root extracts. J Pharm 2:157–161
68. Attarde DL, Chaudhari BJ, Bhambar RS (2011) Phytochemical investigation and in vitro antioxidant activity of extracts from leaves of *Limonia acidissima linn*. (Rutaceae). J Pharm Res 4(3):766–768
69. Thapa N, Thapa P, Bhandari J, Niraula P, Shrestha N, Shrestha BG (2016) Study of phytochemical, antioxidant and antimicrobial activity of *Artocarpusheterophyllus*. Nepal J Biotechnol 4:47–53
70. Cheplick S, Kwon YI, Bhowmik P, Shetty K (2010) Phenolic linked variation in strawberry cultivars for potential dietary management of hyperglycemia and related complications of hypertension. Bioresour Technol 101:404–413
71. Fernando MR, Wickramasinghe SM, Thabrew MI, Ariyaratne PL, Karunanayake EH (1991) J Ethnopharmacol 31:277–282
72. Chackrewarthy S, Thabrew MI, Weerasuriya M, Jayasekera S (2010) Evaluation of the hypoglycemic and hypolipidemic effects of an ethyl acetate fraction of *Artocarpus heterophyllus* (jak) leaves in streptozotocin-induced diabetic rats. Phcog Mag 6:186–190
73. Sathyavathi GV, Gupta AK, Tandon N (1987) Medicinal plants of India. ICMR, New Delhi
74. Ajaiya Kumar SK, Merlin RS, Athira CR (2017) Control of blood glucose level ond glycated Haemoglobin (Hbalc) with dietary jackfruit meal taken by type-II diabetic patients. Int J Sci Res 6(3):43–46
75. Hettiaratchi UPK, Ekanayake S, Welihinda J (2011) Nutritional assessment of a jackfruit (*Artocarpusheterophyllus*) meal. Ceylon Med J 56:54–58
76. Sajilata M, Singhal S, Kulkarni P (2006) Resistant starch – a review. Compr Rev Food Sci Food Saf 5(1):1
77. Atkinson F, Foster-Powell K, Brand-Miller (2008) International tables of glycaemic index and glycaemic load values. Diabetes Care 31:2281–2283
78. Suresh Kumar G, Appukttan PS, Basu DK (1982) D-Galactose – specific lectin from jack fruit seed. J Biosci 4:257–261
79. Pereira-da-Silva GAN, Moreno F, Marques C, Jamur A, Panunto-Castelo MC (2006) Neutrophil activation induced by the lectin KM+ involves binding to CXCR2. Biochim Biophys Acta 1(1):86–94
80. Siddappa GS (1957) Development of products from jackfruit – canned jackfruit, frozen canned jackfruit and jackfruit jam. J Sci Ind Res 11:166–199
81. Esmaillzadeh A, Azadbakht L (2008) Food intake patterns may explain the high prevalence of cardiovascular risk factors among Iranian women. J Nutr 138(8):1469–1475

82. Russo F, Chimienti G, Riezzo G, Pepe G, Petrosillo G, Chiloiro M, Marconi E (2008) Inulin-enriched pasta affects lipid profile and Lp(a) concentrations in Italian young healthy male volunteers. Eur J Nutr 47(8):453–459
83. Fan J, Watanabe T (2003) Inflammatory reactions in the pathogenesis of atherosclerosis. J Atheroscler Thromb 10:63–71
84. Xu S, Touyz RM (2006) Reactive oxygen species and vascular remodeling in hypertension: still alive. Can J Cardiol 22(11):947–951
85. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84
86. FAOSTAT (2002) Database FAO. Food and Agriculture Organisation of the United Nations, Rome, 19.2
87. Betancur-Ancona D, Gallegos-Tintore S, Chel Guerrero L (2004) Wet-fractionation of *Phaseolus lunatus* seeds: partial characterization of starch and protein. Sci Food Agr 84:1193–1201
88. Gebre-Mariam T, Schmidt PC (1996) Isolation and physicochemical properties of Enset starch. Starch-Starke 48:208–214
89. Aldana DLM, Gómez BT, Oca MMM, Ayerdi SGS, Meraz FG, Pérez LAB (2011) Isolation and characterization of Mexican jackfruit (*Artocarpus heterophyllus* L) seeds starch in two mature stages. Starch/Stärke 63:364–372
90. Debjit B, Chiranjib B, Krishna K, Pankaj RMC (2009) Fast dissolving tablet: an overview. J Chem Pharm Res 1(1):163–177
91. Mohanachandran PS, Sindhumol PG, Kiran TS (2011) Superdisintegrants: An overview. Int J Pharm Sci Rev Res 6:105–109
92. Septama AW, Panichayupakaranant P (2015) Antibacterial assay-guided isolation of active compounds from *Artocarpusheterophyllus* heartwoods. Pharm Biol 53(11):1608–1613
93. Wetprasit N, Threesangsri W, Klamklai N, Chulavatnatol M (2000) Jackfruit lectin, properties of mitogenicity and the inhibition of herpesvirus infection. Jpn J Infect Dis 53(4):156–161
94. Fonseca KC, Morais NC, Queiroz MR, Silva MC, Gomes MS, Costa JO (2010) Purification and biochemical characterization of eumiliin from *Euphorbia milii var. hislopii latex*. Phytochemistry 71:708–715
95. Jitendra R, Kalpana S, Shweta S, Kumar MS, Manish B (2014) *Artocarpusheterophyllus* (jackfruit) potential unexplored in dentistry – an overview. Univers J Pharm 03(01):50–55

Sambucus nigra Berries and Flowers Health Benefits: From Lab Testing to Human Consumption

77

Ângelo C. Salvador, Ricardo J. R. Guilherme, Armando J. D. Silvestre, and Sílvia M. Rocha

Contents

Â. C. Salvador
QOPNA, Department of Chemistry, University of Aveiro, Aveiro, Portugal

CICECO- Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal
e-mail: angelomcsalvador@gmail.com

R. J. R. Guilherme · S. M. Rocha (✉)
QOPNA, Department of Chemistry, University of Aveiro, Aveiro, Portugal
e-mail: rjguilherme@ua.pt; smrocha@ua.pt

A. J. D. Silvestre
CICECO- Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal
e-mail: armsil@ua.pt

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_46

Abstract

European elder plant, *Sambucus nigra* L., has been used as an important part of the folk medicine and as multipurpose foods and dietary supplements. The scientific developments in nutrition research, in evaluation of biological activities, and in prospection of bioactive compounds reveal a set of potential health benefits associated with *S. nigra* berries and flowers. This chapter aims to briefly cover aspects linked to elder plant taxonomic classification, geographic distribution, and typologies of products available in the market. Current challenges regarding in vitro and in vivo assays will be also discussed. Despite in vitro studies are often used to predict the health benefits due to the high amount of generated data and their low cost, the use of in vivo assays is crucial to predict such health benefits, as the real contribution of foods, extracts, or specific bioactive compounds to human health is largely modulated by their bioavailability that cannot be accessed at in vitro level. Thus, the main findings regarding the potential health benefits of *S. nigra*-based preparations using in vitro and/or in vivo assays, namely, anti-infective, antioxidant, anti-inflammatory, immunomodulatory, anticancer, and antidiabetic, will be critically revisited. Finally, current challenges related to nutrivigilance systems, which are crucial to ensure consumer health and safety, will be presented.

Keywords

Sambucus nigra L. · Elderflowers · Elderberries · Bioavailability · Health benefits · Nutrivigilance

Abbreviations

ABTS	2,2'-Azino-bis(3-ethyl-benzothialzoline-6-sulfonic acid)
ACN	Anthocyanins
AOx	Antioxidant activity
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
C_{max}	Maximal plasma concentration
COX	Cyclooxygenase
DPPH	2,2-Diphenyl-1-picrylhydrazyl
F	Female
FRAP	Ferric reducing antioxidant power
Glc	Glucose
GSH	Glutathione peroxidase
IL	Interleukins
iNOS	Nitric oxide synthase
LDL	Low-density lipoprotein
M	Male
MDA	Malondialdehyde
NF-*k*B	Nuclear factor *k*B

NO	Nitric oxide
NP	Nanoparticle
ORAC	Oxygen radical absorption capacity
Peo	Peonidin
PI3K	Phosphatidylinositol 3-kinase
PPAR	Proliferator-activated receptor
QR	Quinone reductase
RIP	Ribosomal-inactivating proteins
ROS	Reactive oxygen species
Sam	Sambubioside
SOD	Superoxide dismutase
TE	Trolox equivalents
TNF-α	Tumor necrosis factor α
TPC	Total phenolic content

1 Introduction

Elderberry plant from *Sambucus nigra* L. species is a worldwide-spread plant, from which both flowers and berries have been used on folk medicine for prophylactic and therapeutic purposes, being considered the medicinal "chest" from the days of Hippocrates [1]. Many phytochemicals such as vitamins, phenolic compounds, sterols, and terpenic compounds are documented on elderflowers and berries [2–6], being these compounds often connected with diverse potential health benefits on humans [7–11].

Due to those human health benefits potential and peculiar sensorial characteristics, a wide range of food products and nutraceuticals are homemade and/or commercially available, including elderflowers' infusions and decoctions [12–15], pastry products [16], nonalcoholic cordials and fermented beverages [16, 17], as well as elderberries' decoctions [12, 18], infusions [16], juice and syrup/concentrate [18], extracts, supplements, pies, ice creams, jellies, juices, beverages, beers, wines, liqueurs, fruit bars, and coloring agents [19–21].

Nowadays, new nutritional strategies reflect the way consumer perceives health, as those show an increasing knowledge regarding the impact of diet on regulation at the genetic and molecular levels [22]. Hence, plants and their extracts or isolated components are believed to have a positive human health effect; therefore, the evaluation of the efficacy and safety of these naturally occurring bioactive compounds is a major challenge to scientists [23]. Thus, this chapter aims at giving a general perspective of *S. nigra* L. berries and flowers' potential health benefits, covering aspects ranging from its taxonomic classification and geographic distribution, current applications, and a critical perspective about their health benefits from lab testing to human consumption. The data related to *S. nigra* possible toxicity and drug interaction will be also addressed.

2 Botany

S. nigra L. is a deciduous multistemmed shrub in which their suckering is made from the roots and branching from the base of the main stems stimulating the plant to form dense thickets. Elder shrub can reach up to 9 m in height, with average height values of 2–3 m, and contains large (5–15 cm long) leaves with 5 to 11 leaflets with sharply serrated margins [16, 17, 24, 25]. Elderberry belongs to genus *Sambucus* being recently included on Adoxaceae family [16, 26]. The most common subspecies from elderberry plant are the American elder [*Sambucus nigra* sbsp. *canadensis* (L.) R. Bolli] and black elder or European elder [*S. nigra* sbsp. *nigra* (L.) R. Bolli], being formerly designated as *S. canadensis* and *S. nigra*, respectively [25].

Elderberry leaves emerge around February or March, and the shrubs bloom around May to June, depending on the geographic location and cultivar, generating creamy-white flowers as illustrated on Fig. 1 [17]. Berries' ripening process happens for a period of 1–2 months, starting from a green color, and, when ripe, they became deep purple (Fig. 1) [16].

S. nigra is classified as European temperate with ideal growth conditions in terms of yield and quality on full sun or partially shaded exposures and in moist soils [27]. Different cultivars from *S. nigra* are currently explored for cultivation, for instance, Hachberg and Rubim in Austria; Albida and Bohatka in Czech Republic; Allesoe, Blangstedgaard, and Finn Sam in Denmark; Road Due in England; Alleso, Sampo, and Samyl in Poland; and Bastardeira, Sabugueira, and Sabugueiro in Portugal, among others [28–36].

3 From Folk Medicine to Food Products and *S. nigra* Supplements

S. nigra has been used for centuries, as far back as the Ancient Rome for a wide range of applications, namely, musical instruments as flutes, whistles, or textile artifacts and dyes, and even used on ritual ceremonies [25]. Leaves and inner bark have been used for their purgative, diuretic, laxative, expectorant, and

Fig. 1 *S. nigra* L. flowers and berries

diaphoretic action [25]. Elderflowers and elderberries have been used to alleviate or cure several illnesses in folk medicine and they have many applications in European countries. Elderflowers, for instance, were used to treat bronchial and pulmonary diseases, tumors, and ulcers [17]. Their infusions have been reported as having soothing and laxative properties and were used to "sweat out fever" [12], on asthma treatment [13], and to treat toothache, colic, cold and rheumatic conditions [14]. Their decoctions are used for cold treatment [15] and as expectorant, as well as to treat bronchitis, whooping cough, asthma, hemorrhoids [13], insect bites, and fever [14]. Elderflower's traditional use for the relief of early symptoms of common cold has been found to fulfill the requirement of medicinal use for at least 30 years according to the Committee on Herbal Medicinal Products [37]. As reviewed by the European Medicines Agency [18], dried elderberries were traditionally used as a decoction to act as laxative, and elderberry juice or syrup were used as a diaphoretic. Elderberry decoction was also documented for fever reduction [12], while their infusions were consumed as an antirheumatic and to treat colic in infants [16]. Their juice has also been used to treat sciatica, headache, dental pain, heart pain, and nerve pain, while the syrup was recommended to treat coughs and colds [18].

For the last few decades, a special attention has been given to elderberries and elderflowers. With the increasing number of studies on this plant and with the revealed potential health benefits related with its composition, industries started to develop new food products and dietary supplements using parts of this plant in order to meet consumers' needs. In Europe, countries such as Austria, Germany, Denmark, and Italy are the major producers of elder plant based products [38]. Currently, elderflowers and elderberries are used in a wide range of products, mainly food- and beverage-derived products followed by nutraceuticals/supplements [16, 19, 20]. One of the main uses of elderberries is the production of juices, concentrates, and natural food colorants, due to their high content in phenolic compounds, with remarkable antioxidant capacity compared to other red fruit juice concentrates [39]. Numerous products containing elderberry juice, pureed, or dried elderberries, such as extracts, syrups, supplements, cosmetics, pies, ice creams, jellies and jams, candies, juices, beers, wines, liqueurs, fruit bars, infusions, and coloring agents, are currently available throughout the world [19–21]. The flowers are commonly used as an ingredient of pastry products as pancakes, muffins, or waffles [16]. In England, elderflowers are considered important wild plant resources, commercially exploited mainly for beverage production (e.g., flavored sparkling water and wine), while in other countries, these flowers are soaked in water and sugar with citrus juices to make a nonalcoholic cordial or for the preparation of infusions [16, 17, 40].

In the last years, consumers' demands have changed remarkably, and diet has been considered not only in providing adequate nutrients, but to have a huge role on human health and well-being [23, 41–43]. The growing demand for natural and healthy food products can be explained by several reasons like transitional health, urbanization and its effect, changing demography with aging population, food security, loss of traditional food cultures, and awareness of deterioration in personal health as consequence of busy lifestyles with poor choices of convenience foods, and competitive food market have converged and propelled for development of

functional foods [44, 45]. Also, insufficient exercise, increased incidence of self-medication, increased level of information from health authorities and media on nutrition, link between diet and health, and scientific developments in nutrition research promote the current interest for healthy food products [46]. In this context, the consumption of the so-called functional foods has been considered as a convenient dietary strategy to address the consumers' demands. The functional food designation was firstly used in Japan in 1984, where scientists studied relationships between nutrition, sensory satisfaction, fortification, and modulation of physiological systems [46]. This concept suffered a few changes over the years with specific rules for the approval of a specific health-related food category being announced [43, 47, 48]. Nowadays, a food can be classified as a functional food, "if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way which is relevant to either the state of well-being and health or the reduction of the risk of a disease. Health claims are expected to be authorized for functional foods based either on enhanced function (type A claim) or disease risk reduction (type B claim)" [49]. Since elderberries and elderflowers are rich in bioactive compounds, many food products containing them with potential health benefits can be found in the market and, thus, potential candidates for being designated as functional foods.

4 In Vitro Versus In Vivo Assays: Current Challenges

In the last decades, there was an increasing interest to obtain scientific evidences to support the claimed folk medicine benefits resulting from *S. nigra* consumption. In spite of the huge importance to satisfactorily demonstrate that plant extracts or food product affects beneficially one or more target functions in the human body, a wide range of in vitro [50–53] and in vivo assays using animal models [3, 54] were performed. Nonetheless, the information regarding the use of clinical trials is scarce [53].

In vitro studies are often used to predict the health benefits of plants and their formulations. These studies are widely used due to the high amount of results they can provide in a short period, their considerably lower cost when compared with in vivo studies, and also by overcoming the legal and ethical restriction associated with the use of animal models and humans [55, 56]. A striking difference between in vitro and in vivo assays is the absence, in the former, of processes related to absorption, metabolism, and excretion [55]. Additionally, in in vitro studies, the hormonal and nutritional factors, the interaction with other cells, and hypoxia conditions are not usually considered [57]. Efforts have been done to overcome these limitations as in vitro digestion models are able to estimate the stability of phytochemicals under gastrointestinal conditions [58, 59], and, despite their inability to reproduce the complexity of the gastrointestinal tract, they have been used to study the changes in the dietary components throughout digestion [60–64]. In vitro digestion models face great chanllenges that include simulation of oral, stomach, and intestinal conditions, as, for instance, the simulation of the peristalsis and realistic shape and

motility of gastric and small intestines, as well as to evaluate the interactions between ingested compounds and microbiota [60].

Animal in vivo trials resort on different animals to evaluate the toxicological effects and/or potential health benefits. Those are frequently performed with mammals and most often with rat or mice. Animal trials raise ethical concerns, and researchers need to follow ethical codes and decrease the number of animals being used [57]. Although in in vivo animal trials there is a more complex system, these models represent specific aspect of a given human disease and not all of its characteristics [65], and since there is a different body weight/body volume ratio and biokinetic properties, the obtained data does not provide a correlation but gives an estimation of the activity of the extracts in humans [57]. It is worth mentioning that allometric scaling for dose conversion from animal to human studies is often applied, despite being controversial, considering the differences in body surface area [66]. Likewise, interspecies correlations are often used in order to sustain and establish a possible correlation between different models [67].

Despite expensive and time-consuming, human trials offer important and complete outcomes about an extract or compound over human health. Different assays can be performed that range from a basic (experimental) research to clinical and epidemiological research, either designed as interventional or noninterventional ones [68]. Interventional human trials are able to demonstrate the effects of a compound or a preparation, to establish possible side effects, considering the absorption, distribution, metabolism, or elimination parameters [68]. This is achieved by appropriate measures, particularly by random allocation of the patients to the groups, thus avoiding bias in the result. Interventional clinical studies are subject to a variety of legal and ethical requirements, including the Medicines Act and the Law on Medical Devices [68]. Clinical studies ideally include randomization of the patients in order to maximize the homogeneity between groups and to ensure that the patients will be allocated to the different groups in a balanced manner and that possible confounding factors, such as risk factors, comorbidities, and genetic variabilities [68, 69]. Single and double blinding are also suitable methods to avoid bias, in which the former the patient is unaware which treatment he is receiving, while with double blinding, neither the patient nor the investigator knows which treatment is planned [68, 69]. A control group is included in most clinical studies, by receiving another treatment regimen and/or placebo.

To study the contribution of foods, extracts, or specific bioactive compounds in the human health, it is important to analyze the impact of the digestion process, as it will affect the bioavailability and therefore the bioactivity. Bioavailability is defined as the relative amount of an administrated dose that reaches the general circulation and the rate that occurs [70]. This comprises a complex network of phases and parameters. For instance, for absorption to take place, the target molecule must first pass through the membrane that separates the lumen of the stomach and the intestine from the systemic circulation. This membrane is a complex structure made of lipids, proteins, lipoproteins, and polysaccharides that is selectively permeable. Thus, active transport and passive diffusion may take place. After absorption, distribution (to tissue and/or organs), metabolism, and elimination processes occur. Thus, in vivo

studies, either in animal or human assays, share the particularity of considering the phytochemical bioavailability, as their biological activities could be altered by digestive processes. Actually, bioactive components or related metabolites from a diet must be bioavailable to achieve a biological effect in a target organ or tissue (except for the gastrointestinal tract) [71]. Hence, considerations about the bioavailability of *S. nigra* extracts and constituents must be highlighted [72]. Most of the research done so far has been focused on the absorption, metabolism, and urinary excretion of elderberry anthocyanins, as systematically described in Table 1.

To sum up Table 1 data, it can be concluded that research has been mainly focused on the absorption and urinary excretion of the anthocyanin constituents from elderberry formulations upon a single-dose oral administration. Elderberry anthocyanins were absorbed from the intestine to the blood, with low recoveries reported in urine [73–83]. Anthocyanins are both absorbed in their unchanged form or might be hydrolyzed in the gut and metabolized in the liver by glycosylation to increase the solubility prior to excretion via the kidneys [74, 75, 78, 80, 81].

The absorption of phenolic compounds takes place mostly in the small intestines before the microbial degradation. However, these compounds can be partially degraded under gastric fluid conditions leading to a decrease in the extension of their absorption [84]. In respect to glycosylated flavonoids, bacteria in the colon hydrolyze glycosidic bonds, releasing the corresponding aglycones [85]; nonetheless, anthocyanins as cyanidin-3-glucoside or cyanidin-3-sambubioside from elderberry have been also detected in human blood and urine, showing that they can resist to bacterial hydrolysis and be absorbed in the glycoside forms (Table 1).

Sugar moieties of the elderberry phenolic compounds play an important role, as the addition of sugar to elderberry juice delayed anthocyanins excretion [82]. The authors suggested that cyanidin group might be drawn into the enterocyte by its glucose moiety, which is transported by the glucose carrier, and the sugar addition may lead to a saturation of the glucose transporter, therefore the intake of anthocyanins is reduced [82]. Additionally, studies that used standards instead of elderberry formulations concluded that cyanidin-3-glucoside and peonidin-3-glucoside were more bioavailable, stable, and/or cleared more slowly than either the galactosides or arabinosides of cyanidin and peonidin [86, 87]; also, quercetin glycosides were found in plasma much more rapidly following the ingestion of the glucoside form compared with the rutinoside [84].

Most of the plant constituents, and specifically phenolic compounds, are at least partially water soluble, and so their bioavailability is conditioned by the ability to cross the membranes of the intestine [88]. Parameters like partition coefficient; chemical structure, including the number and type of sugar molecules; and microflora seem to play an effective role in the absorption of phenolics [89]. The anthocyanin bioavailability is usually low (recoveries in urine of 0.01–0.4% of the oral dose), and their transport/absorption efficiency is commonly lower than other aglycone phenolics, even though it was reported that structures with more free hydroxyl groups and less methoxy groups can show a decrease of their bioavailability [87], or higher hydrophobic nature of aglycones might increase partitioning into cells and tissues [71].

Table 1 Follow-up of *S. nigra* berry's anthocyanin human bioavailability during one day, after single-dose administration

Population	Product and dose[a]	Dosage (g)	Urine recovery (%)[b]	Main results	Kinetics	Metabolites	Ref.
7: 4M, 3F	4 g of spray-dried juice	0.5	0.05	Low urinary ACN excretion and the low serum levels suggest low ACN bioavailability	Max. excretion after 3–4 h	Detected but not identified	[73]
1M	25 g of extract[c]	1.5	–	ACN absorbed in their glycosidic forms	–	–	[74]
4F	12 g of extract[c]	0.720	0.077	Low rates of ACN absorption and excretion	Max. excretion after 4–6 h	Peo-3-glc, peo-3-sam, peo-glucuronide, cy-3-glc glucuronide	[75]
6	30 or 200 mL of extract[c]	0.278 or 1.852	0.39 and 0.27	Dose-proportional in plasma of major ACN	Total renal clearance of ACN was 196 and 169 $mL.min^{-1}$	–	[76]
4F	12 g of extract[c]	0.720	0.055	The two main ACN detected unchanged in plasma and urine; ACN excreted in urine within 4 h	97.4 $nmol.L^{-1}$ of C_{max} in 72 min; First-order kinetics of ACN plasma elimination	Detected but not identified	[77]
7: 6F, 1M	150 mL of concentrate	3.57	0.053	Only 0.003% of the ingested ACN was excreted as cy-glucuronide	First-order excretion kinetics; Max. excretion after 1 h	Glucuronides ACN derivatives	[78]
6	30 mL of extract[c]	0.147	0.037	ACN absorption was higher comparing intake of elderberry with blackcurrant extract	$t_{1/2}$ of 1.74 h of urinary excretion	–	[79]
4F	12 g of extract[c]	0.720	0.055	Most ACN were excreted in urine during the first 4 h	Elimination of plasma ACN was 1st-order kinetics; $t_{1/2}$ 132.6 min	ACN are absorbed in their unchanged forms	[80]

(*continued*)

Table 1 (continued)

Population	Product and dose[a]	Dosage (g)	Urine recovery (%)[b]	Main results	Kinetics	Metabolites	Ref.
7	150 mL concentrate	3.57	0.06	The low urinary excretion of ACN indicated that a large proportion of ACN are metabolized before entry into the circulation	$t_{1/2}$ of 1.35 h of urinary excretion	ACN metabolized before entry into the circulation	[81]
16: 8F, 8M	11 g of concentrate	1.9	0.012	ACN detected unchanged in urine; sucrose ingestion reduced excretion	Maximum excretion occurred after 1–3 h	Detected but not identified	[82]
8: 4F, 4M	200–400 mL of juice	0.361–0.722	0.033–0.040	Dose-independent urinary excretion of elderberry ACN	–	–	[83]

[a]Single-dose administration
[b]Percentage of the ingested amount
[c]Solvent for extraction not described, *M* male, *F* female, *ACN* anthocyanins, *Cy* cyanidin, *Peo* peonidin, *Glc* glucose, *Sam* sambubioside, C_{max} maximal plasma concentration

Concluding, phenolic compound absorption is mainly modulated by the chemical structure of the aglycones and by the sugar side chains, to which the aglycones are often bound in plants [90, 91]. Also, the vehicle of administration, antecedent diet, sex and gender, and colon microbial population may interfere on bioavailability phenomena [72]. In respect to metabolism of phenolic compounds, the major conjugates derived from kidneys and liver enzymes activities are glucuronides, sulfates, and conjugates with both glucuronide and sulfate moieties [85], being the former the most reported after elderberry oral ingestion (Table 1).

5 Potential Health Benefits

The scientific developments in nutrition research, in evaluation of biological activities, and in prospection of bioactive compounds from natural products, demonstrated by several published evidences, indicate a set of potential health benefits associated with the *S. nigra* berries and flowers [18, 37]. These have been conducted aiming to sustain the empirical knowledge that folk medicine claimed and to identify the molecules that might contribute for disease prevention or even management through a dietary strategy that includes *S. nigra*-based products. It is important to note that when a deregulation occurs in a biological system, several processes are affected. For instance, epidemiological evidences relate diets rich in fruits and vegetables with a reduction in the risk of cardiovascular and neurodegenerative diseases, metabolic processes, and some types of cancer, all of which involve inflammation and oxidative stress [92]. Thus, despite the following topics are discussed separately, it is important to bear in mind that a disease is the outcome of diverse imbalances on a complex network of biochemical mechanisms. This is an advantage in a nutrition research point of view, as the consumption of *S. nigra* bioactive components may act and have a preventive role on a broad spectrum of processes.

Anti-infective, antioxidant, anti-inflammatory, immune-active, colorectal cancer modulation, and antidiabetic activities were selected by the fact that those are of major concern worldwide, being projected millions of affected people (for instance, diabetes, with 300 million before 2025 [93]), but also, these processes are recurrently studied on elderberry and elderflower matrices [93–95].

5.1 Anti-Infective Activity

Several in vitro assays revealed the antimicrobial effects (fungi, bacteria) of aqueous elderberry polar extracts (phenolic-type extracts), against pathogenic microorganisms to humans. For instance, the antimicrobial activity of elderberry extracts was assessed against the growth of common nosocomial Gram-positive and Gram-negative pathogens, as *Streptococcus* from Groups C and G, *Staphylococcus aureus* (methicillin-resistant and methicillin-sensitive), *Streptococcus mutans*, *Streptococcus pyogenes*, *Haemophilus influenza*, *Haemophilus parainfluenzae*, *Branhamella*

catarrhalis, and *Helicobacter pylori* [96, 97]. The results indicated that for the applied concentrations (50–200 mg of standardized elderberry aqueous extract, Rubini®, per mL of liquid broth media), the elderberry extract exhibited antimicrobial activity against the tested bacterial pathogens. At an extract concentration of 100 mg extract.mL^{-1}, the bacterial strains in liquid culture media decreased their growth by >70% in comparison with untreated samples, while at 200 mg.mL^{-1}, it resulted in bacterial development below 1 percent of the originally measured values. Elderflower polar (80% ethanol) extracts (phenolic-type extracts) showed activity against *Bacillus subtilis*, *S. aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, being those activities linked to the presence of chlorogenic and caffeic acids [37, 98]. These studies indicated the potential of *S. nigra* extracts to inhibit certain pathogens in vitro, being required more studies, including on humans, to fully confirm the real impact of such effects in vivo, which bacterial and fungal pathogens are susceptible [99], and also their mechanisms of action.

One of the most studied applications of *S. nigra* berries is related with their potential to inhibit the influenza virus. Influenza virus A or B causes an acute, febrile illness that occurs in outbreaks of varying severity almost every winter. Standardized elderberry extracts reduced hemagglutination and inhibited replication of various human and animal influenza viruses A and B in in vitro assays, as schematically illustrated on Fig. 2 [53, 100].

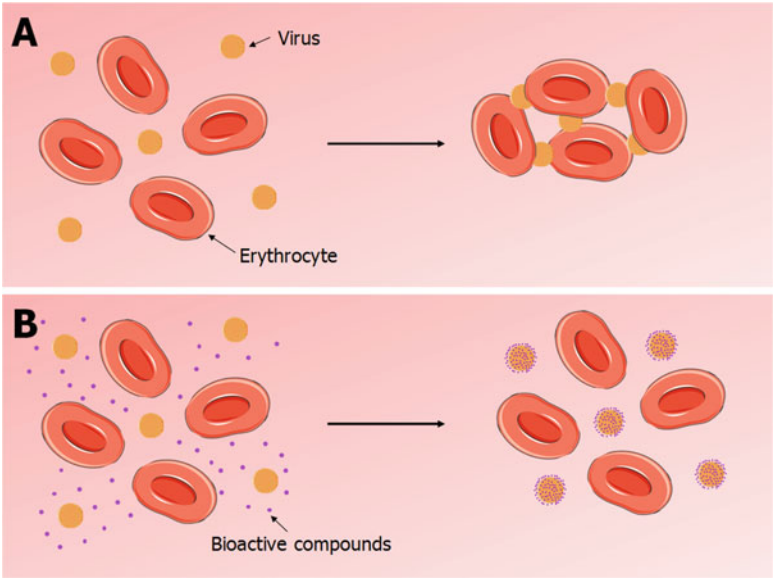


Fig. 2 Virus hemagglutination (**a**) is inhibited by elderberry bioactive compounds (possibly phenolic compounds) that bind to hemagglutinin structure of virus, inhibiting hemagglutination and replication of influenza virus by blocking their ability to infect the host cell (**b**)

Antiviral effects were also reported against HIV-infected peripheral lymphocytes and herpes simplex virus 1-infected human diploid fibroblasts and buffalo green monkey cells [101]. Flavonoids, including quercetin, cyanidin and petunidin, and proanthocyanidins, identified in European elderberry extracts blocked HIV-1 entry and infection in host cells. The action of flavonoids is exerted through binding to HIV-1 virions, thus effectively blocking their ability to infect host cells (Fig. 2b) [52, 102]. *S. nigra* flowers, *Hypericum perforatum* aerial components, and *Saponaria officinalis* root infusions inhibited HIV-1 in vitro and influenza A and B in vitro and in vivo animal trials [99, 103], while methanolic flower extracts exhibited anti-dengue virus serotype-2 activity [104], illustrating the potential of elderflowers' formulations on antiviral activity.

In vivo studies performed on chimpanzees supported the in vitro findings of a standardized elderberry aqueous extract, reporting a reduction of flu-like symptoms by two thirds [105]. Additionally, clinical studies revealed promising results as the flu-related symptoms were reduced [53, 100, 106], as well as a higher inhibition of the hemagglutination on treated groups [53]. The suggested mechanisms of action of elderberry extract on influenza virus are that its bioactive constituents (i) stimulate the immune system, (ii) inhibit the hemagglutination of the influenza virus and thus prevent the adhesion of the virus to the cell receptors (Fig. 2), and (iii) present anti-inflammatory effect. In summary, the antiviral activity of aqueous elderberry polar extracts (phenolic-type extracts) in vitro, in animal models, but also on human trials (standardized aqueous extracts, Sambucol®, at daily doses of 60 mL during 5 days and 2–4 spoons of daily doses during 3 days) indicates possible effectiveness for flu treatment/management in two controlled clinical studies (20 and 40 individuals) [53, 100]. The absence of side effects of this *S. nigra* preparation offers a possibility for a safe treatment for influenza [53, 100], as these results highlight positive indicators on flu prevention and management through a diet rich in *S. nigra*-based formulations.

5.2 Antioxidant Activity

Oxidative stress is a result of an imbalance from the pro-oxidant-antioxidant equilibrium in favor of the pro-oxidants [107]. Tissue destruction and degeneration can result in increased oxidative damage, by such processes as metal-ion release, phagocyte activation, lipoxygenase activation, and disruption of mitochondrial electron transport chains, being accompanied by increased formation of reactive oxygen species [108]. Oxidative stress may result in an increased lipid peroxidation, DNA damage, GSH depletion, and protein damage [108], being a number of disturbs linked to these processes, namely, arteriosclerosis, different types of carcinoma, diabetes, respiratory diseases, and aging, accompanied with inflammatory processes, among others [107].

Antioxidants are believed to protect cells against the detrimental effect of reactive oxygen species (ROS), and the measurement of the antioxidant activity is a common practice for the determination of the potential inhibition or scavenging capacity of

foods against ROS. Several in vitro assays including 2,2'-azino-bis(3-ethylbenzothialzoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorption capacity (ORAC), among others, are frequently used to estimate antioxidant capacities in fresh fruits and vegetables [109]. A systematic screening of the total antioxidants in dietary plants reported that elderberry had an overall mean of 4.31 mmol Trolox equivalents (TE).100 g^{-1} fresh fruit being comparable to wild raspberry 3.97 and wild/cultivated blackberry (6.13/5.07, respectively) [110]. Another survey showed that elderberries presented a high antioxidant activity (ORAC value of 14697 μmol TE.100 g^{-1}), which might be linked to the high content of total phenolic compounds but also with the presence of other components as minerals, selenium, vitamins (namely, ascorbic acid and vitamin E), and carotenoids [93, 111–113].

S. nigra bioactive compounds, including anthocyanins, may act as preventive compounds of diverse diseases via antioxidant mechanisms, although the epidemiological evidence is still insufficient, illustrating the need of more research on this topic [114]. Table 2 summarizes the main findings regarding the antioxidant activity of elderflowers and elderberries preparations.

Table 2 Relevant findings regarding antioxidant activity documented on *S. nigra* berry and flower preparations

Preparation	Scope	Main results	Proposed bioactive compounds	Ref.
Elderflowers				
In vitro studies				
Ethanol–water mixture (80:20 v/v): 20–200 °C	AOx of alcoholic extracts of DPPH and β-carotene/linoleic acid methods	No direct correlation between the level of flavonoids in the extracts and their AOx	Flavonols (rutin)	[115]
Aqueous extracts	Ability to inhibit the pro-inflammatory activity of periodontal pathogens	AOx connected with inhibition of the oxidative burst of neutrophils	Flavonols	[116]
Extract (solvent not described)	AOx to neutralize DPPH and hydroxyl radicals	Greater AOx when compared to standards (rutin, quercetin, BHT, and BHA)	Rutin and other phenolic compounds	[117]
Extract (methanol)	Effect of altitudinal variation on the AOx (DPPH)	Samples collected at 670 m had lower IC_{50} values than extracts from 1000 m	Flavonols and hydroxycinnamic acids	[118]

(*continued*)

Table 2 (continued)

Preparation	Scope	Main results	Proposed bioactive compounds	Ref.
Extracts (water and ethanol)	AOx using DPPH	Higher AOx using hot water when compared to ethanol	–	[119]
Methanol:water (80:20, v/v) extracts	Extract and components AOx on-line by cupric reducing antioxidant capacity	Water-soluble groups such as sugars attached to position 3 were less effected on the AOx	Phenolic acids and flavonols	[120]
Aqueous extracts	Antioxidant properties (ABTS, FRAP)	Prolonged extraction in water solvent caused higher antioxidant activity	Correlated with total phenolic content	[121]
Elderberries				
In vitro studies				
Spray-dried juice	Effects of copper and peroxyl-radical-mediated LDL oxidation	Pro-oxidant activity is probably marginal	ACN	[122]
Concentrate and isolated ACN and anthocyanidins	Extracellular and intracellular AOx	The intracellular steady state of oxidized DNA bases was not altered, although AOx was observed extracellularly	ACN or anthocyanidins	[114]
Extract (solvent not described)	ACN potential benefits against various oxidative stressors	ACN conferred significant protective effects in endothelial cells	ACN	[123]
Juice	Hydrogen-donating ability, reducing power, chelating ability, and total AOx	AOx correlated with the TPC	ACN	[112]
Hexane/dichloromethane (1:1) and acetone/water/acetic acid (70:29.5:0.5) extracts	Lipophilic and hydrophilic antioxidant capacities (ORAC)	TPC in general paralleled hydrophilic AOx	ACN	[124]
Purified ACN extract from elderberry concentrate	Radical scavenging activities (DPPH)	Radical scavenging activity weaker than positive control, Trolox	Not necessarily parallel with the anthocyanidin amount	[125]

(*continued*)

Table 2 (continued)

Preparation	Scope	Main results	Proposed bioactive compounds	Ref.
Juice	Scavenging capacity against peroxyl and hydroxyl radicals and peroxynitrite	Nonlinear correlations between sample concentration and AOx were observed	ACN	[126]
In vivo (animal trials) experiments				
Ethanolic extract	One-month diet containing 4% of extract on acute colitis in rats	Lower response to oxidative stress was documented and lower level of primary products of lipoperoxidation-conjugated dienes in the liver and colon	Flavonoids	[127]
Ethanol extract	Dietary supplementation of 8-week diet (dosage of 0.046 $g.kg^{-1}$ body weight), on total AOx levels and blood pressure parameters	AOx was lower after extract supplementation. Blood pressure with induced hypertension was reduced by polyphenolic extract	Phenolic compounds	[128]
Concentrate and cyanidin-3-glucoside	Effects of dietary concentrate and cyanidin-3-glucoside on male rats for 4 weeks	ACN may act synergistically with vitamin C and the antioxidant defense system in sparing vitamin E	ACN	[54]
Human trials				
Spray-dried juice (400 mg or 4000 mg of anthocyanins)	AOx in a cohort of young volunteers (34)	Minor effect AOx capacity	ACN	[129]
400 mL of juice	Elderberry juice intake effects on postprandial plasma AOx in healthy humans (8)	Plasma AOx and TPC were significantly increased 1 h after ingestion	ACN	[83]

ACN anthocyanins, *AOx* antioxidant activity, *TPC* total phenolic content.

Several studies analyzed the antioxidant activity of *S. nigra* preparations using in vitro and in vivo studies (Table 2) [54, 83, 112, 114–127, 129]. These activities are often linked to phenolic compounds, although it is important to point out possible interactions between extract components, including non-phenolic compounds,

influence of extraction parameters [115, 121], and pre- and postharvest conditions [118, 130], which play a critical role on the outcome of the antioxidant data.

Despite the complex composition of elderberry and elderflower matrices, and the possible contribution of other families to the antioxidant action as mentioned above, phenolic compounds, and particularly anthocyanins and flavonols, are assumed to be the key players related to antioxidant activity. The possible protective mechanisms in mammalian cells of elderberry anthocyanin-rich extracts were compared to their extracellular and intracellular antioxidative potential in vitro (ferric reducing ability assay) and in human colon tumor cells [114]. Although extracellularly, these compounds showed strong antioxidant activity; however, the intracellular steady state of oxidized DNA bases was not altered by anthocyanins or anthocyanidins [114]. In this context, the protection of cells may occur through the extracellular reduction of exposure to oxidative and carcinogenic factors, which, indirectly, might also protect the intracellular steady state [114]. Additionally, other authors concluded that the incorporation of elderberry anthocyanins into the plasma membrane and cytosol of endothelial cells conferred significant protective effects against oxidative stressors [123]. These results show that vascular endothelial cells can incorporate anthocyanins into the membrane and cytosol, conferring protective effects against oxidative species.

Dietary phenolic compounds may facilitate homeostasis of oxidative stress once consumed, although during digestion their stability and bioavailability might be compromised [131]. Despite there was a reported loss of elderberry bioactive compounds due to the in vitro digestion process (e.g., losses of anthocyanins of ca. 44%), the colon-digested aqueous extract (resuspended freeze-dried elderberry powder in water, 50 $mg.mL^{-1}$, treated with pepsin, pancreatic-bile salts, and human fecal bacterial culture) was able to reduce the excessive intracellular ROS production (22%) and oxidative DNA damage (46%) in the colon cells at a dose of 1 mg of freeze-dried elderberry $powder.mL^{-1}$ [132]. Concluding, gastrointestinal in vitro digestion of elderberry aqueous extracts decreased radical scavenging activity and oxidative DNA damage, inhibited the oxidant-induced mutagenicity, and inhibited pro-inflammatory pathway in LPS-stimulated macrophages, and regarding the levels of phenolic compounds, there was not a clear consensus, whether a significant reduction or a no effect on phenolic compound levels were reported [131–133].

Beyond the oxidation of lipoproteins, oxidative stress may also play an important role in the etiology of inflammatory bowel diseases. The effect of 1-month diet containing 4% of 70% ethanol elderberry polar extract (phenolic-type extract) in rats with induced acute colitis was evaluated [127]. A decrease on the disposition to colitis was observed at macroscopic level, being the damage score and the myeloperoxidase activity in the experimental group one half of that observed in the control group [127]. Higher activities of lysosomal enzymes (acid phosphatase, cathepsin D) found in the group fed with elderberry diet were considered to be an indication of higher integrity of the colonic mucosa [127]. The lower response to oxidative stress in the experimental group was also documented by a lower level of primary products of lipoperoxidation-conjugated dienes in the liver and colon, accompanied by a higher level of colonic reduced glutathione, and by higher

activities of glutathione-S-transferase in the erythrocytes [127]. The results indicate that long-term diet with the antioxidant active elderberry extract provided a protective effect on the rat colon exposed to acetic acid-induced colitis. Furthermore, it is important to highlight that the nutritional stimulation of the antioxidative defense of the organism could also have beneficial effect on the course of inflammation [127], suggesting that the bioactive compounds present on elderberry and elderflower matrices may have different (indirect) mechanisms to prevent or regulate illness that alter the inflammatory status. As previously stated, a disease is currently accompanied by diverse imbalances, namely, on inflammatory and pro-oxidant processes, which might be managed or prevented through a balanced diet.

5.3 Anti-Inflammatory and Immunological Activities

Inflammation is an important factor involved in different human diseases, including, among others, asthma, diabetes, allergy, multiple sclerosis, cardiovascular diseases, neurodegenerative disorders, metabolic syndrome, hypertension, and some types of cancer [92, 133, 134]. An important process of organism self-protection involves inflammation, aiming to remove harmful stimuli including damaged cells, irritants, or pathogens [135]. Often, the inflammation process involves the activation of monocytes and/or macrophages (located in various tissues), which plays a central role in the regulation of inflammation through the release of several inflammatory cytokines, such as interleukins (IL), tumor necrosis factor (TNF-α), and inflammatory mediators including reactive oxygen species (ROS), nitric oxide (NO), and prostaglandin E2 [135], which are generated by inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2) [133] (Fig. 3). In this perspective, research conducted on elderberry and elderflower's formulations often connects anti-inflammatory and immunomodulatory activities, being for this reason discussed on the same topic.

Cytokines as TNF-α and interleukins (ILs) are multipotential mediators of the cellular immune system, having a wide variety of biological activities, and they can have favorable or unfavorable effects on the host immune response, depending on their local concentration, where the balance between the production of inflammatory and anti-inflammatory cytokines will be responsible for the outcome and the duration of the immune response [139]. Elderberry aqueous extracts downregulated the expression of IL-1, IL-1β, IL-6, and TNF-α, as illustrated on Fig. 3 [133, 136]. The effect on the induction of pro-inflammatory cytokines' expression by human serum in cell culture (in vitro and ex vivo assays) was also studied. A decrease in the pro-inflammatory activity of blood serum was observed, and the cytokine expression was suppressed for 8 h after a single treatment. An aqueous polar elderberry extract, Sambucol®, modulated the production of four inflammatory cytokines (IL-1β, TNF-α, and IL-6 and IL-8) and one anti-inflammatory cytokine (IL-10) using blood-derived monocytes from 12 healthy donors [139, 140]. An increase of the production of five cytokines (1.3-fold) compared to the control was revealed, which allows to

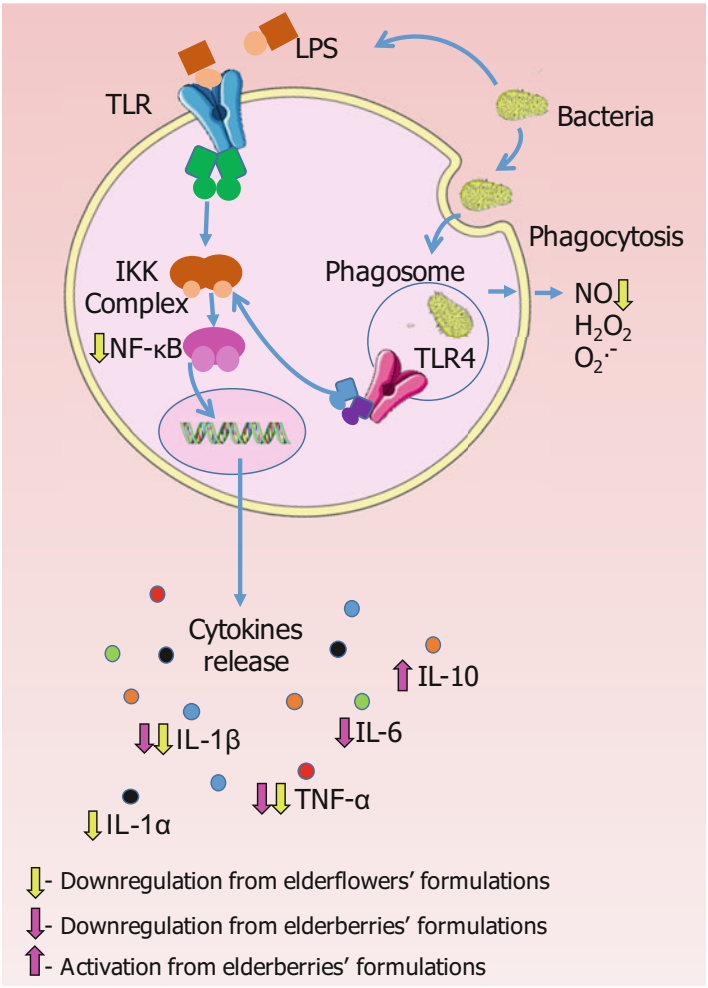


Fig. 3 Inflammation process involving the activation of monocytes and/or macrophages caused by pathogens or another harmful stimulus. Pathogen-derived molecules, including lipopolysaccharide (*LPS*), activate macrophages toll-like receptors (*TLR*) signaling which leads to the production of inflammatory cytokines as interleukins (IL-1β, IL-6 and IL-8) and tumor necrosis factor (*TNF-α*), via activation of IκB kinase (*IKK*) complex and release of nuclear factor nuclear factor κB (*NF-κB*). Elderflower and elderberry bioactive compounds may regulate the expression of marked inflammation mediators [116, 133, 135–137] (Inflammation mechanism adapted with permission from [138])

infer that the increase on inflammatory cytokines production may promote the immune system activation.

Concerning elderflowers, it was reported that aqueous extracts inhibited the macrophage release of pro-inflammatory cytokines and suppressed the activation of neutrophils (as illustrated on Fig. 3) [116]. These effects were linked to the

inhibition of activation of NF-κB and phosphatidylinositol 3-kinase (PI3K), an enzyme that plays a central role in the immunity and inflammation processes. The active ingredients responsible for the anti-inflammatory action of elderflower aqueous extract are unknown, although the ability of aqueous extract to inhibit P13K has been suggested to be mediated at least partially through quercetin. Another study revealed that methanolic and hexane extracts possessed a medium to low inhibitory effect on the biosynthesis of IL-1α, IL-1β, and TNF-α (Fig. 3) [137].

Modulation of NO production by macrophages and dendritic cells may have therapeutic value in inflammatory diseases [135]. Ethanolic elderflower extracts showed an inhibitory activity on NO production in RAW cells and dendritic cells (Fig. 3) [135]. Moreover, phenolic compounds in the range of 0.1–100 μM showed a dose-dependent inhibition of NO production, with quercetin, rutin, and kaempferol as the most potent ones [135]. Complement fixating activity and macrophage-stimulating activity were also observed on pectic elderflower polysaccharides [141, 142]. In vivo studies corroborated the potential elderflower anti-inflammatory, as an 80% ethanol extract had moderate anti-inflammatory activity in rats, inhibiting carrageenan-induced footpad [143].

The available information regarding *S. nigra* berries and flowers' anti-inflammatory and immunological effects on human models is still scarce. As far as we know, only three studies are available, including a few dozen of healthy individuals, and significant alterations were not observed on inflammatory biomarkers (as C-reactive protein, IL-6, and TNF-α), as well as serum lipidic profile, serum antioxidant levels, and cardiovascular disease risk biomarkers between 2 and 12 weeks of treatment with different elderberry-derived formulations with daily doses of 200 mL elderberry concentrate enriched with elderflower 90% ethanol polar extract, 400 mg spray-dried juice, and 500 mg of aqueous polar Artemis® extract [129, 144, 145]. Although, studies conducted on humans with vegetarian diets revealed positive results related to inflammatory and immunological diseases [146], namely, a vegetarian diet during 2 months ameliorated atopic dermatitis by reduction in eosinophils and neutrophils and PGE2 production, on an open-trial study carried out in 20 patients with atopic dermatitis [147]. This indicates that the increased consumption of fruit and vegetables would reduce inflammation and improve clinical outcomes in inflammation-related diseases.

5.4 Colorectal Cancer Modulation

Colorectal cancer is the third most common cancer and the third leading cause of cancer death among men and women, and it is assumed that dietary factors are responsible for 70–90% of the occurrences and diet optimization may prevent most cases [148]. Phenolic components, fiber, minerals, and other bioactive components present in plants may have a positive effect on intestinal cancer prevention, through modulation of DNA damage, oxidative stress, angiogenesis, inflammatory processes, and probably gut microbiota [148], allowing to infer the potential of *S. nigra* on intestinal cancer prevention and/or management.

In vitro assays using a human colorectal adenocarcinoma (HT29) cell line were performed to evaluate the inhibition of colon cancer cell proliferation [149]. Aqueous standardized elderberry polar extract (Artemis International®) suppressed HT29 cell growth with reported growth inhibition (GI_{50}) of 130.3 μg of cyanidin-3-glucoside eq.mL^{-1}. The chemical structure of anthocyanins is known to have a significant impact on their biological activity, and data suggest that non-acylated monoglycosylated anthocyanins are more potent inhibitors of colon cancer cell growth proliferation [149]. Also, the 3,5-glycosylation pattern on anthocyanidins might indicate lower biological activities as compared to 3-monoglycosylation. The chemopreventive potential of berry aqueous acetone extracts was also reported through the induction of quinone reductase (QR) and inhibition of cyclooxygenase-2 (COX-2), which is indicative of anti-initiation and antipromotion properties, respectively [150]. The elevation of phase II enzymes, such as NAD(P)H:quinone reductase, was already linked with the protection against chemical-induced carcinogenesis in animal models, in the stage of promotion and initiation [151]. The *S. nigra* polar extract produced from the 70% aqueous acetone extraction did not exhibit significant activity in either COX assay, though different fractions were screened for inhibition against both COX-1 and COX-2, and those showed great percentage of inhibition of those related inflammatory enzymes [150]. The elderberry fractions containing phenolic compounds (quercetin, quercetin monoglucoside, proanthocyanidins, and epigallocatechin) and non-phenolic ones (i.e., iridoid monoterpene glycosides, sesquiterpenes, and phytosterols, among other unidentified compounds) were active against the initiation and promotion stages of carcinogenesis.

The in vitro studies conducted so far indicate the dietary relevance of *S. nigra* preparations to positively modulate colorectal cancer, by modulation of inflammatory factors, initiation and promotion of tumoral factors, and oxidation processes. The presence of anthocyanins, flavonols, flavan-3-ols, but also non-phenolic compounds, as monoterpene glycosides, sesquiterpenes, and sterols, among other unidentified compounds, was related to the reported activities. The dietary *S. nigra* on cancer prevention and management highlights the need for in vivo studies but also for studies that are able to understand the underlying anticancer mechanisms.

5.5 Antidiabetic (Types 1 and 2) Activity and Related Complications

The term *diabetes mellitus* describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both [152]. Along with hypertension, dyslipidemia, and obesity, these clinical traits are the main characteristics of this metabolic syndrome. Diabetes is mainly caused by a combination of insulin resistance and β-cell failure (pancreatic cells) and can be treated with insulin-sensitizing drugs that target the nuclear receptor peroxisome proliferator-activated receptor (PPARγ) [153]. Various extracts of elderflowers (hexane, dichloromethane, methanol, ethyl acetate, and water) showed in vitro activation of PPAR(α, δ or γ),

between 20 and 250 μg extract.mL^{-1} without the stimulation of adipocyte differentiation [153, 154]. These extracts had a beneficial effect on insulin-stimulated glucose uptake suggesting that elderflowers produce compounds with bioactivities similar to those of partial PPARγ agonists [154]. Dietary supplementation with elderflower aqueous extracts (100–200 mg.kg^{-1} body weight) and kaempferol (32–64 mg.kg^{-1} body weight) revealed acute (6 h) and sub-chronic (8 days) antidiabetic potential in alloxan-induced diabetic mice by lowering blood glucose and increasing mice weight and catalase serum levels [155]. Elderflower lipophilic extract (dichloromethane extract from 20 mg extract.L^{-1} of solvent) and polar extract (aqueous extract from 250 mg elderflowers.L^{-1} of solvent) led to an increase of the glucose uptake in the presence and absence of insulin (on mouse abdominal muscle), reduction of fat accumulation (*Caenorhabditis elegans* model) [156], and insulin secretion (clonal pancreatic β-cells) [51]. Naringenin and 5-*O*-caffeoylquinic acid partially explained the increase in glucose uptake in primary porcine myotube cultures, while naringenin and kaempferol were linked to the reduction of fat accumulation on *C. elegans* model. Insulin secretion was not stimulated by rutin, lupeol, and *β*-sitosterol [51], while PPARγ (without stimulating adipocyte differentiation) was activated by the fatty acids α-linolenic and linoleic acid, as well as the flavanone naringenin [153, 154]. Quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, and 5-*O*-caffeoylquinic acid were unable to activate PPARγ [153, 154].

Elderflower aqueous extracts also led to diuresis in rats observed from 2 to 24 h [157]. Likewise, the intragastric administration of an elderflower infusion (20 mL.kg^{-1} body weight) or of an extract rich in potassium and flavonoid of the elderflower extract had a diuretic effect in rats [37]. These studies indicate that elderflowers may have diuretic effects in rats, but this has not been established in humans. Diabetes is also often associated with excess body sodium and frequently accompanied by hypertension, which could be reduced by this potential diuretic effect.

Antidiabetic potential of elderberry extracts was also evaluated through in vivo assays using STZ-induced diabetic rats' dietary supplemented with acidified (0.5% HCl) methanol polar extracts (phenolic-type extract), with doses ranging from 28 to 350 mg of extract.kg^{-1} body weight, and dichloromethane extracts (lipophilic-type extracts) with doses of 190 mg of extract.kg^{-1} body weight, during from 4 to 16 weeks of treatment [3, 158–166]. The serum lipidic, glycemic, inflammatory, oxidative, and immunological status of the diabetic rats and control group was monitored. On the tested conditions, no remarkable alterations were observed on the hematological indices, sera, and tissular trace element homeostasis, and the blood sera aminotransferases remained unaltered [3]. In vitro assays revealed that the toxicity of these extracts (9–1995 mg.L^{-1}) using the *Aliivibrio fischeri* bioluminescence toxicity model (light output reflects the cells' metabolic rate) had only a slight impact in the viable bacterial activity [3]. The reduction of the glycemic serum levels and pro-inflammatory interleukin levels (as IL-6 and IL-1β) was documented with the supplementation with these extracts when compared to the diabetic/not supplemented group [3, 158, 160–163]. Hypolipidemic and hypocholesterolemic effects were also reported, through the significant reduction of total and LDL

cholesterol and triglycerides and increase of HDL cholesterol during long dietary supplementations (12–16 weeks) [158, 163], although for supplementations of 4 weeks no alterations were observed on lipidic pattern [3]. Serum oxidative status was altered by increasing the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH) enzymes and serum total antioxidant activity and reducing uric acid content when compared to diabetic group/not supplemented group [158, 160, 161, 163]. The ability of phenolic compounds, namely, elderberry anthocyanins, to decrease lipid peroxidation and LDL oxidation might be mediated through the uptake of lipid-peroxyl radicals and lipoxygenase activity reduction [158, 166], which is corroborated by the significant decrease of the serum malondialdehyde content (an index of lipid peroxidation) on diabetic rats/supplemented group [160, 161]. Acidified methanol (0.5% HCl) elderberry polar extracts (phenolic-type extracts) also affected the immune system imbalances on diabetic rats, in which the authors beyond linked the potential antidiabetic activity to diabetes mellitus type 2, also connected with type 1, as the latter is an auto-immune illness mediated by the T cells [159, 161–163, 165]. The increment of the population of lymphocytes (T CD3+ and T helper naive CD4+CD45RA+) and the levels of cytokines (TNF-α and IFN-γ), and reduction of monocytes population and levels of fibrinogen (a cardiac risk factor [164]), was reported, when compared to the group of diabetic and not supplemented rats [159, 161–163, 165]. Recent studies, resorted on the use functionalized nanoparticles (NPs) with elderberry extract (acetone:water) on diabetic rats, revealed that functionalized NP administration (tube feeding) increased the muscle and systemic GSH/GSSG ratio and decreased malondialdehyde (MDA) levels and COX-2 expression and metalloproteinases activity also decreased after pretreatment with NPs [167].

Atherogenesis is assumed to be related to the oxidation of low-density lipoprotein (LDL) in the arterial wall, a common complication on obesity that is another component of the metabolic syndrome diabetes, along with hypertension or dyslipidemia. Dietary antioxidants may be helpful in relieving symptoms and complications observed in diabetes patients and inherent complications due to their positive action against the oxidative damage caused by dysregulation of glucose metabolism [168]. Despite the fact that hyperglycemia by itself does not cause diabetic complications, a common endpoint of hyperglycemia-dependent cellular changes is the generation of reactive oxygen intermediates and the presence of elevated oxidative stress, thereby suggesting that oxidative stress plays a crucial role in the pathogenesis of late diabetic complications [169]. The known biochemical mechanisms of hyperglycemia-induced tissue and cell damages come along with, among others, the production of reactive oxygen species inside the aortic endothelial cells [169, 170]. Thus, strategies that reduce the production of reactive oxygen species, or increase its degradation, such as with antioxidant supplementation, could be a possibility to slow down the development and progression of diabetes and its complications [171]. Oxidation resistance of LDL and also the antioxidant potential of plasma and whole blood play an important role in the assessment of the risk for developing atherosclerosis [122]. Elderberry anthocyanin glucosides gave considerable antioxidant protection, both from copper-induced LDL

oxidation and from the attack of peroxyl radicals, and the pro-oxidative properties of the extract were neglected [122].

6 Nutrivigilance System to Improve Consumer Health and Safety

Food industry faces an era of rapid and constant innovation and development of new products, being resorted on the use of innovative ingredients and technologies, and creation of dietary supplements, medical foods, functional foods, and beverages. Within this food industry trend, a nutrivigilance platform that aims to establish "the implementation of the vigilance system" which refers to "novel foods, food supplements, foods that are added to nutritional or physiological substances" and to identify risk situations upon the consumption of these products should be established [172, 173]. Nutrivigilance allows a better management of benefit/risks related to food supplements, fortified foods, and novel foods by identifying possible adverse effects as chemical and toxicological effects; it also assesses their health impacts, and thus, it strengthens the consumer health and safety. This information is the basis for preventive and corrective risk management measures. This section focuses on the nutrivigilance of *S. nigra* formulations at the level of possible toxic components from this matrix, as well as possible drug-botanical interaction. The criteria for these two themes are solely based on the available literature, thus highlighting the need for more data about other parameters covered by nutrivigilance systems.

The reported potential toxicity from *S. nigra* matrix is mainly related with two chemical families, the cyanogenic glucosides and the ribosomal-inactivating proteins. Elderberry leaves, stems, bark, roots, flowers, and unripe fruits contain the cyanogenic glycosides sambunigrin, prunasin, holocain, and zierin (Fig. 4) [6, 174].

Cyanogenic glycosides are considered toxic due to their decomposition into hydrogen cyanide [175]. The consumption of uncooked berries or nonthermally processed elderberry products can result in gastrointestinal disorders, such as vomiting and diarrhea but also nausea, weakness, and dizziness [101, 175]. However this risk is normally easily circumvented since elderberry-based products most often involve thermal processing steps that lead to the degradation of those cyanogenic compounds and elimination of hydrogen cyanide [6, 18, 176, 177].

Those assumptions point out to elderberry extract safety. However, adverse events have been reported, namely, abdominal pain after oral administration of elderberry syrup and knee pain after oral administration of elderberry food supplement. However, those formulations were homemade using not only raw elderberries but also leaves and branches, and the cyanogenic glucosides from branches and/or leaves have been associated with the reported episodes [18]. Despite the lack of enough information on elderberry and flowers toxicity during fetal development, pregnancy, or lactation, the Committee on Herbal Medicinal Products, European Medicines Agency recommendation is to avoid plant parts other than the flowers and ripe berries by pregnant and lactating women, as well as children and adolescents under 18 years of age [18, 37].

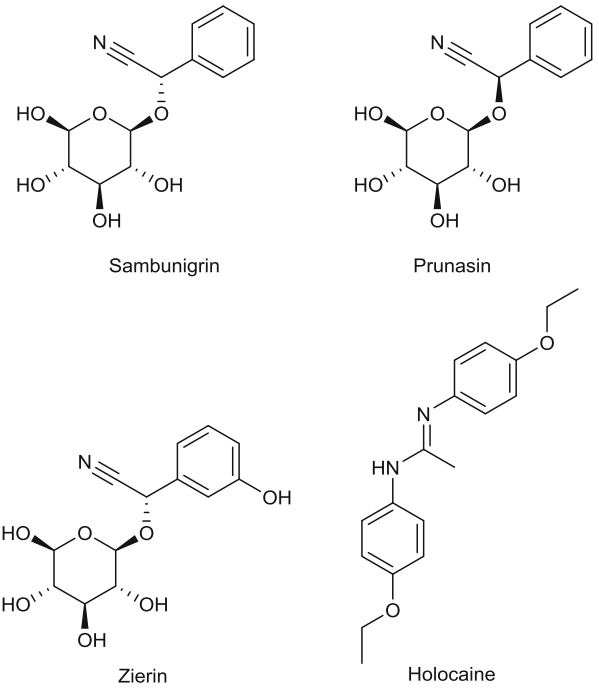


Fig. 4 Cyanogenic glucosides from *S. nigra* L

Another class of components with potential toxicity documented on *S. nigra* are the ribosomal-inactivating proteins, as, for example, agglutinin (SNA-I), a plant glycoprotein reported on elderberries with interesting features on sugar binding sites as it binds reversibly with specific sugars [178–180]. SNA-I is unique among lectins in recognizing sialic acid residues, being a very important probe to detect cell surface sugars, enzymes, and immunoglobulins, leading to many uses in medicine and physiology [178–180]. These include studies in the development of cancer, in the understanding of immunological and allergic disorders, and in the study of the cell surfaces of normal tissues compared to the surface properties of normal and diseased tissues and used to facilitate gene transfer into epithelial cells [17]. However, these proteins may also show some allergenicity, as demonstrated in a study where about 1% of 3668 patients tested ribosomal-inactivating proteins (RIPs) were connected with type-1 allergy to *S. nigra* as evidenced by positive-skin prick or RAST test [181].

The knowledge regarding interactions between dietary supplements and pharmaceutical drugs is rather vague, especially among patients with chronic diseases [182]. Plants, vitamins, and other dietary supplements may augment or antagonize the actions of drugs [183], and thus, drug interactions with foods or dietary supplements may also raise precautions upon *S. nigra* consumption. So far, only a few studies about the interaction of elderberry or flowers (or their extracts) with drugs have been reported, namely, with antidiabetic, analgesic, hypnotic, diuretic, and immune-active drugs [6, 18, 37, 183]. The ability of *Sambucus* flower aqueous polar extracts

(phenolic-type extracts) to potentiate diuresis in in vivo animal models [37, 157] may result in hypokalemia, especially in the case of excessive or prolonged use of any elderflower-derived formulation, including tea. Moreover, elderberry and elderflower decoctions administered orally (2 $mL.kg^{-1}$) to rats caused a reduction of the sleep induction time of pentobarbitone and increased sleeping time when compared with rats administered only with pentobarbitone [183]. No significant effect was observed on the analgesic activity of morphine taken in combination with elderberry or elderflower decoctions for the administrated doses (2 mL of decoction per kg of body weight). According to the Committee on Herbal Medicinal Products, the evidence of any interaction between elderberry (decoctions) and pentobarbitone appears to be limited to this study, being concluded that it is not known whether this effect will occur in humans, but even if it does, it is unlikely to be clinically relevant [18]. To sum up, no robust data is still available to support *S. nigra* drug-botanical interactions.

7 Final Remarks

S. nigra plant parts have been used in folk medicine since ancient times to treat a wide variety of diseases only based on experience and empirical knowledge; however the scientific advances improve the knowledge related with the detailed chemical composition of elderberries and flowers and the systematic in vitro assays allowed to unambiguously demonstrate their potential, as antibacterial, antiviral, antioxidant, anti-inflammatory, antidiabetic, and anticancer agents. It is important to point out that the quantities are necessary to promote the reported biological effect, i.e., the dose is effect-dependent, and for the studies reported above, the doses are in the order of few $\mu g.mL^{-1}$ to $mg.mL^{-1}$, depending on the biological effect and type of tested extract.

Nonetheless, the extension of these biological effects for animal testing (which, with allometric scaling would allow a better extrapolation to humans) is limited, and the extension to human clinical trials is even more scarce. These would be fundamental steps in future research to prove the efficiency of the *S. nigra*-related extracts or products on human health and well-being, strongly supported in scientific evidence rather than in folk knowledge. Finally, the use of *S. nigra*-based products will require a careful nutrivigilance system that would allow maximizing the understanding of their benefits by the adequate tuning of formulations, to prevent any problems arising from the presence of cyanogenic or allergenic compounds.

Acknowledgments Thanks are due to FCT/MEC for the financial support to the QOPNA research Unit (FCT UID/QUI/00062/2013) and CICECO (FCT UID/CTM/50011/2013), through national funds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement. Â. Salvador thanks the grant AgroForWealth: Biorefining of agricultural and forest by-products and wastes – integrated strategic for valorisation of resources toward society wealth and sustainability (CENTRO-01-0145-FEDER-000001), funded by Centro2020, through FEDER and PT2020.

References

1. Dawidowicz AL, Wianowska D, Gawdzik J, Smolarz DH (2003) Optimization of ASE conditions for the HPLC determination of rutin and isoquercitrin in *Sambucus nigra* L. J Liq Chromatogr Relat Technol 26:2381–2397
2. Salvador ÂC, Silvestre AJ, Rocha SM (2017) Unveiling elderflowers (*Sambucus nigra* L.) volatile terpenic and norisoprenoids profile: effects of different postharvest conditions. Food Chem 229:276–285
3. Salvador ÂC, Król E, Lemos VC, Santos SAO, Bento FPMS, Costa CP, Almeida A, Szczepankiewicz D, Krejpcio Z, Silvestre AJD, Rocha SM (2017) Effect of elderberry (*Sambucus nigra* L.) extract supplementation in STZ-induced diabetic rats fed with a high-fat diet. Int J Mol Sci 18:1–19
4. Salvador ÂC, Rocha SM, Silvestre AJD (2015) Lipophilic phytochemicals from elderberries (*Sambucus nigra* L.): influence of ripening, cultivar and season. Ind Crop Prod 71:15–23
5. Salvador ÂC, Rudnitskaya A, Silvestre AJD, Rocha SM (2016) Metabolomic-based strategy for fingerprinting of *Sambucus nigra* L. berry volatile terpenoids and norisoprenoids: influence of ripening and cultivar. J Agric Food Chem 64:5428–5438
6. Sidor A, Gramza-Michałowska A (2014) Advanced research on the antioxidant and health benefit of elderberry (*Sambucus nigra*) in food – a review. J Funct Foods 18:941–958
7. Vinholes J, Rudnitskaya A, Gonçalves P, Martel F, Coimbra MA, Rocha SM (2014) Hepatoprotection of sesquiterpenoids: a quantitative structure-activity relationship (QSAR) approach. Food Chem 146:78–84
8. De Cássia R, Andrade LN, de Sousa DP (2013) A review on anti-inflammatory activity of monoterpenes. Molecules 18:1227–1254
9. Pollak OJ (1953) Reduction of blood cholesterol in man. Circulation 7:702–706
10. Romeo JT (1998) Phytochemicals in human health and plant defense, vol 33. Springer Science+Business Media, LLC, Tampa
11. Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 99:191–203
12. Pieroni A, Giusti ME, Münz H, Lenzarini C, Turković G, Turković A (2003) Ethnobotanical knowledge of the Istro-Romanians of Žejane in Croatia. Fitoterapia 74:710–719
13. Kültür Ş (2007) Medicinal plants used in Kırklareli Province (Turkey). J Ethnopharmacol 111:341–364
14. Passalacqua NG, Guarrera PM, De Fine G (2007) Contribution to the knowledge of the folk plant medicine in Calabria region (Southern Italy). Fitoterapia 78:52–68
15. Camejo-Rodrigues J, Ascensão L, Bonet MÀ, Vallès J (2003) An ethnobotanical study of medicinal and aromatic plants in the Natural Park of "Serra de São Mamede" (Portugal). J Ethnopharmacol 89:199–209
16. Charlebois D, Byers PL, Finn CE, Thomas AL (2010) Elderberry: botany, horticulture, potential. In: Janick J (ed) Horticultural reviews, vol 37. Wiley, Hoboken, pp 213–280
17. Atkinson MD, Atkinson E (2002) *Sambucus nigra* L. J Ecol 90:895–923
18. CHMP Committee on Herbal Medicinal Products (2013) Assessment report on *Sambucus nigra* L. Fructus 44:1–26
19. Charlebois D, Richer C (2005) Le sureau: exigences de la production, cultivars et potentiel de mise en marché. Agri-Réseau 1:1–8
20. Cernusca M, Gold M, Godsey L (2009) Elderberry market research – report based on research performed in 2009. The Center for Agroforestry. University of Missouri, USA
21. Zafrilla P, Valero A, García-Viguera C (1998) Stabilization of strawberry jam colour with natural colourants. Food Sci Technol Int 4:99–105
22. Biesalski H, Dragsted LO, Elmadfa I, Grossklaus R, Muller M, Schrenk D, Walter P, Weber P (2009) Bioactive compounds: definition and assessment of activity. Nutrition 25:1202–1205
23. Biesalski H, Dragsted LO, Elmadfa I, Grossklaus R, Muller M, Schrenk D, Walter P, Weber P (2009) Bioactive compounds: safety and efficacy. Nutrition 25:1206–1211

24. Finn CE, Thomas AL, Byers PL, Serce S (2008) Evaluation of American (*Sambucus canadensis*) and European (*S. nigra*) elderberry genotypes grown in diverse environments and implications for cultivar development. HortSci 43:1385–1391
25. Charlebois D (2007) Elderberry as a medicinal plant. In: Janick J, Whipkey A (eds) Issues in new crops and new uses. ASHS Press, Alexandria
26. Donoghue MJ, Bell CD, Winkworth RC (2003) The evolution of reproductive characters in *Dipsacales*. Int J Plant Sci 164:S453–S464
27. Janick J, Paull RE (2008) The encyclopedia of fruit & nuts. CABI, Oxfordshire
28. Kaack K (2008) Processing of aroma extracts from elder flower (*Sambucus nigra* L.) Eur Food Res Technol 227:375–390
29. Vítová E, Divišová R, Sůkalová K, Matějíček A (2013) Determination and quantification of volatile compounds in fruits of selected elderberry cultivars grown in Czech Republic. J Food Nutr Res 52:1–11
30. Lee J, Finn CE (2007) Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. J Sci Food Agric 87:2665–2675
31. Jensen K, Christensen LP, Hansen M, Jørgensen U, Kaack K (2001) Olfactory and quantitative analysis of volatiles in elderberry (*Sambucus nigra* L.) juice processed from seven cultivars. J Sci Food Agric 81:237–244
32. Veberic R, Jakopic J, Stampar F, Schmitzer V (2009) European elderberry (*Sambucus nigra* L.) rich in sugars, organic acids, anthocyanins and selected polyphenols. Food Chem 114:511–515
33. Kaack K, Austed T (1998) Interaction of vitamin C and flavonoids in elderberry (*Sambucus nigra* L.) during juice processing. Plant Foods Hum Nutr 52:187–198
34. Kaack K, Christensen L, Hughes M, Eder R (2006) Relationship between sensory quality and volatile compounds of elderflower (*Sambucus nigra* L.) extracts. Eur Food Res Technol 223:57–70
35. Kaack K, Christensen LP (2010) Phenolic acids and flavonoids in tea processed from flowers of black elder (*Sambucus nigra* L.) stored in different packing materials. Eur J Hortic Sci 75:214–220
36. Neto FC (2007) Sabugueiro – suas potencialidades. DRAP-Norte 1:1–16
37. CHMP Committe on Herbal Medicinal Products (2008) *Sambucus nigra* L., flos. European Medicines Agency Evaluation of Medicines for Human Use, London, pp 1–24
38. Mohebalian PM, Cernusca MM, Aguilar FX (2012) Discovering Niche Markets for Elderberry Juice in the United States. HortTechnology 22:556–566
39. Bermúdez-Soto MJ, Tomás-Barberán FA (2004) Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. Eur Food Res Technol 219:133–141
40. Sanderson H, Prendergast DV (2002) Commercial uses of wild and traditionally managed plants in England and Scotland. Centre for Economic Botany and Royal Botanical Gardens, Kew/Richmond, pp 1–133
41. Mollet B, Rowland I (2002) Functional foods: at the frontier between food and pharma. Curr Opin Biotechnol 13:483–485
42. Menrad K (2003) Market and marketing of functional food in Europe. J Food Eng 56:181–188
43. Roberfroid MB (2000) A European consensus of scientific concepts of functional foods. Nutrition 16:689–691
44. Zegler J (2016) Food & drink trends 2017. Mintel Group Ltd, London, UK
45. Roberfroid MB (2000) Concepts and strategy of functional food science: the European perspective. Am J Clin Nutr 71:1660–1664
46. Siró I, Kápolna E, Kápolna B, Lugasi A (2008) Functional food. Product development, marketing and consumer acceptance – a review. Appetite 51:456–467
47. Burdock GA, Carabin IG, Griffiths JC (2006) The importance of GRAS to the functional food and nutraceutical industries. Toxicology 221:17–27

48. Kwak N, Jukes DJ (2001) Functional foods. Part 1: the development of a regulatory concept. Food Control 12:99–107
49. Roberfroid MB (2002) Functional foods: concepts and application to inulin and oligofructose. Br J Nutr 87:139–143
50. Uncini Manganelli RE, Zaccaro L, Tomei PE (2005) Antiviral activity *in vitro* of *Urtica dioica* L., *Parietaria diffusa* and *Sambucus nigra* L. J Ethnopharmacol 98:323–327
51. Gray AM, Abdel-wahab YHA, Flatt PR, Al GET (2000) Biochemical and molecular action of nutrients the traditional plant treatment, *Sambucus nigra* (elder), exhibits insulin-like and insulin-releasing actions in vitro. J Nutr 130:15–20
52. Roschek B, Fink RC, McMichael MD, Li D, Alberte RS (2009) Elderberry flavonoids bind to and prevent H1N1 infection in vitro. Phytochemistry 70:1255–1261
53. Zakay-Rones Z, Varsano N, Zlotnik M, Manor O, Regev L, Schlesinger M, Mumcuoglu M (1995) Inhibition of several strains of influenza virus in vitro and reduction of symptoms by an elderberry extract (*Sambucus nigra* L.) during an outbreak of influenza B Panama. J Altern Complement Med 1:361–369
54. Frank J, Kamal-Eldin A, Lundh T, Määttä K, Törrönen R, Vessby B (2002) Effects of dietary anthocyanins on tocopherols and lipids in rats. J Agric Food Chem 50:7226–7230
55. Yoon M, Campbell JL, Andersen ME, Clewell HJ (2012) Quantitative in vitro to in vivo extrapolation of cell-based toxicity assay results. Crit Rev Toxicol 42:633–652
56. Higuera GA, Hendriks JAA, van Dalum J, Wu L, Schotel R, Moreira-Teixeira L, van den Doel M, Leijten JCH, Riesle J, Karperien M, van Blitterswijk CA, Moroni L (2013) In vivo screening of extracellular matrix components produced under multiple experimental conditions implanted in one animal. Integr Biol (Camb) 5:889–898
57. Rezaee R, Abdollahi M (2017) The importance of translatability in drug discovery. Expert Opin Drug Discovery 12:237–239
58. Chen Y, Li C, Long T, Che T, Min J (2014) Bioavailability of cranberry bean hydroalcoholic extract and its inhibitory effect against starch hydrolysis following in vitro gastrointestinal digestion. Food Res Int 64:939–945
59. Marhuenda J, Alemán MD, Gironés-vilaplana A, Pérez A, Caravaca G, Figueroa F, Mulero J, Zafrilla P (2016) Phenolic composition, antioxidant activity, and in vitro availability of four different berries. J Chem 2016:1–6
60. Etienne-mesmin L, Denis S (2012) Relevance and challenges in modeling human gastric and small intestinal digestion. Trends Biotechnol 30:591
61. Lake ALB, Tewart DES (2005) Assessing potential bioavailability of raspberry anthocyanins using an in vitro digestion system. J Agric Food Chem 53:15–17
62. Gil-izquierdo A, Gil I, Ferreres F, Toma FA (2001) In vitro availability of flavonoids and other phenolics in orange juice. J Agric Food Chem 49:1035–1041
63. Azzari MAF, Ukumoto LANAF, Azza GIM, Ivrea MAAL, Esoriere LUT, Arco LUDIM (2008) In vitro bioavailability of phenolic compounds from five cultivars of frozen sweet cherries (*Prunus avium* L.) J Agric Food Chem 56:3561–3568
64. Pérez-Vicente A, Gil-Izquierdo A, García-Viguera C (2002) In vitro gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. Food Chem 50:2308–2312
65. Doke SK, Dhawale SC (2015) Alternatives to animal testing: a review. Saudi Pharm J 23:223–229
66. Nair A, Jacob S (2016) A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 7:27–31
67. Devillers J, Pandard P, Thybaud E, Merle A (2009) Interspecies correlations for predicting the acute toxicity of xenobiotics. In: Devillers J (ed) Ecotoxicology modeling, vol 2. Springer Science+Business Media, LLC, New York, pp 84–115
68. Röhrig B, du Prel J-B, Wachtlin D, Blettner M (2009) Types of study in medical research. Dtsch Arztebl Int 106:262–268
69. Charles C (1995) Epidemiology faces its limits. Science 269:164–169

70. APhA (1972) Guidelines for biopharmaceutical studies in man. American Pharmaceutical Association Academy of Pharmaceutical Sciences, Washington, DC
71. McGhie TK, Ainge GD, Barnett LE, Cooney JM, Jensen DJ (2003) Anthocyanin glycosides from berry fruit are absorbed and excreted unmetabolized by both humans and rats. J Agric Food Chem 51:4539–4548
72. Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 13:572–584
73. Murkovic M, Mulleder U, Adam U, Pfannhauser W (2001) Detection of anthocyanins from elderberry juice in human urine. J Sci Food Agric 81:934–937
74. Cao GH, Prior RL (1999) Anthocyanins are defected in human plasma after oral administration of an elderberry extract. Clin Chem 45:574–576
75. Wu XL, Cao GH, Prior RL (2002) Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. J Nutr 132:1865–1871
76. Frank T, Janssen M, Netzel G, Christian B, Bitsch I, Netzel M (2007) Absorption and excretion of elderberry (*Sambucus nigra* L.) anthocyanins in healthy humans. Methods Find Exp Clin Pharmacol 29:525–533
77. Milbury PE, Cao G, Prior RL, Blumberg J (2002) Bioavailability of elderberry anthocyanins. Mech Ageing Dev 123:997–1006
78. Bitsch R, Netzel M, Sonntag S, Strass G, Frank T, Bitsch I (2004) Urinary excretion of cyanidin glucosides and glucuronides in healthy humans after elderberry juice ingestion. J Biomed Biotechnol 2004:343–345
79. Bitsch I, Janssen M, Netzel M, Strass G, Frank T (2004) Bioavailability of anthocyanidin-3-glycosides following consumption of elderberry extract and blackcurrant juice. Int J Clin Pharmacol Ther 42:293–300
80. Cao G, Muccitelli HU, Sanchez-Moreno C, Prior RL (2001) Anthocyanins are absorbed in glycated forms in elderly women: a pharmacokinetic study. Am J Clin Nutr 73:920–926
81. Frank T, Sonntag S, Strass G, Bitsch I, Bitsch R, Netzel M (2005) Urinary pharmacokinetics of cyanidin glycosides in healthy young men following consumption of elderberry juice. Int J Clin Pharmacol Res 25:47–56
82. Mulleder U, Murkovic M, Pfannhauser W (2002) Urinary excretion of cyanidin glycosides. J Biochem Biophys Methods 53:61–66
83. Netzel M, Strass G, Herbst M, Dietrich H, Bitsch R, Bitsch I, Frank T (2005) The excretion and biological antioxidant activity of elderberry antioxidants in healthy humans. Food Res Int 38:905–910
84. Wiseman H (1999) The bioavailability of non-nutrient plant factors: dietary flavonoids and phyto-oestrogens. Proc Nutr Soc 58:139–146
85. de Beer D, Joubert E, Gelderblom WCA, Manley M (2002) Phenolic compounds: a review of their possible role as in vivo antioxidants of wine. S Afr J Enol Vitic 23:48–61
86. Milbury PE, Vita JA, Blumberg JB (2010) Anthocyanins are bioavailable in humans following an acute dose of cranberry juice. J Nutr 140:1099–1104
87. Yi W, Akoh CC, Fischer J, Krewer G (2006) Absorption of anthocyanins from blueberry extracts by Caco-2 human intestinal cell monolayers. J Agric Food Chem 54:5651–5658
88. Kesarwani K, Gupta R (2013) Bioavailability enhancers of herbal origin: an overview. Asian Pac J Trop Biomed 3:253–266
89. Karakaya S (2017) Bioavailability of phenolic compounds bioavailability of phenolic compounds. Crit Rev Food Sci Nutr 44:453–464
90. Koli R, Erlund I, Jula A, Marniemi J, Mattila P, Alfthan G (2010) Bioavailability of various polyphenols from a diet containing moderate amounts of berries. J Agric Food Chem 58:3927–3932
91. Hollman P, Katan M (1997) Absorption, metabolism and health effects of dietary flavonoids in man. Biomed Pharmacother 51:305–310

92. Mena P, Domínguez-Perles R, Gironés-Vilaplana A, Baenas N, García-Viguera C, Villaño D (2014) Flavan-3-ols, anthocyanins, and inflammation. Int Union Biochem Mol Biol 66:745–758
93. Smith RE, Tran K, Richards KM, Ryan S, Luo R, Salvador ÂC, Silvestre AJD, Rocha SM (2014) Elderberry juice composition and health benefits. In: Elder KE (ed) Fruit juices: types, nutritional composition and health benefits – nutrition and diet research progress. Nova Science Publishers, Inc, New York, pp 1–20
94. Cunha S, Meireles D, Machado J, Cunha S, Machado J (2016) Sambucus nigra – a promising natural source for human health. Exp Pathol Heal Sci 8:59–66
95. Salvador ÂC, Silvestre AJD, Rocha SM (2016) *Sambucus nigra* L.: a potential source of health-promoting components. In: Atta-ur-Rahman F (ed) Frontiers in natural product chemistry, vol 2. Bentham Science Publishers, Ltd., Sharjah, pp 343–392
96. Krawitz C, Abu Mraheil M, Stein M, Imirzalioglu C, Domann E, Pleschka S, Hain T (2011) Inhibitory activity of a standardized elderberry liquid extract against clinically-relevant human respiratory bacterial pathogens and influenza A and B viruses. BMC Complement Altern Med 11:1–6
97. Chatterjee A, Yasmin T, Bagchi D, Stohs SJ (2004) Inhibition of *Helicobacter pylori in vitro* by various berry extracts, with enhanced susceptibility to clarithromycin. Mol Cell Biochem 265:19–26
98. Izzo AA, Di Carlo G, Biscardi D, De Fusco R, Mascolo N, Borrelli F, Capasso F (1995) Biological screening of Italian medicinal plants for antibacterial activity. Phytother Res 9:281–286
99. Porter RS (2017) A review of the antiviral properties of black elder (*Sambucus nigra* L.) products. Phytother Res 554:533–554
100. Zakay-Rones Z, Thom E, Wollan T, Wadstein J (2004) Randomized study of the efficacy and safety of oral elderberry extract in the treatment of influenza A and B virus infections. J Int Med Res 32:132–140
101. Vlachojannis JE, Cameron M, Chrubasik S (2010) A systematic review on the Sambuci fructus effect and efficacy profiles. Phytother Res 24:1–8
102. Fink RC, Roschek BJ, Alberte RS (2009) HIV type-1 entry inhibitors with a new mode of action. Antivir Chem Chemother 19:243–255
103. Serkedjieva J, Manolova N, Zgórniak-Nowosielska I, Zawilińska B, Grzybek J (1990) Antiviral activity of the infusion (SHS-174) from flowers of *Sambucus nigra* L., aerial parts of *Hypericum perforatum* L., and roots of *Saponaria officinalis* L. against influenza and herpes simplex viruses. Phytother Res 4:97–100
104. Castillo-maldonado I, Moreno-altamirano MMB, Serrano-gallardo LB (2017) Anti-dengue serotype-2 activity effect of *Sambucus nigra* leaves-and flowers-derived compounds. Virol Res Rev 1:1–5
105. Burge B, Mumcuoglu M, Simmons T (1999) The effect of Sambucol on flu-like symptoms in chimpanzees: prophylactic and symptom-dependent treatment. Int Zoo News 46:16–19
106. Kong F (2009) Pilot clinical study on a proprietary elderberry extract: efficacy in addressing influenza symptoms Fan-kun. J Pharmacol 5:32–43
107. Sies H (1991) Oxidative stress: from basic research to clinical application. Am J Med 91:31S–38S
108. Halliwell B (1991) Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 91:14S–22S
109. Jacobo-Velázquez DA, Cisneros-Zevallos L (2009) Correlations of antioxidant activity against phenolic content revisited: a new approach in data analysis for food and medicinal plants. J Food Sci 74:R107–R113
110. Halvorsen BL, Holte K, Myhrstad MCW, Barikmo I, Hvattum E, Remberg SF, Wold A-B, Haffner K, Baugerød H, Andersen LF, Moskaug Ø, Jacobs DR, Blomhoff RA (2002) Systematic screening of total antioxidants in dietary plants. J Nutr 132:461–471
111. Haytowitz DB, Bhagwat S (2010) USDA database for the oxygen radical absorbance capacity (ORAC) of selected foods, release 2; Maryland

112. Lugasi A, Hóvári J (2003) Antioxidant properties of commercial alcoholic and nonalcoholic beverages. Nahrung/Food 47:79–86
113. Espín JC, Soler-Rivas C, Wichers HJ, García-Viguera C (2000) Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. J Agric Food Chem 48:1588–1592
114. Pool-Zobel BL, Bub A, Schröder N, Rechkemmer G (1999) Anthocyanins are potent antioxidants in model systems but do not reduce endogenous oxidative DNA damage in human colon cells. Eur J Nutr 38:227–234
115. Dawidowicz AL, Wianowska D, Baraniak B (2006) The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. (antioxidant properties of extracts). LWT Food Sci Technol 39:308–315
116. Harokopakis E, Albzreh MH, Haase EM, Scannapieco FA, Hajishengallis G (2006) Inhibition of proinflammatory activities of major periodontal pathogens by aqueous extracts from elder flower (*Sambucus nigra*). J Periodontol 77:271–279
117. Stoilova I, Wilker M, Stoyanova A, Krastanov A, Stanchev V (2007) Antioxidant activity of extract from elder flower (*Sambucus nigra* L.) Herba Pol 53:45–54
118. Rieger G, Muller M, Guttenberger H, Bucar F (2008) Influence of altitudinal variation on the content of phenolic compounds in wild populations of *Calluna vulgaris*, *Sambucus nigra*, and *Vaccinium myrtillus*. J Agric Food Chem 56:9080–9086
119. Buřičová L, Réblová Z (2008) Czech medicinal plants as possible sources of antioxidants. Czech J Food Sci 26:132–138
120. Çelik SE, Özyürek M, Güçlü K, Çapanoğlu E, Apak R (2014) Identification and anti-oxidant capacity determination of phenolics and their glycosides in elderflower by on-line HPLC-CUPRAC method. Phytochem Anal 25:147–154
121. Mikulic-Petkovsek M, Samoticha J, Eler K, Stampar F, Veberic R (2015) Traditional elderflower beverages: a rich source of phenolic compounds with high antioxidant activity. J Agric Food Chem 63:1477–1487
122. Abuja PM, Murkovic M, Pfannhauser W (1998) Antioxidant and prooxidant activities of elderberry (*Sambucus nigra*) extract in low-density lipoprotein oxidation. J Agric Food Chem 46:4091–4096
123. Youdim KA, Martin A, Joseph JA (2000) Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress. Free Radic Biol Med 29:51–60
124. Wu XL, Gu LW, Prior RL, McKay S (2004) Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. J Agric Food Chem 52:7846–7856
125. Nakajima J, Tanaka I, Seo S, Yamazaki M, Saito K (2004) LC/PDA/ESI-MS profiling and radical scavenging activity of anthocyanins in various berries. J Biomed Biotechnol 2004:241–247
126. Lichtenthäler R, Marx F (2005) Total oxidant scavenging capacities of common European fruit and vegetable juices. J Agric Food Chem 53:103–110
127. Bobek P, Nosal'Ova V, Cerna S (2001) Influence of diet containing extract of black elder (*Sambucus nigra*) on colitis in rats. Biol (Bratisl) 56:643–648
128. Ciocoiu M, Badescu M, Badulescu O, Badescu L (2017) The beneficial effects on blood pressure, dyslipidemia and oxidative stress of Sambucus nigra extract associated with renin inhibitors. Pharm Biol 54:3063–3067
129. Murkovic M, Abuja PM, Bergmann AR, Zirngast A, Adam U, Winklhofer-Roob BM, Toplak H (2004) Effects of elderberry juice on fasting and postprandial serum lipids and low-density lipoprotein oxidation in healthy volunteers: a randomized, double-blind, placebo-controlled study. Eur J Clin Nutr 58:244–249
130. Sadowska K, Andrzejewska J, Kl Ł (2017) Influence of freezing, lyophilisation and air-drying on the total monomeric anthocyanins, vitamin C and antioxidant capacity of selected berries. Int J Food Sci Technol 52:1246–1251
131. Zhou N, Zhu W, Yang F, Kequan Z (2016) In vitro gastrointestinal digestion model to monitor the antioxidant properties and bioavailability of phenolic antioxidants from elderberry. React Oxyg Species 2:421–431

132. Olejnik A, Olkowicz M, Kowalska K, Rychlik J, Dembczyn R, Myszka K, Juzwa W, Białas W, Pat M (2016) Gastrointestinal digested *Sambucus nigra* L. fruit extract protects *in vitro* cultured human colon cells against oxidative stress. Food Chem 197:648–657
133. Olejnik A, Kowalska K, Olkowicz M, Rychlik J, Juzwa W, Myszka K (2015) Anti-inflammatory effects of gastrointestinal digested *Sambucus nigra* L. fruit extract analysed in co-cultured intestinal epithelial cells and lipopolysaccharide-stimulated macrophages. J Funct Foods 19:649–660
134. Debnath T, Kim DH, Lim BO (2013) Natural products as a source of anti-inflammatory agents associated with inflammatory bowel disease. Molecules 2:7253–7270
135. Thanh G, Ho T, Wangensteen H, Barsett H (2017) Elderberry and elderflower extracts, phenolic compounds, and metabolites and their effect on complement, RAW 264.7 macrophages and dendritic cells. Int J Mol Sci 18:584
136. Gorchakova T, Suprun I, Sobenin I, Orekhov A (2007) Use of natural products in anticytokine therapy. Bull Exp Biol Med 143:316–319
137. Yeşilada E, Üstün O, Sezik E, Takaishi Y, Ono Y, Honda G (1997) Inhibitory effects of Turkish folk remedies on inflammatory cytokines: interleukin-1α, interleukin-1β and tumor necrosis factor α. J Ethnopharmacol 58:59–73
138. Moresco EMY, Lavine D, Beutler B (2011) Toll-like receptors. Curr Biol 21:R488–R493
139. Barak V, Birkenfeld S, Halperin T, Kalickman I (2002) The effect of herbal remedies on the production of human inflammatory and anti-inflammatory cytokines. Isr Med Assoc J 4:919–922
140. Barak V, Halperin T, Kalickman I (2001) The effect of Sambucol, a black elderberry-based, natural product, on the production of human cytokines: I. Inflammatory cytokines. Eur Cytokine Netw 12:290–296
141. Thanh G, Ho T, Zou Y, Aslaksen TH, Wangensteen H, Barsett H (2016) Structural characterization of bioactive pectic polysaccharides from elderflowers (Sambuci flos). Carbohydr Polym 135:128–137
142. Thanh G, Ho T, Zou Y, Wangensteen H, Barsett H (2016) RG-I regions from elderflower pectins substituted on GalA are strong immunomodulators. Int J Biol Macromol 92:731–738
143. Mascolo N, Autore G, Capasso F, Menghini A, Fasulo MP (1995) Biological screening of Italian medicinal plants for anti-inflammatory activity. Phytother Res 9:281–286
144. Curtis PJ, Kroon PA, Hollands WJ, Walls R, Jenkins G, Kay CD, Cassidy A (2009) Cardiovascular disease risk biomarkers and liver and kidney function are not altered in postmenopausal women after ingesting an elderberry extract rich in extract rich in anthocyanins for 12 weeks. J Nutr 139:2266–2271
145. Chrubasik C, Maier T, Dawid C, Torda T, Schieber A, Hofmann T, Chrubasik S (2008) An observational study and quantification of the actives in a supplement with *Sambucus nigra* and *Asparagus officinalis* used for weight reduction. Phytother Res 22:913–918
146. Singh A, Holvoet S, Mercenier A (2011) Dietary polyphenols in the prevention and treatment of allergic diseases. Clin Exp Allergy 41:1346–1359
147. Tanaka T, Kouda K, Kotani M, Takeuchi A, Tabei T (2001) Vegetarian diet ameliorates symptoms of atopic dermatitis through reduction of the number of peripheral eosinophils and of PGE2 synthesis by monocytes. J Physiol Anthropol Appl Hum Sci 20:353–361
148. Pericleous M, Mandair D, Caplin ME (2013) Diet and supplements and their impact on colorectal cancer. J Gastrointest Oncol 4:409–423
149. Jing P, Bomser JA, Schwartz SJ, He J, Magnuson BA, Giusti MM (2008) Structure−function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell crowth. J Agric Food Chem 56:9391–9398
150. Thole JM, Kraft TFB, Sueiro LA, Kang Y-H, Gills JJ, Cuendet M, Pezzuto JM, Seigler DS, Lila MA (2006) A comparative evaluation of the anticancer properties of European and American elderberry fruits. J Med Food 9:498–504
151. Cuendet M, Oteham CP, Moon RC, Pezzuto JM (2006) Quinone reductase induction as a biomarker for cancer chemoprevention. J Nat Prod 69:460–463

152. Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15:539–553
153. Christensen KB, Petersen RK, Kristiansen K, Christensen LP (2010) Identification of bioactive compounds from flowers of black elder (*Sambucus nigra* L.) that activate the human peroxisome proliferator-activated receptor (PPAR) gamma. Phytother Res 24:S129–S132
154. Christensen KB, Minet A, Svenstrup H, Grevsen K, Zhang H, Schrader E, Rimbach G, Wein S, Wolffram S, Kristiansen K, Christensen LP (2009) Identification of plant extracts with potential antidiabetic properties: effect on human peroxisome proliferator-activated receptor (PPAR), adipocyte differentiation and insulin-stimulated glucose uptake. Phytother Res 23:1316–1325
155. Raafat K, El-Lakany A (2015) Acute and subchronic in-vivo effects of *Ferula hermonis* L. and *Sambucus nigra* L. and their potential active isolates in a diabetic mouse model of neuropathic pain. BMC Complement Altern Med 15:1–14
156. Bhattacharya S, Christensen KB, Olsen LCB, Christensen LP, Grevsen K, Færgeman NJ, Kristiansen K, Young JF, Oksbjerg N (2013) Bioactive components from flowers of *Sambucus nigra* L. increase glucose uptake in primary porcine myotube cultures and reduce fat accumulation in *Caenorhabditis elegans*. J Agric Food Chem 61:11033–11040
157. Beaux D, Fleurentin J, Mortier F (1999) Effect of extracts of *Orthosiphon stamineus benth*, *Hieracium pilosella* L., *Sambucus nigra* L. and *Arctostaphylos uva-ursi* (L.) spreng. in rats. Phytother Res 13:222–225
158. Ciocoiu M, Mirón A, Mares L, Tutunaru D, Pohaci C, Groza M, Badescu M (2009) The effects of *Sambucus nigra* polyphenols on oxidative stress and metabolic disorders in experimental diabetes mellitus. J Physiol Biochem 65:297–304
159. Groza M, Jitaru D, Badescu L, Ciocoiu M, Badescu M, Descu MBĂ (2011) Evaluation of the immune defense in diabetes mellitus using an experimental model. Rom Biotechnol Lett 16:5971–5979
160. Badescu L, Badulescu O, Badescu M, Ciocoiu M (2012) Mechanism by *Sambucus nigra* extract improves bone mineral density in experimental diabetes. Evid Based Complement Alternat Med 2012:848269
161. Ciocoiu M, Badescu L, Badulescu O, Tutunaru D, Badescu M (2012) Protective intervention of *Sambucus nigra* polyphenols in the diabetic heart. Ann Rom Soc Cell Biol 17:312–317
162. Groza M, Ciocoiu MBL, Oana B, Magda B, Tudent PHDS (2010) The effects of the *Sambucus nigra* vegetal extracts on the immune system dysfunction in the diabetes mellitus. Ann Rom Soc Cell Biol 15:241–246
163. Ciocoiu M, Tutunaru D, Badescu L, Furnica R, Badescu M (2003) Beneficial effects of various plant polyphenols on diabetic angiopathy. Ann Rom Soc Cell Biol 14:193–198
164. Bembde AS (2012) A study of plasma fibrinogen level in type-2 diabetes mellitus and its relation to glycemic control. Indian J Hematol Blood Transfus 28:105–108
165. Badescu M, Badulescu O, Badescu L, Ciocoiu M (2015) Effects of *Sambucus nigra* and *Aronia melanocarpa* extracts on immune system disorders within diabetes mellitus. Pharm Biol 53:533–539
166. Johansen JS, Harris AK, Rychly DJ, Ergul A (2005) Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol 11:1–11
167. Opris R, Tatomir C, Olteanu D, Moldovan R, Moldovan B, David L, Nagy A, Decea N, Ludovic M, Adriana G (2017) The effect of *Sambucus nigra* L. extract and phytosynthesized gold nanoparticles on diabetic rats. Colloids Surf B: Biointerfaces 150:192–200
168. Ruhe RC, McDonald RB (2001) Use of antioxidant nutrients in the prevention and treatment of type 2 diabetes. J Am Coll Nutr 20:363S–369S
169. Mohamed KA, Bierhaus A, Schiekofer S, Tritschler H, Ziegler R, Nawroth PP (1999) The role of oxidative stress and NF-κB activation in late diabetic complications. Biofactors 10:157–167
170. Dewanjee S, Maiti A, Sahu R, Dua TK, Mandal V (2011) Effective control of type 2 diabetes through antioxidant defense by edible fruits of *Diospyros peregrina*. Evid Based Complement Alternat Med 2011:1–7

171. Huynh K, Bernardo BC, McMullen JR, Ritchie RH (2014) Diabetic cardiomyopathy: mechanisms and new treatment strategies targeting antioxidant signaling pathways. Pharmacol Ther 142:375–415
172. Lehmann H, Pabst J-Y (2016) La phytovigilance: impératif médical et obligation légale. Ann Pharm Fr 74:49–60
173. Maixent J (2015) Opinion paper food supplements: the European regulation and its application in France. Thoughts on safety of food supplements. Cell Mol Biol 58:OL1720–OL1729
174. Jensen S, Nielsen B (1973) Cyanogenic glucosides in *Sambucus nigra* L. Acta Chem Scand 27:2661–2685
175. Senica M, Stampar F, Veberic R, Mikulic-Petkovsek M (2017) The higher the better? Differences in phenolics and cyanogenic glycosides in *Sambucus nigra* leaves, flowers and berries from different altitudes. J Sci Food Agric 97:2623–2632
176. Williamson E, Driver S, Baxter K (eds) (2009) Stockley's herbal medicines interactions. Pharmaceutical Press, London
177. Senica M, Stampar F, Veberic R, Mikulic-Petkovsek M (2016) Processed elderberry (*Sambucus nigra* L.) products: a beneficial or harmful food alternative? LWT Food Sci Technol 72:182–188
178. Shahidi-Noghabi S, Van Damme EJM, Smagghe G (2008) Carbohydrate-binding activity of the type-2 ribosome-inactivating protein SNA-I from elderberry (*Sambucus nigra*) is a determining factor for its insecticidal activity. Phytochemistry 69:2972–2978
179. Shibuya N, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ (1987) The elderberry (*Sambucus nigra* L.) bark lectin recognizes the Neu5Ac(alpha 2-6)Gal/GalNAc sequence. J Biol Chem 262:1596–1601
180. Van Damme EJM, Peumans WJ, Barre A, Rouge P (1998) Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. Crit Rev Plant Sci 17:575–692
181. Förster-Waldl E, Marchetti M, Schöll I, Focke M, Radauer C, Kinaciyan T, Nentwich I, Jäger S, Schmid E, Boltz-Nitulescu G, Scheiner O, Jensen-Jarolim E (2003) Type I allergy to elderberry (*Sambucus nigra*) is elicited by a 33.2 kDa allergen with significant homology to ribosomal inactivating proteins. Clin Exp Allergy 33:1703–1710
182. Gardiner P, Phillips R, Shaughnessy A (2008) Herbal and dietary supplement–drug interactions in patients with chronic illnesses. Am Fam Physician 77:73–78
183. Jakovljevic V, Popovic M, Mímica-Dukic N, Sabo J (2001) Interaction of *Sambucus nigra* flower and berry decoctions with the actions of centrally acting drugs in rats Pharmaceutical. Pharm Biol 39:142–145

Bioactive Compounds and Health Benefits of Jamun (*Syzygium cumini*)

78

Shalini S. Arya, Kakoli Pegu, and Prajakta D. Sadawarte

Contents

Abstract

Jamun (*Syzygium cumini*) which is indigenous to India has been used as medicine for century in Unani and Ayurveda. The presence of bioactive compounds such as alkaloids, tannins, phenols, lipids, flavonoids in its leaves, barks, fruits, stems,

S. S. Arya (✉) · K. Pegu · P. D. Sadawarte
Food Engineering and Technology Department, Institute of Chemical Technology, Mumbai, India
e-mail: shalu.ghodke@gmail.com; ss.arya@ictmumbai.edu.in; kpegu0714@gmail.com; prajaktasadawarte9@gmail.com

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6_56

and roots contributes to rich source for nutrition and medicine. Due to the presence these compounds, they have pharmacological effects with antioxidant, antimicrobial, antidiabetic, central nervous system activity (CNS), chemo preventive, anti-inflammatory, antiallergic, hepatoprotective, etc. properties. Jamun is commonly known for its antidiabetic activity as it has been proved to be the most promising nutraceutical value. Bioactive compounds are result of evolution, which may be due to specific requirement of plant by mutualistic or antagonistic interaction with another organism. The structure, action, metabolism, and health benefits of bioactive compounds in Jamun have been discussed in this article.

Keywords
Anthocyanin · Bioactive compounds · Biosynthetic pathway · Human anatomy · Pharmacology · *Syzygium cumini* · Bioavailability

1 Introduction

Bioactive compounds are secondary metabolites, natural products that originate from plant, animal, marine, and microorganisms. The early day's plant extracts have been used for the treatment of various diseases. And it is the secondary metabolites of plants that have pharmacological and toxicological effects in animal and human being. Secondary metabolites are produced in the phase subsequent to plant growth and development besides primary biosynthesis. They are not needed for daily functioning of plant and hence are regarded as side tracks. Several of them are found to hold various types of important functions in the living plants such as protection, attraction, or signaling. Metabolic pathways of plant leading to the formation of bioactive compounds are shown in Fig. 1 following the erythrose-4-phospahtes and pyruvate primary pathways. Production of secondary metabolites by plants is a result of evolution of mutualistic (volatiles, pigments, and floral scent for attracting pollinators) or antagonistic (synthesis of toxic compounds as a function of defense against pathogens and herbivores) interaction [1]. They are classified in three classes: (i) phenolics are aromatic components that are synthesized through acetyl-coA and erythrose-4-phosphate; (ii) terpenes are organic compounds that have the properties that contribute in scent, flavor, and color; and (iii) nitrogen-containing secondary metabolites (e.g., alkaloids) that are synthesized primarily from amino acids. It has been proposed that by utilizing the bioactive properties of secondary metabolites by animal can provide a "treatment" against various challenges that perturb homeostasis in animals [2]. Secondary metabolites that are toxic at higher dose have pharmacological effects on humans and animals. Thus, bioactive compounds in plants benefit humans and animals.

Jamun is plum fruit with sour-sweet taste, dark purple in color, and round-oval shape. The color is due to the presence of anthocyanins and astringency taste due to tannins. Jamun is known by many names: botanical name (i.e., *Syzygium cumini*), Indian blackberry, jambu, mahaphala, javaplum, Malabar plum, duhat, jambolana, mesegerak, jamelonguier, jamblang, jambolana, and kavika [3]. Jamun is native to

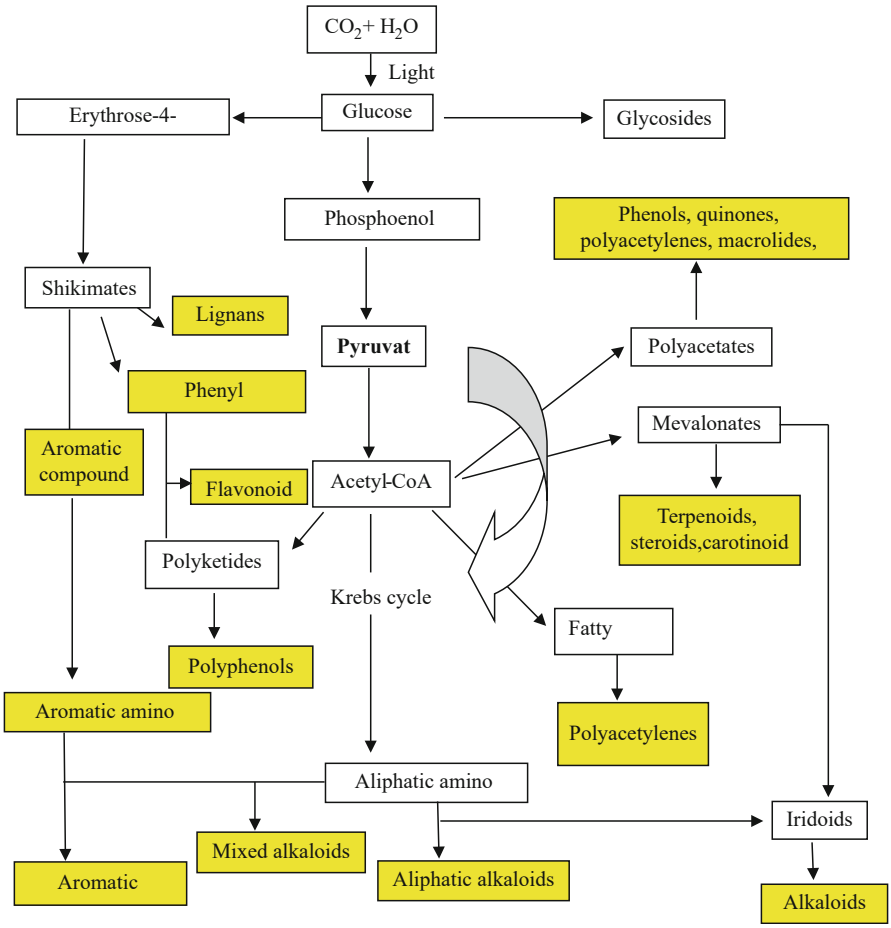


Fig. 1 Biosynthetic pathways of plants. The highlighted compounds are the bioactive compounds produced during plant metabolism [5]

Asian subcontinent, adjoining regions of Southeast Asia, China, and Queensland and nowadays is found growing throughout Eastern Africa, South America, and Madagascar [4]. The extracts of Jamun parts have been in use for the prevention and curing of various diseases and today it is commonly known for its antidiabetic property. Parts of Jamun having therapeutic effects are shown in Fig. 2. It has the ayurvedic characteristics such as RASA: Kasaya (astringent), amal (sour), madhura (sweet); ANURASA (aftertaste): kasaya (astringent); VIRYA (potency): sheeta (cold); VIPAKA (resultant): katu (pungent). The extracts of Jamun parts have been in use for prevention and curing of various diseases for centuries and today it is commonly known for its anti-diabetic property. Essential oils like lauric acid, phytochemicals, lipids, phenols with their antioxidant property, present in Jamun, have medicinal effects.

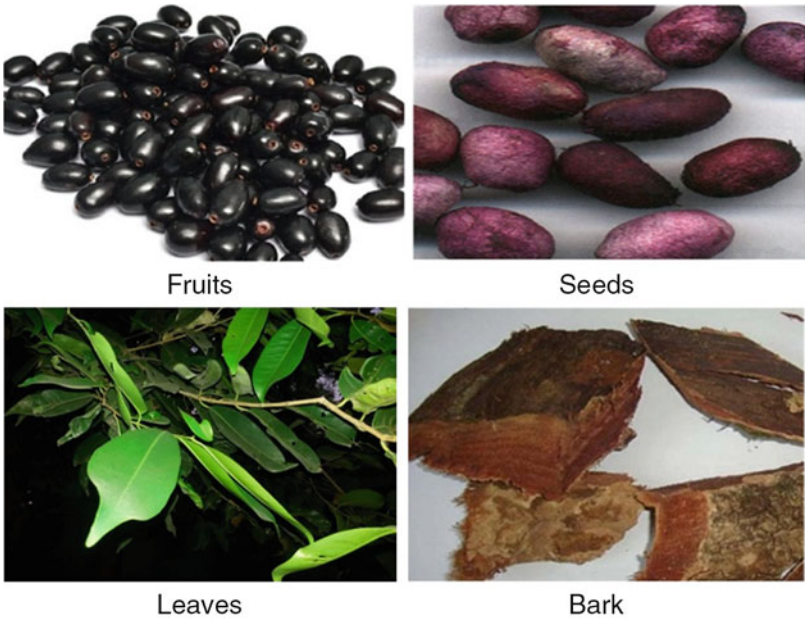


Fig. 2 Parts of Jamun (*S. cumini*)

2 Phytochemistry of Jamun

Jamun is a rich source of phytochemicals in its different parts: leaves, fruit, seed, and bark. Studies have showed the presence of phenols, flavonoids, alkaloids, glycosides, steroids, cardiac glycosides, saponins, terpenoid, and tannins in the Jamun leave extract [6, 7]. The abundant constituents of the oils in Jamun leaves are: α-pinene (32.32%), β-pinene (12.44%), trans-caryophyllene (11.19%), 1, 3, 6-octatriene (8.41%), delta-3-carene (5.55%), α-caryophyllene (4.36%), and α-limonene (3.42%) [8]. *Syzygium cumini* seed oil was found to contain lauric (2–8%), myristic (31.7%), palmitic (4–7%), stearic (6.5%), oleic (32.2%), linoleic (16.1%), malvalic (1.2%), sterculic (1.8%), and vernolic (3.0%) acids [9]. Chemicals present in different parts of Jamun are given in Table 1.

References [10] and [11] have reviewed the potential food application of Jamun and its health benefits and found that seed and pulp have antidiabetic, antimicrobial, and antioxidant effect, leaf buds have laxative effects, and bark have wound healing effects. They also suggested that these beneficial contributions of Jamun parts are due to the phytochemicals present in them. References [12] and [13] have reported that phenolic, flavonoid, and anthocyanin content of Jamun have antioxidant activity that involves inhibition of α-glucosidase which is vital in management of diabetes mellitus and prevention of oxidative cell damage.

Table 1 Phytochemicals present in the Jamun plant

Sr. No	Plant part	Chemicals present
1	Seeds	Jambosine, gallic acid, ellagic acid, corilagin, 3,6-hexahydroxy diphenoylglucose, 1-galloylglucose, 3-galloylglucose, quercetin, *β*-sitosterol, 4,6 hexahydroxydiphenoylglucose
2	Stem bark	Friedelin, friedelan-3-*α*-ol, betulinic acid, *β*-sitosterol, kaempferol, *β*-sitosterol-D-glucoside, gallic acid, ellagic acid, gallotannin and ellagitannin, and myricetin
3	Flowers	Oleanolic acid, ellagic acids, isoquercetin, quercetin, kaempferol, and myricetin
4	Fruit pulp	Anthocyanins, delphinidin, petunidin, malvidin-diglucosides
5	Leaves	*β*-sitosterol, betulinic acid, mycaminose, crategolic (maslinic) acid, n-hepatcosane, n-nonacosane, n-hentriacontane,noctacosanol, n-triacontanol, n-dotricontanol, quercetin, myricetin, myricitrin and the flavonol glycosides myricetin 3-O-(4″-acetyl)-*α* L-rhamnopyranosides
6	Essential oils	*α*-Terpineol, myrtenol, eucarvone, muurolol, *α*-myrtenal, 1, 8-cineole, geranyl acetone, *α*-cadinol and pinocarvone

3 Bioactive Compounds Present in Jamun

3.1 Terpenes

Terpenes are organic compounds that consist of multiple isoprenes units. They have neuroprotective, antitumorigenic, anti-inflammatory, antimicrobial, antifungal, antiviral, antihyperglycemic, and antiparasitic activities [14, 15]. They also have pleasant odor; thus, they are used in fragrance, as additives in food, and pharmaceutical industries. Terpenes present in *S. cumini* are monoterpenoids (1,8-cineole, linalool oxide, mysterol, nerol, terpinolene, α-terpeneol, α –terpenene, β-phellandrene, β-pineme, citronellol, eugenol) and triterpenoids (acetyl oleanolic acid, betulinic acid, oleanolic acid, β-sitosterol), and some of the structures are shown in Fig. 3. 2- and 3-Hydroxylated products of 1,8-cineole catalyzed by the isoenzyme CYP3A4 were seen in human liver microsome and also in human urine, and two other hydroxylated products 7- and 9-hydroxy-1,8-cineole are also identified [16].

3.2 Flavonoids

The basic structure of flavonoids is made up of skeletal diphenylpropane, namely, two benzene rings linked by a three-carbon chain that forms a closed pyran ring (heterocyclic ring containing oxygen) with benzenic ring. Therefore, their structure is also referred to as C_6-C_3-C_6 and is subdivided into six group by the nature of C_3 elements: Flavones, isoflavones, flavanones, flavanols, anthocyanidins, and flavan-3-ols [17]. Flavonoids are known for its antioxidant with anti-inflammatory activity in human and its bioavailability, metabolism, and biological activity of flavonoids depend upon the

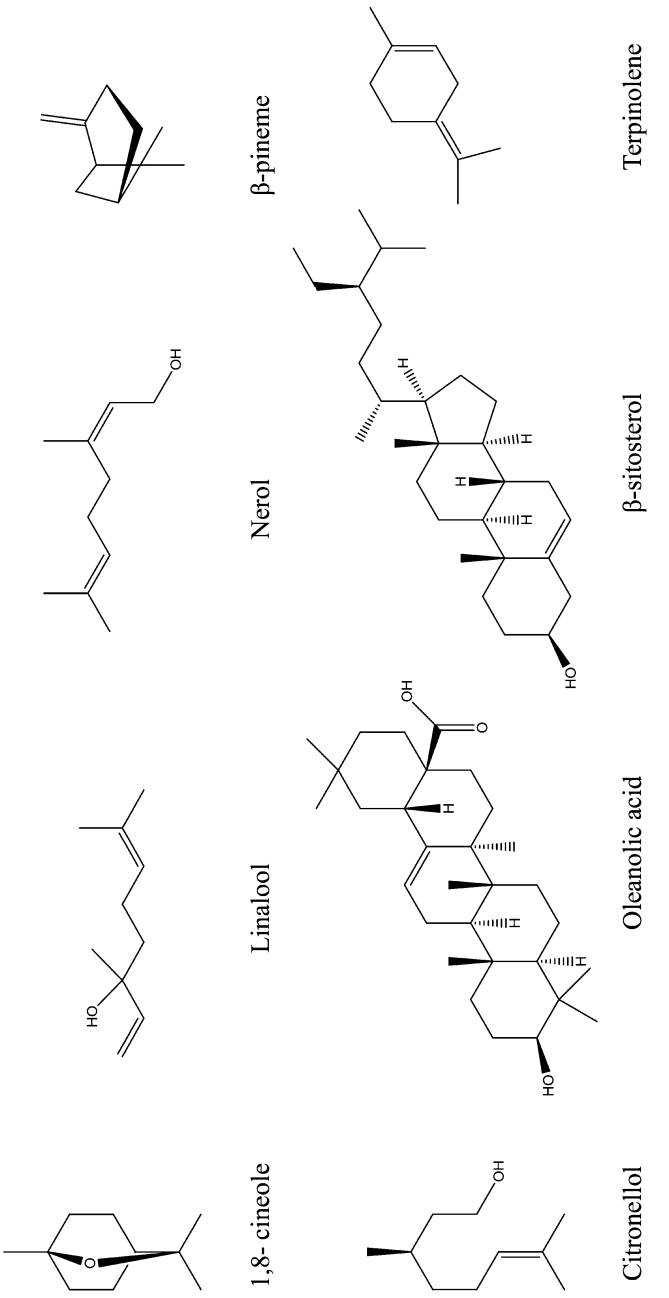


Fig. 3 Structures of terpenes found in Jamun (*S. cumini*)

configuration, total number of hydroxyl groups, and substitution of functional groups about their nuclear structure [18]. Flavonoids present in *S. cumini* are isoquercetin, kaempferol, malvidin (anthocyanin), malvidin-3-O-L-d-laminaribioside (anthocyanin), malvidin-diglucosides (anthocyanins), myricetin, petunidin, quercetin (flavanol), anthocyanins, cyanidin diglycoside, delphinidin-3-o-ß-d-gentiobiosid (anthocyanin), and delphinidin-3-gentiobioside (anthocyanin) as shown in Fig. 4. Of all the flavonoids present, anthocyanin is the most important one as they are present in bulk and the color of *S. cumini* fruit is the result of this. Reginold jebitta and Jeyanth allwin [19] have studied the phytochemicals content of *S. cumini* pulp powder considering different drying methods and found that freeze drying at −40 °C giving high antioxidant property (70.4–75.8%) and total flavonoids (104.8 mg quercetin equivalents (QE)/g), total phenolic (13.99 mg GA equivalents/g), and anthocyanin (7.56 mg/g) content. Five anthocyanins are yield on hydrolysis of anthocyanin (0.08%) as detected by HPLC and confirmed by mass spectral analysis namely delphinidin (20.3%), cyanidin (6.6%), petunidin (24.6%), peonidin (2.8%), and malvidin (44.2%) [20].

Kay [21], Clifford, and Brown [22] have describe the metabolism of anthocyanin in human by flavonoid metabolism as reference. The flavonoid found in food may be either in glycosylated (e.g., aglycones) or nonglycosylated form or in both form. The nonglycosylated flavonoids are absorbed through passive diffusion, and the glycosylated form may follow either of the two routes: sodium-glucose co-transporter (transport of intact glucoside, e.g., anthocyanin) in the enterocytes or at the brush border via lactate phlorizin hydrolase. And the metabolites that are not hydrolyzed by these enzymes are absorbed in the colon but influence the liberated aglycones.

3.3 Other Bioactive Compounds in Jamun (*S. cumini*)

Other bioactive compounds in Jamun are: lipid (lauric acid, linoleic acid, N-hentriacontane, N- nanocosane, stearic acid), alkane (malic acid and citric acid); phenols like ferulic acid and caffeic acid, and phenylpropanoid (cinnamaldehyde, cinnamyl acetate, cinnamyl alcohol, coniferyl alcohol) as shown in Fig. 5.

3.4 Summary of General Metabolic Pathway of Bioactive Compounds in Human Anatomy

When we talk about metabolism of bioactive compounds in human body, we come across two terms: bioavailability and bioefficacy. Bioavailability refers to the extent and rate at which the active moiety (drug or metabolite) enters systemic circulation, thereby accessing the site of action. Bioefficacy is a unit of measure to show levels of effectiveness by measuring changes to anatomy and physiology. Bioavailability of bioactive compounds is affected by its structure (high molecular weight compounds need to be broken down so that they can pass through intestinal cells) and transport mechanism (facilitated diffusion, passive diffusion, and active transport) [23]. In order to gain beneficial effects of bioactive compounds, they must have to be bioavailable.

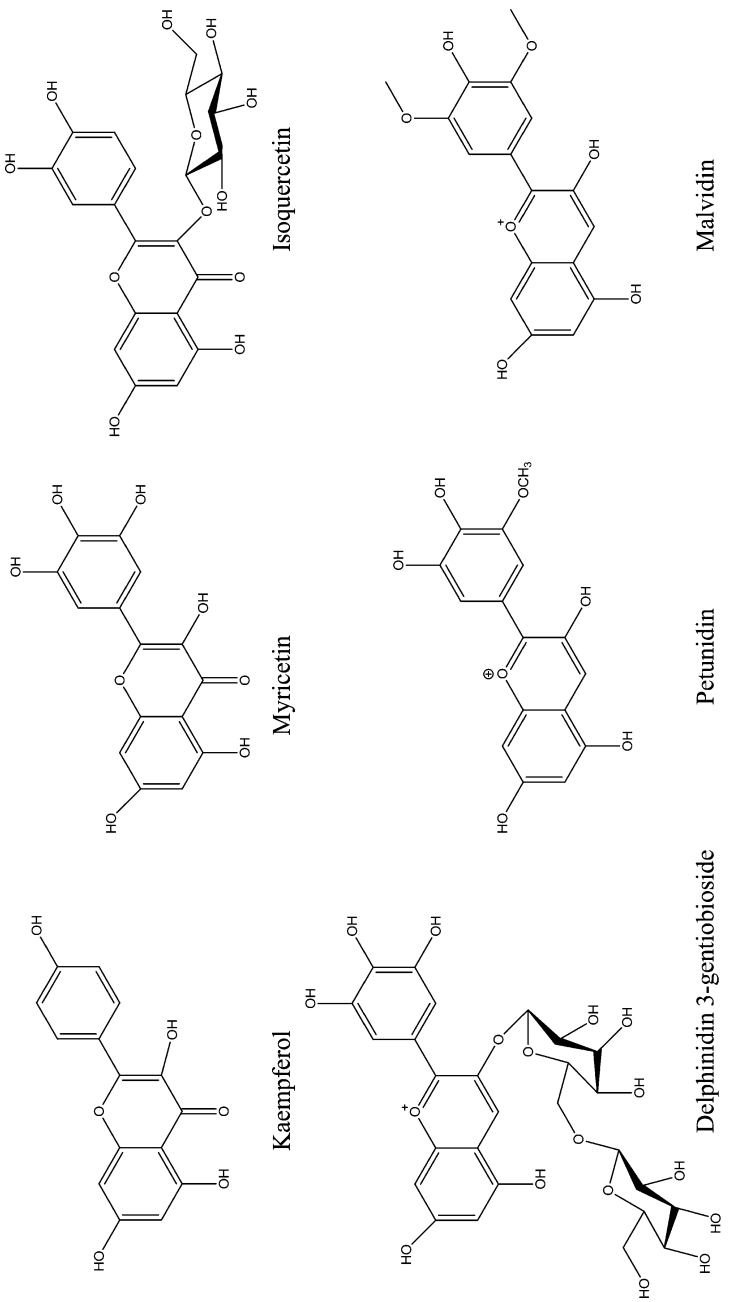


Fig. 4 Structures of some flavonoids found in Jamun (*S. cumini*)

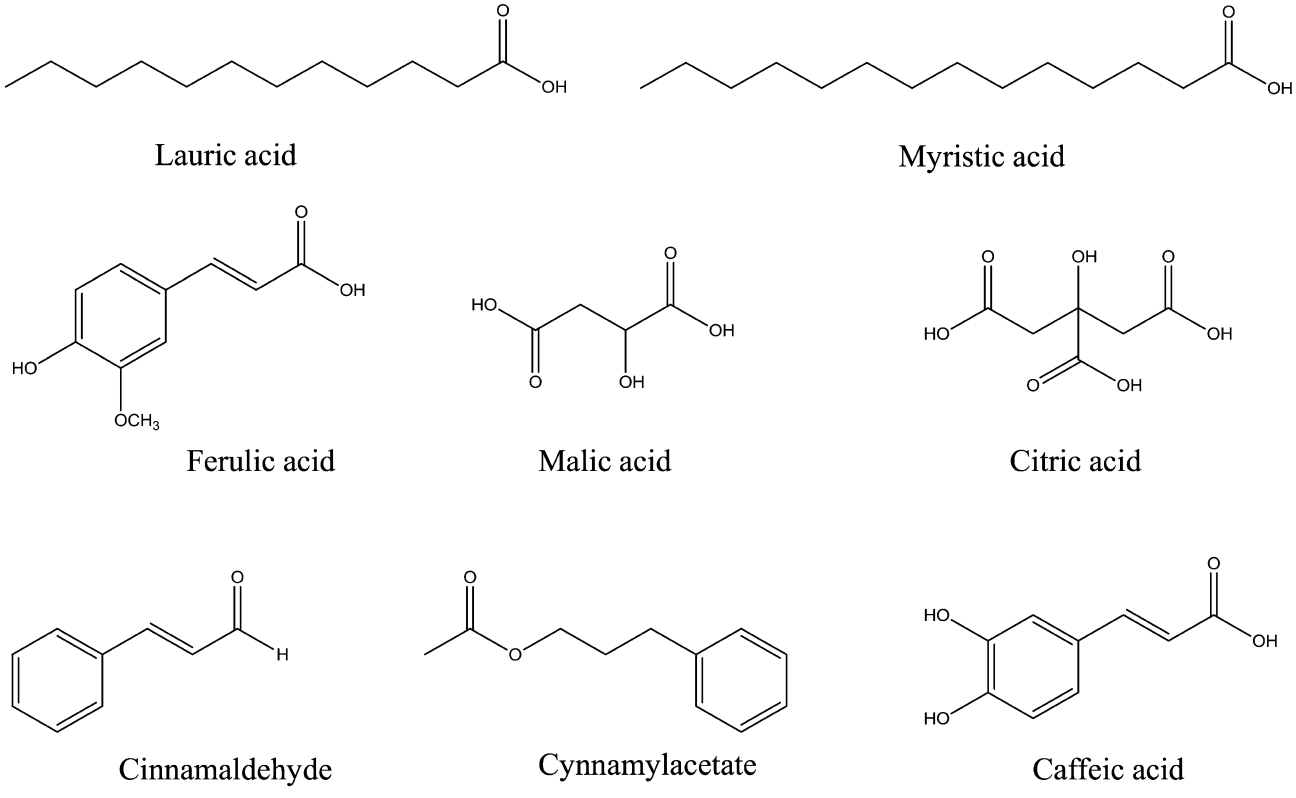


Fig. 5 Structure of other some bioactive compound present in Jamun (*S. cumini*)

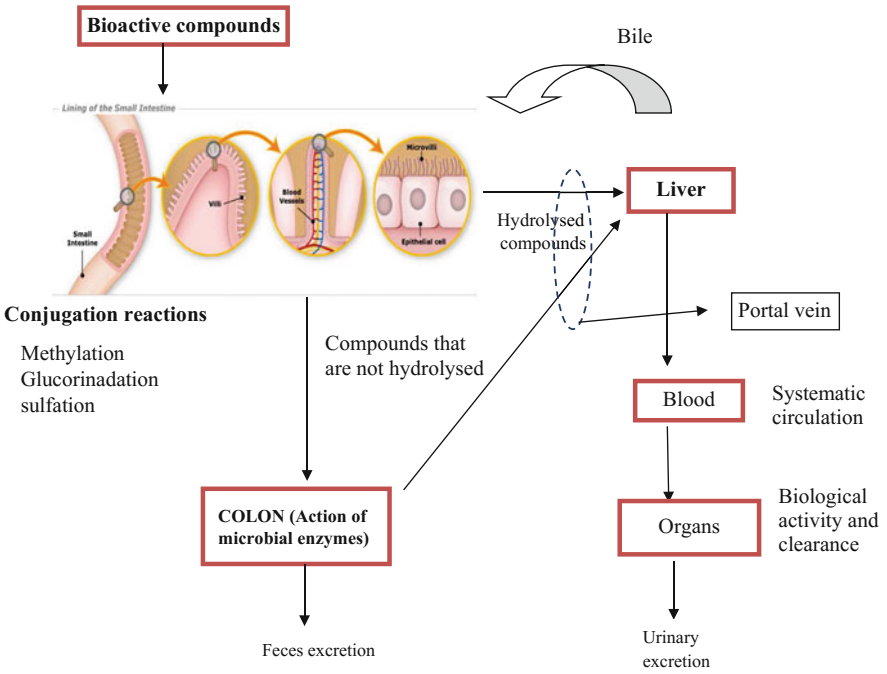


Fig. 6 General metabolic pathway of bioactive compounds in lower human anatomy [25, 26]

Fig. 6 shows the general metabolism in human anatomy. Vinicyus Teles Chagas et al. [24] have reported that some bioactive compounds target different metabolic pathway, thus acting as potential pharmacological tools. In contrast to this they have taken the example of compounds like myricetin, quercetin, rutin, ellagic, and gallic acids act as potential multitargeted drugs on distinct pathways of cardiometabolic disorder. Quercitin and rutin act as an antihypergylcemic by blocking L-type calcium channels on pancreatic beta cell, thus affecting the stimulus of insulin secretion. Vasorelaxant and antihypertensive properties of quercetin and myricetin follow the same path. Myceritin also acts on GLUT-4 expression in both skeletal muscles and adipose tissue and thus improves glucose homeostasis. Phenolic acid such as ellagic acid and gallic shows antihyperlipidemic properties by inhibiting 3-hydroxy-3 methly-glutaryl (HMG)- CoA reductase in liver.

4 Health Benefits of Jamun

4.1 Antimicrobial Activity

Patel and Rao [27] have studied on the antimicrobial activity of Jamun pulp. Four grams positive bacterial cultures, namely, MTCC-430 *Bacillus cereus* (BC), MTCC-121 *Bacillus subtilis* (BS), MTCC-106 *Micrococcus luteus* (ML), and MTCC-435

Staphylococcus epidermidis (SE), and four grams negative bacterial cultures, namely, MTCC-443 *Escherichia coli* (EC), MTCC-109 *Klebsiella pneumoniae* (KP), MTCC-735 *Salmonella paratyphi* (SP), and MTCC-734 *Salmonella typhi* (ST) were used. They found that the diethyl ether extracts gave high percentage of inhibition against the organisms tested followed by methanol, water, acetone, and ethyl acetate fractions. The activity of the extracts varied along with the fruits maturity, signifying the role of maturity indices in accumulation of bioactive compounds.

Gowri SS et al. [6] have reported that the Jamun leaves extracted in methanol and water have inhibitory activity against clinical isolates of the gram negative bacteria such as Salmonella *enteritidis*, *Salmonella typhi*, *Salmonella typhi A*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa*, and *Escherichia coli* and Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* using the disc diffusion method. And the methanol extract was more potent than the aqueous extract.

The activity of leaf oil and leaf methanol extract was found to be quite comparable with the standard antibiotics screened under similar conditions as shown in Table 2. They can be considered as good sources of natural antioxidants and antimicrobial compounds and can be incorporated into the drug formulations [7].

Table 2 Inhibition zones formed by *S. cumini* leaf essential oil and leaf extracts compared with standard antibiotic [7]

	Diameter of inhibition zones (mm/50 µL)									
	S. cumini leaf oil		Leaf extracts				Standard antibiotics			
Microorganisms	5%	1%	A	B	C	D	Tob 10 µg	Gen 10 µg	Oflo 10 µg	Cip 10 µg
1. *Bacillus cereus*	20	18	18	14	12	10	28	32	34	30
2. *Enterobacter Faecalis*	22	20	24	14	11	10	26	32	32	26
3. *Salmonella paratyphi*	20	18	22	16	11	10	25	30	28	30
4. *Staphylococcus aureus*	26	24	26	15	11	10	26	28	24	24
5. *Escherichia coli*	14	10	25	16	14	12	30	36	32	34
6. *Proteus Vulgaris*	24	20	24	18	12	11	26	30	24	32
7. *Klebsiella pneumonia*	20	18	20	14	12	11	26	32	32	36
8. *Pseudomonas Aeruginosa*	24	22	22	20	16	14	26	24	32	28
9. *Serratia Marcescens*	20	16	24	20	14	12	24	32	30	30

A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether
Tob: tobramycin; Gen: gentamicin sulfate; Oflo: ofloxacin; Cip: ciprofloxacin
Used concentrations: 50 µL of 5% and 1% essential oil samples in DMSO and 50 µL of 10 mg/mL of plant extracts

4.2 Antidiabetic Activity

Diabetes is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar, or glucose) or when the body cannot effectively use the insulin it produces. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. Diabetes caused 1.5 million deaths in 2012. Higher-than-optimal blood glucose caused an additional 2.2 million deaths, by increasing the risks of cardiovascular and other diseases.

Ethanol Jamun seed extract showed dose-dependent decrease in blood glucose level in streptozotocin-induced diabetes in rats and also reported the antiulcer activity of *S. cumini* against both physical (4 h pylorus ligation and 2 h cold restraint stress)- and chemical (aspirin and alcohol)-induced gastric ulcers in rats [28].

Glycemic control is beneficial in preventing the incidence and progression of diabetic retinopathy, loss of vision, and need for photocoagulation treatment (Diabetes Control and Complications Trial Research Group, 1993). Treatment with *S. cumini* (200 mg/day of lyophilized powder for 8 weeks) prevented the development of diabetic cataract even after 4 months of alloxan (120 mg/kg, s.c) administered in rats. Rathi et al. [29] and Kumar et al. [30] have reported antidiabetic activity against streptozotocin-induced diabetes in rats by isolated compound "mycaminose" from *S. cumini* and suggested to have same the mechanism of action of glibenclamide.

Oral administration of 50 and 100 mg/kg of the aqueous and methanol extracts of roots, leaves, seeds, and barks of *S. cumini* in alloxan monohydrate (150 mg/kg i.p.) induced diabetic male Sprague Dawley (SD) rats for 21 days resulted in the significant reduction in blood glucose level and biochemical parameters in dose-dependent manner [31].

4.3 Cardioprotective Effects

Researchers have found that when blood sugars are abnormally high (hyperglycemia), this activates a biological pathway that causes irregular heartbeats – a condition called cardiac arrhythmia – which is linked to heart failure and sudden cardiac death. According to the World Heart Federation, people who suffer from diabetes are two to four times more likely to develop cardiovascular disease, compared with people who do not have diabetes.

Diabetic heart disease is caused by complex interactions that result from overlapping mechanisms. The driving forces are related to the phenotypic alterations associated with diabetes – in particular hyperglycemia, dyslipidemia, hypertension, and possibly insulin resistance. A vicious circle develops, leading to increased oxidative stress and enhanced glycosylation of several humoral and vessel wall proteins, which cause endothelial damage and structural changes in coronary arteries. In turn, damaged endothelial cells can become a source of ROS and reactive nitrogen species in addition to other factors, sustaining the proatherosclerotic

process. Production of reactive oxygen takes place through oxidative and non-oxidative phosphorylation with increase in glucose levels [32].

Traditionally, seeds of *S. cumini* have been using in Ayurveda and Unani to fight against diabetes. Neha Atale et al. [33] have studied the antiglycoxidative potential of *S. cumini* and the effect of *S. cumini* on glucose-induced cardiac stress was observed, and they found that the methanol seed extract showed maximum potential compared to aqueous and ethanol extract and significantly suppresses the glucose-induced stress on H9C2 cardiac cell lines by inhibiting glycation event.

4.4 Anti-Inflammatory and Wound Healing Activity

It is found that the formulations (10% ointment) of crude ethanolic extract of *S. cumini* bark have accelerated healing effect than the control Nitrofurazone ointment (0.2% w/w, Smithkline – Beecham) in deep burn wound model in Albino rats [34].

Kumar et al. [35] have studied on the anti-inflammatory effect of ethyl acetate and methanol extract of Jamun seed. The extracts did not exhibit any mortality up to the dose level of 2000 mg/kg and were found to significantly inhibit the carrageenan-induced rat paw edema, a test which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation. The methanol extract at the dose of 400 mg/kg showed high significant anti-inflammatory activity at 4 h, where it caused 62.6% inhibition, as compared to that of 5 mg/kg of diclofenac sodium.

The root bark ethanol extract that are successively fractionated into petroleum ether fraction, chloroform fraction, n-butanol fraction, and methanol fraction was evaluated for the antinociceptive activity by acetic acid-induced writhing test and formalin-induced nociception test, and anti-inflammatory activity was screened by carrageenan-induced rat paw edema, cotton pellet-induced granuloma formation, and adjuvant-induced arthritis in rat models [36]. At 400 mg/kg b.w., p.o. dose petroleum ether fraction significantly inhibited 54.28% writhing response and 73.77% formalin induced nociception in mice. The fraction with same dose showed significant 79.31% inhibition of carrageenan-induced rat paw edema, 57.78% antiproliferative effect, and 77.93% inhibition of adjuvant-induced arthritis. And the fractions were isolated with three compounds, namely, β-sitosterol, stigmasterol, and lupeol, which may be the bioactive compound responsible for anti-inflammatory and antinociceptive activity.

Leaves extract of Jamun showed the presence of chemical compounds with hydroxyl, ester, carbonyl, and olefin functionalities, exhibited dose-dependent anti-inflammatory activity in acute and chronic models, and showed anti-inflammatory activity in acute carrageenan paw edema and chronic granuloma pouch model administration in albino rats [37]. Considering that anthocyanins decrease cellular lipid peroxidation and oxidative stress, Donepudi et al. [38] hypothesized that Jamun fruit extract administration could protect against cholestatic liver injury and inflammation in mice; they found that Jamun fruit phytochemicals decreased hepatic inflammation and oxidative stress and protected against hepatocellular injury in mice.

4.5 Chemo Preventive

Chemotherapy used in prevention of and treatment for tumorigenesis is likely to cause many side effects. Extracts of Jamun can be used as an alternative for chemotherapy as they have shown to have cancer preventive properties and antioxidant potential as in Table 3.

4.6 Antiviral Activity

The leave extracts of *S. cumini* were studied for antiviral activity against two emerging and re-emerging contagious diseases: buffalo pox and goat pox. An outbreak of buffalo pox in domestic buffaloes, with high morbidity and significant production loss, was recorded in the Aurangabad district of Maharashtra State in India in November 2003 associated with several cases of human infection, particularly in milkers working with the affected herd [22]. Goat pox (SGPX) is probably the most serious infectious disease of small ruminants in many parts of the world. The disease inflicts substantial losses in terms of reduced productivity and lower quality of wool and leather [39]. *Syzygium cumini* leaves at their maximum nontoxic concentrations 1999.73 ± 0.50 μg/ml had inhibition 99.92% and 98.52%, in all cytopathic effect (CPE) inhibition assays for goat pox and buffalo pox, respectively [40, 41].

Influenza virus is a major health concern as they are significant human respiratory pathogens that cause both seasonal, endemic infections and periodic, unpredictable pandemics [42]. Annual outbreaks of influenza occur regularly in temperate regions of the world with remarkable seasonality, defined by peak incidence in the colder months of the year [43]. Avian influenza virus (H5N1), an RNA virus that belongs to the family *Orthomyxoviridae*, emerged in Hong Kong in 1997, causing severe human disease. It is the serotype that causes bird flu in 2004. Hot and cold aqueous leave and bark extracts of *S. cumini* showed significant virucidal activity (100% inhibition) that was further confirmed in virus yield reduction assay (~98–99% reduction) and by egg based in ovo assay against avian influenza virus (H5N1 serotype) [44].

4.7 Antiallergic Activity

HPLC analysis revealed that hydrolyzable tannins and flavonoids are the major components of the extract. Aqueous extract of *S. cumini* showed antiallergic effect and indicate that its antiedematogenic effect is due to the inhibition of mast cell degranulation and of histamine and serotonin effects, whereas the inhibition of eosinophil accumulation in the allergic pleurisy model is probably due to an impairment of CCL11/eotaxin and IL-5 production [45].

4.8 Central Nervous System (CNS) Activity

Kumar et al. [46] have worked on the Central Nervous System Activity of ethyl acetate and methanol extracts of Jamun (*S. cumini)* seed on Albino mice in rota rod

Table 3 Chemo preventive effects of phytochemicals in Jamun and its mechanism

Sr. No	Agent	Chemo preventive effects and the mechanisms operating
1	Oleanolic acid	(1) inhibits tumor promotion in mouse skin; (2) inhibits azoxymethane (AOM)-induced colonic aberrant crypt foci and multicrypt aberrant crypt/foci in a dose-dependent manner; and (3) suppress preneoplastic lesions induced by 1, 2-dimethylhydrazine in rat colon
2	Ellagic acid	(1) inhibitor of benzo[a]pyrene-induced pulmonary adenoma and 7,12-dimethyl benz[a]anthracene-induced skin tumorigenesis in Swiss mice; (2) topical application as well as oral feeding of ellagic acid rendered protection against 3-methylcholanthrene-induced skin tumorigenesis in mice and decreased tumor incidence, number of tumors, tumors per mouse, and tumors per tumor bearing animal; (3) topical application of ellagic acid and oral before a tumor-initiating by B[a]P 7,8-diol-9,10-epoxide-2 and promotion with 12-O-tetradecanoylphorbol-13-acetate inhibits the number of skin tumors per mouse; (4) ellagic acid applied topically to female CF-1 mice 20 min before each 12-O-tetradecanoylphorbol-13-acetate (TPA) treatment inhibits the inductions of epidermal ornithine decarboxylase activity, hydroperoxide production, and DNA synthesis and also inhibits the promotion of skin papillomas and carcinomas in the two-step initiation-promotion protocol; (5) topical application of ellagic acid simultaneously with phorbol-12-myristate-13-acetate (PMA) or mezerein results in significant protection against 7, 12-dimethyl-benz[a]anthracene-induced skin tumors in mice; (6) the levels of aryl hydrocarbon hydroxylase (AHH) activity in skin and liver and the extent of 3H–BP-binding to skin, liver, and lung DNA are decreased; (7) it is a potent inhibitor of benzo[a]pyrene metabolism and its subsequent glucuronidation, sulfation, and covalent binding to DNA in cultured BALB/C mouse keratinocytes; and (8) inhibits the epidermal microsomal aryl hydrocarbon hydroxylase (AHH) activity and of benzo[a]pyrene (BP)-binding to both calf thymus DNA in vitro and to epidermal DNA in vivo
3	Gallic acid	(1) inhibits the TPA-induced inductions of epidermal ornithine decarboxylase activity, hydroperoxide production, and DNA synthesis and also inhibits the promotion of skin papillomas and carcinomas in the two-step initiation promotion protocol; (2) administering (0.3% to 1%) for 20 consecutive weeks from 4 weeks of age to the male TRAMP mice (a transgenic mice develops prostate tumor) caused decrease in tumor cells with decreasing the proliferative index with a concomitant increase in the apoptotic cells which were due to decrease in the expression of Cdc2, Cdk2, Cdk4, Cdk6, cyclin B1, and E
4	Quercetin	(1) possesses chemopreventive effects against 4-nitroquinoline 1-oxide-induced and its administration during both initiation and post-initiation phases caused a significant reduction in the frequency of tongue carcinoma in rats. It reduced the polyamine levels and the proliferation; (2) prevents N-nitrosodiethylamine-induced lung tumorigenesis in mice; (3) prevents 20-methyl cholanthrene-induced cervical neoplasia in virgin Swiss albino mice by increasing the antioxidant enzymes, decreasing DNA damage, and the lipid peroxidation; (4) decreases DMBA-induced DNA damage; (5) in a bioengineered human gingival epithelial tissue, quercetin was observed to inhibit BaP-DNA binding, a precursor for mutagenesis and carcinogenesis; (6) quercetin supplementation prevents benzo(a) pyrene-induced carcinogenesis by modulating the antioxidants and decreasing lipid peroxidation, aryl hydrocarbon hydroxylase, gamma glutamyl transpeptidase, 5′-nucleotidase, lactate dehydrogenase, and adenosine deaminase

(*continued*)

Table 3 (continued)

Sr. No	Agent	Chemo preventive effects and the mechanisms operating
5	Myricetin	(1) inhibits epidermal growth factor (EGF)-activated cell transformation of JB6 cells by modulating DNA binding and transcriptional activity of STAT3, and mitogen-activated protein kinase (MEK)and inhibitor of neoplastic cell transformation and MEK1; (2) prevents TPA-induced transformation, PKC activation, and c-jun expression in mouse fibroblast cells; (3) suppresses UVB-induced skin cancer by targeting Fyn in JB6 cells. Inhibits Akt survival signaling and induces bad-mediated apoptosis in immortalized human keratinocytes (HaCaT cells); (4) inhibits matrix metalloproteinase 2 protein expression and enzyme activity in colorectal carcinoma cells and also down-regulates phorbol ester-induced cyclooxygenase-2 expression in mouse epidermal cells by blocking activation of nuclear factor kappa B; and (5) inhibits polycyclic aromatic hydrocarbon-DNA adduct formation in epidermis and lungs of SENCAR mice
6	Kaempferol	(1) possess inhibitory effects on phosphatidylinositol 3-kinase and inhibits the neoplastic transformation
7	Betulinic acid	(1) topical application of betulinic acid inhibited the TPA-induced inflammation and decreased the levels of ornithine decarboxylase; and (2) markedly inhibited the 7, 12-dimethylbenz[a]anthracene and TPA promoted skin tumor formation in mice
8	β- sitosterol	(1) topical application of β-sitosterol inhibited the TPA-induced inflammation; (2) induces dose-dependent growth inhibition, induces apoptosis, and suppresses the expression of β-catenin and PCNA antigens in human colon cancer cells (COLO 320 DM cells); and (3) β-sitosterol supplementation reduced the number of aberrant crypt and crypt multiplicity in DMH-initiated rats in a dose-dependent manner with no toxic effects
9	Delphinidin	(1) suppresses 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cell transformation and activator protein-1 transactivation in the JB6 cells by blocking the phosphorylation of protein kinases in the extracellular signal-regulated protein kinase (ERK) and the c-Jun N-terminal kinase (JNK) signaling pathways; (2) possess chemopreventive effects against prostate carcinogenesis in both in vitro and vivo study models; and (3) suppresses ultraviolet B-induced cyclooxygenases-2 expression through inhibition of MAPKK4 and PI-3 kinase

Source: Farrukh Aqil et al. [20], Swami et al. [10]

and actophotometer at the dose level of 200 mg/kg and 400 mg/kg. They found that the safety dose for animals to be 2000 mg/kg body weight and both the extracts significantly decrease the spontaneous loco-motor activity in mice thus, indicating central depressant effect.

4.9 Hepatoprotective Activity

Medicine or drugs that are consumed during medication to treat various diseases may get accumulated in the liver and cause damage, for example, paracetamol if given as overdose causes acute liver damage. Das and Sarma [47] have studied hepatoprotective effect in albino rats by ethanol extract of Jamun pulp (*S. cumini*). Hepatotoxin used was paracetamol. They found that the rats (induced with paracetamol) have significant hepatoprotective activity against hepatotoxin when 100 and 200 mg/kg/day of Jamun pulp extract was given, thus showing reduction in the serum levels of all liver enzymes and total bilirubin and an increase in the total protein.

5 Conclusion

Studies have suggested the usefulness of the bioactive compounds present in Jamun (*S. cumini*) in enhancing the various ailments associated with cardiac, gastrointestinal, and nervous system. Their pharmacological effects are attributed due to the presence of flavonoids, terpenes, alkaloids, phenyl propanoids, tannins, and lipids. Out of all the pharmacological effects, the antidiabetic activity is important. Based on their pharmacological property, this traditional medicinal plant with rich source of bioactive compounds must be carried out for further studies on phytochemical and clinical research for safer drugs that can be used for treating various diseases.

References

1. Pichersky E, Gang DR (2000) Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends Plant Sci 5:439–445. https://doi.org/10.1016/S1360-1385(00)01741-6
2. Forbey JS, Harvey AL, Huffman MA et al (2009) Exploitation of secondary metabolites by animals: a response to homeostatic challenges. Integr Comp Biol 49:314–328. https://doi.org/10.1093/icb/icp046
3. Mukhopadhyay K, Chaudhary B (2012) *Syzygium cumini* (L.) SKEELS: A POTENTIAL SOURCE OF NUTRACEUTICALS. Int J Pharm Bio Sci 2:2230–7605
4. Warrier P, Nambiar V, Ramankutty C (1996) Indian medical plants, vol 5. Orient Longman Ltd, Hyderabad, pp 225–228
5. Anulika NP, Ignatius EO, Raymond ES et al (2016) The chemistry of natural product : plant secondary metabolites the chemistry of natural product : plant secondary. Meta 4:0–8
6. Shyamala Gowri S, Vasantha K (2010) Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. Int J PharmTech Res 2:1569–1573

7. Reddy J, Jose B (2013) Evaluation of antibacterial and DPPH radical scavenging activities of the leaf extracts of Cassia fistula Linn from South India. Open Access Sci Reports 2:2–5
8. Mohamed AA, Ali SI, El-Baz FK (2013) Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* leaves. PLoS One. https://doi.org/10.1371/journal.pone.0060269
9. Mahmood C, Daulatabad JD, Mirajkar AM et al (1988) Epoxy and cyclopropenoid fatty acids in *Syzygium cuminii* seed oil. J Sci Food Agric 43:91–94
10. Swami SB, Thakor NSJ, Patil MM, Haldankar PM (2012) Jamun (*Syzygium cumini* L.): a review of its food and medicinal uses. Food Nutr Sci 3:1100–1117. https://doi.org/10.4236/fns.2012.38146
11. Sadawarte P, Pujari K, Sonawane S (2016) Potential food applications and health benefits of Jambhul (*Syzygium cuminii* L.) Indian J. https://doi.org/10.21048/ijnd.2016.53.3.5340
12. Sonawane S, Arya SS (2013) Antioxidant activity of jambhul, wood apple, ambadi and ambat chukka: an indigenous lesser known fruits and vegetables of India. Adv J Food Sci Technol 5:270–275
13. Vasi S, Austin A (2009) Antioxidant potential of Eugenia jambolana Lam. Seeds. J Biol Sci 9:894–898. https://doi.org/10.3923/jbs.2009.894.898
14. Paduch R, Kandefer-Szerszeń M, Trytek M, Fiedurek J (2007) Terpenes: substances useful in human healthcare. Arch Immunol Ther Exp 55:315–327. https://doi.org/10.1007/s00005-007-0039-1
15. Cho KS, Lim YR, Lee K et al (2017) Terpenes from forests and human health. Toxicol Res 33:97–106. https://doi.org/10.5487/TR.2017.33.2.097
16. Jäger W, Höferl M (2016) Metabolism of terpenoids in animal models and humans. In: Hüsnü Can Baser K, Buchbauer G (eds) Handbook of essential oil scince, technology and applications, 2nd edn. CRC Press/Taylor & Francis Group, Boca Raton, pp 253–280. https://doi.org/10.1201/b19393-10
17. Clifford MN, Brown JE (2006) Dietary flavonoids and health. In: Anderson OM, Markham KR (eds) Flavonoids: chemistry, biochemistry and applications. CRC Press/Taylor & Francis Group, Boca Raton, pp 319–370
18. Shashank K, Abhay K (2013) Review article chemistry and biological activities of flavonoids: an overview. Sci World J 4:32–48. https://doi.org/10.1155/2013/162750
19. Reginold Jebitta S, Jeyanth Allwin S (2016) Antioxidant activity, total phenol, flavonoid, and anthocyanin contents of Jamun (*Syzygium cumini*) pulp powder. Asian J Pharm Clin Res 9:361–363
20. Aqil F, Jeyabalan J, Gupta A, Sharma RJ, Sidana J, Singh IP, Gupta RC (2010). Chemopreventive potential of 'jamun' (Indian blackberry) against estrogen-mediated mammary carcinogenesis. In: Proceedings of the 101st annual meeting of the American Association for Cancer Research; 2010 Apr 17-21; Washington, DC. Philadelphia (PA): AACR; Cancer Res 2010;70 (8 Suppl): Abstract nr 5688
21. Kay CD (2006) Aspects of anthocyanin absorption, metabolism and pharmacokinetics in humans. Nutr Res Rev 19:137. https://doi.org/10.1079/NRR2005116
22. Singh RK, Hosamani M, Balamurugan V et al (2006) An outbreak of buffalopox in buffalo (Bubalus bubalis) dairy herds in Aurangabad, India. Rev Sci Tech 25:981–987
23. Rein MJ, Renouf M, Cruz-Hernandez C et al (2013) Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. Br J Clin Pharmacol 75:588–602. https://doi.org/10.1111/j.1365-2125.2012.04425.x
24. Chagas VT, França LM, Malik S, Paes AM de A (2015) *Syzygium cumini* (L.) skeels: a prominent source of bioactive molecules against cardiometabolic diseases. Front Pharmacol 6:1–8. https://doi.org/10.3389/fphar.2015.00259
25. Marín L, Miguélez EM, Villar CJ, Lombó F (2015) Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. Biomed Res Int. https://doi.org/10.1155/2015/905215
26. van Duynhoven J, Vaughan EE, Jacobs DM et al (2011) Metabolic fate of polyphenols in the human superorganism. Proc Natl Acad Sci 108:4531–4538. https://doi.org/10.1073/pnas.1000098107

27. Patel PR, Rao TVR (2012) Antibacterial activity of underutilized fruits of Jamun (*Syzigium cumini*). Intl J Curr Pharmaceut 4:36–39
28. Diamante L, Li S, Xu Q, Busch J (2013) Effects of apple juice concentrate, blackcurrant concentrate and pectin levels on selected qualities of apple-blackcurrant fruit leather. Foods 2:430–443. https://doi.org/10.3390/foods2030430
29. Rathi SS, Grover JK, Vikrant V, Biswas NR (2002) Prevention of experimental diabetic cataract by Indian Ayurvedic plant Extractst. Phyther Res 16:774–777. https://doi.org/10.1002/ptr.1064
30. Kumar A, Ilavarasan R, Jayach T et al (2013) Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. J Med Plant Res 2:246–249
31. Deb L, Bhattacharjee C, Shetty SR, Dutta A (2013) Evaluation of anti-diabetic potential of the *Syzygium cuminii* (Linn) Skeels by reverse pharmacological approaches. Bull. Pharm Res 3:135–145
32. Leiva Díaz E, Giannuzzi L, Giner SA (2009) Apple pectic gel produced by dehydration. Food Bioprocess Technol 2:194–207. https://doi.org/10.1007/s11947-007-0035-9
33. Atale N, Chakraborty M, Mohanty S et al (2013) Cardioprotective role of *Syzygium cumini* against glucose-induced oxidative stress in H9C2 cardiac myocytes. Cardiovasc Toxicol 13:278–289. https://doi.org/10.1007/s12012-013-9207-1
34. Nandagopal PD, Subramonian SJ, Ganthi AS, Sankar S (2011) WOUND HEALING ACTIVITIES OF EUGENIA JAMBOLANA LAM . BARK EXTRACTS IN ALBINO RATS. Abstract: Wound healing is physiological process , which takes place by body's natural regenerative capacity. Due to various reasons there may be delay in healing and thi. pp 112–116
35. Kumar A, Ilavarasan R, Jayachandran T et al (2008) Anti-inflammatory activity of *Syzygium cumini* seed. Afr J Biotechnol 7:941–943. https://doi.org/10.1016/S0367-326X(00)00325-7
36. Saha S, Subrahmanyam EVS, Kodangala C et al (2013) Evaluation of antinociceptive and anti-inflammatory activities of extract and fractions of Eugenia jambolana root bark and isolation of phytoconstituents. Brazilian J Pharmacogn 23:651–661. https://doi.org/10.1590/S0102-695X2013005000055
37. Pavan KK, Dharani PP, Narayana RA et al (2010) Anti-inflammatory activity of Eugenia jambolana in albino rats. Int J Pharm Bio Sci 1:8–11
38. Donepudi AC, Aleksunes LM, Driscoll MV et al (2012) The traditional ayurvedic medicine, Eugenia jambolana (Jamun fruit), decreases liver inflammation, injury and fibrosis during cholestasis. Liver Int 32:560–573. https://doi.org/10.1111/j.1478-3231.2011.02724.x
39. Babiuk S, Bowden TR, Boyle DB et al (2008) Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. Transbound Emerg Dis 55:263–272. https://doi.org/10.1111/j.1865-1682.2008.01043.x
40. Bhanuprakash V, Hosamani M, Balamurugan V, Singh RK, Swarup D (2007) In vitro antiviral activity of Eugenia Jambolana plant extract on buffalopox virus: conventional and QPCR methods. Int J Trop Med 2:3–9
41. Bhanuprakash V, Hosamani M, Balamurugan V et al (2008) In vitro antiviral activity of plant extracts on goatpox virus replication. Indian J Exp Biol 46:120–127
42. Rock KL, Kono H (2008) The inflammatory response to cell death. Annu Rev Pathol Dis 3:67–97. https://doi.org/10.1146/annurev.path
43. Meigs JB (2010) Epidemiology of type 2 diabetes and cardiovascular disease: translation from population to prevention – the Kelly west award lecture 2009. Diabetes Care 33:1865–1871. https://doi.org/10.2337/dc10-0641
44. Sood R, Swarup D, Bhatia S et al (2012) Antiviral activity of crude extracts of Eugenia jambolana Lam. Against highly pathogenic avian influenza (H5N1) virus. Indian J Exp Biol 50:179–186
45. Quideau S (2006) Flavonoids. Chemistry, biochemistry and applications. Edited by Øyvind M. Andersen and Kenneth R. Markham. Angew Chem Int Ed. https://doi.org/10.1002/anie.200685399
46. Kumar A (2007) Central nervous system activity of *Syzygium cumini* seed. Pakistan J6:698–700
47. Das S, Sarma G (2009) Study of the hepatoprotective activity of the ethanolic extract of the pulp of Eugenia Jambolona (Jamun) in albino rats. J Clin Diagn Res 3:1466–1474

Index

J.-M. Mérillon, K. G. Ramawat (eds.), *Bioactive Molecules in Food*, Reference Series in Phytochemistry, https://doi.org/10.1007/978-3-319-78030-6

Q